A mechanism emerges for the critical period hypothesis for estrogen treatment

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During the past few decades, a significant body of literature has emerged from the basic science community demonstrating neuroprotective effects and synaptic enhancements induced by estrogen in brain regions that mediate memory and cognition, such as the hippocampus (1). A parallel but less consistent literature emerged from clinical studies suggesting that estrogen treatment (ET) is protective against cognitive decline in surgically and naturally menopausal women (2, 3). However, the Women's Health Initiative (WHI) studies published in 2003 not only failed to support such positive effects, but, in contrast, reported an increased risk of cognitive decline and dementia with hormone therapy (HT) (4, 5). Although the WHI results generated great concern in the clinical research and basic science communities, they also galvanized both groups to address conflicting reports on benefits vs. risks of HT and inconsistencies between the preclinical and clinical findings (6). Recent analyses of the clinical literature (2, 3, 7) have supported a hypothesis that was initially proposed by Gibbs based on animal studies (8); ET or HT has to be administered soon after estrogen depletion as a result of a "window of opportunity" during which estrogen could exert positive effects. Currently, there is strong support in both the basic science and clinical research communities for what is now referred to as the critical period hypothesis, which posits that ET or HT must be administered relatively soon after ovarian estrogen is depleted to be neuroprotective and exert positive effects on brain circuitry and cognitive function (7). Although there are extensive data supporting the critical period hypothesis, the cellular mechanism(s) underlying the decreased response to estrogen with time are poorly understood. The report by Brann and colleagues in PNAS (9) offers compelling evidence for a mechanism underlying the critical period that centers on sustaining sufficient levels of estrogen receptor (ER)-α. In addition, Brann and colleagues link the same mechanism to underlying events that occur with natural aging, which further extends the translational relevance of their findings (9).

CHIP-mediated Degradation of ER-α

The authors have targeted the CA1 region of hippocampus, as it is known to be highly responsive to ET with respect to synaptic effects, cognitive enhancement, and neuroprotection (1). Estrogen exerts its cellular effects through binding to ERs, with ER-α and ER-β activating multiple signaling cascades through regulation of transcription in the nucleus and direct effects on synaptic structure and transmission (10). Brann's group demonstrated previously that long-term estrogen depletion (LTED) resulted in a failure of ET to provide neuroprotection against cerebral ischemia in CA1, which was accompanied by decreased levels of ER-α in CA1 (11). Following up on a previous study

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implicating a ligase referred to as the carboxyl terminus of Hsc70-interacting protein (CHIP) as the mediator of ubiquitin-proteasome degradation pathway for ER-α (12), they hypothesized that this pathway might be implicated in decreased ER-α in CA1. The logic behind their hypothesis was that, as CHIP binds only to unliganded ER-α (i.e., ER-α not bound to estrogen) and LTED results in an environment for ER-α that lacks sufficient estrogen for binding, then CHIP-mediated degradation of ER-α should be accelerated, leading to decreased levels of ER-α in CA1. They demonstrated that this is precisely what happens following LTED for 10 wk in young rats, with increased ubiquitination and degradation of ER-α mediated by CHIP, decreased ER-α levels, and a loss of estrogen-induced neuroprotection (9). If estrogen was administered to the rats throughout the 10 mo postovareictomy phase, neuroprotection was retained, CHIP-mediated degradation of ER-α was not elevated, and ER-α levels were sustained. Furthermore, if ET was given after 10 wk of LTED, it had no neuroprotective effects. Inhibiting proteasomal activity reversed the LTED-induced decrease in ER-α, further supporting the central role of this degradation pathway in the loss of estrogen effects beyond a critical period. In addition, they demonstrated that CHIP-mediated degradation of ER-α was similarly accelerated in normal aging, which explained the age-related loss of estrogen-induced neuroprotection observed in young and middle middle-aged rats.

Implications for the Critical Period Hypothesis

There are several important implications of these findings. First, they have revealed a molecular mechanism for the critical period hypothesis that provides potential therapeutic targets for sustaining healthy levels of ER-α in key brain regions. In addition, the mechanistic focus on ER-α is key, as we know that synaptic ER-α decreases with age in rat CA1, and is directly correlated with cognitive performance in nonhuman primates (13). Second, they have extended the notion of a critical period following ovariectomy in young animals to natural aging, which enhances the relevance of such a mechanism to the events immediately surrounding menopause in women. Their findings on aging also enhance the relevance of these data to the unexpected increase in vulnerability to dementia linked to HT in the WHI studies. In addition, their observed increase in mortality in the estrogen-treated aged animals is consistent with the notion that healthy cells respond positively to estrogen, whereas cells that are compromised by aging might actually be negatively affected by estrogen (14). Third, they demonstrate that this pathway is tissue-specific in that it occurs in CA1, but not uterus (9), suggesting that the brain has unique pathways and patterns of vulnerability that should inform therapies directed at the aging brain and cognitive decline. These data provide a strong molecular framework for both the loss of estrogen effects when the animal has an extended period in a low-estrogen environment, as well as retained beneficial effects if a sustained period of low

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estrogen is avoided through ET, i.e., a critical period.

Challenges and Unresolved Issues

Although this study provides a compelling mechanistic basis for the critical period hypothesis, there are several remaining issues that need to be addressed, particularly if we are to link these findings to cognitive aging and treatment strategies. First, we do not know why ER-α is being targeted for CHIP-mediated ubiquitination and proteasomal degradation. This process is generally observed when the target protein is pathologically phosphorylated or has an abnormal conformation, such as with CHIP-mediated degradation of tau (15). Apparently, only unliganded ER-α is vulnerable to this pathway, but is there an age-related pathological alteration of the structure of ER-α in the unligated state? If so, what is the nature of the structural pathology, and can it be prevented or reversed? Second, the data of Brann and colleagues (9) demonstrate increased ER-α degradation through this pathway with both LTED and natural aging, but the approaches used do not resolve which pools of ER-α are depleted and to what extent they are depleted. We do not know the degree to which the increased degradation affects ER-α–mediated regulation of nuclear transcription, synaptic events mediated directly by ER-α in CA1 synapses, or both. This is a critically important distinction, as synaptic ER-α decreases with age in rat CA1 (16) and has been directly linked to cognitive performance in prefrontal cortex of female rhesus monkeys (17). It is probable that nuclear and synaptic ER-α are decreased through CHIP-mediated degradation, but the link to cognitive aging is likely to depend primarily on alterations of synaptic ER-α, and targeting synaptic ER-α with novel therapeutic approaches will present unique challenges. Third, although the authors are to be commended for extending their model to natural aging, there are still important issues regarding species specificity to be resolved. For example, although synaptic ER-α decreases in rat CA1 with age (16), this is not the case for monkey CA1 or prefrontal cortex (17), Although we do not know what age-related alterations of ER-α occur in humans, the differences between rat and monkey suggest we should be conservative in extending the rat findings directly to menopause in women. Fourth, there are also important issues surrounding the targeted brain region. The difference between uterus and CA1 with respect to CHIP-mediated ER-α degradation suggests that there is a high degree of tissue specificity with respect to this pathway. Thus, brain regions other than CA1 need to be analyzed as well, such as prefrontal cortex. Such studies will extend the relevance of this pathway to humans, as prefrontal cortex is perhaps more sensitive to aging than CA1 and highly responsive to estrogen in nonhuman primates (13). Finally, this study further highlights the importance of ER-α, and to a lesser degree ER-β, in therapeutic strategies for hormone treatments. A fairly detailed picture of the signaling cascades activated by these receptors is emerging (10), which should provide multiple therapeutic targets that go beyond current approaches that are designed simply to replace estrogen.

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