

17 β -Estradiol activates ICI 182,780-sensitive estrogen receptors and cyclic GMP-dependent thioredoxin expression for neuroprotection¹

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SPECIFIC AIMS

This new research idea on estrogen was derived from our recent discovery that preconditioning-induced neuroprotection is mediated by cGMP-dependent new protein synthesis—in particular, expression of the redox protein thioredoxin (Trx). The original aim was to investigate receptor-mediated, cGMP-dependent induction of cytoprotective proteins as the possible molecular mechanism underlying estrogen-induced neuroprotection at physiological concentrations (<10 nM).

PRINCIPAL FINDINGS

It is known that 17 β -estradiol induces NOS1 and NOS3, increases the synthesis of •NO, and activates cGMP-dependent PKG signaling. However, it is not clear whether activation of NOS1 in neurons by estrogen would lead to an increase in Trx expression or play a role in estrogen-mediated neuroprotection. After confirming that 17 β -estradiol can concomitantly induce NOS1 and neuroprotection against oxidative stress in human SH-SY5Y cells at low nanomolar concentrations (<10 nM), we make the following interesting observations.

1. 17 β -Estradiol (<10 nM), the most potent estrogen, induced NOS1 and activated cGMP-dependent PKG and its signal transduction pathway to increase expression of the redox protein Trx for enhancing cell viability

2. In addition to induction of NOS1 and Trx, 17 β -estradiol induced MnSOD but not BDNF or HO-2

3. 17 β -Estradiol-induced antioxidative and cytoprotective effects were blocked by ICI 182,780, a selective inhibitor of estrogen receptor (ER), and by pharmacological agents against NOS1, guanylyl cyclase, PKG, and Trx reductase

4. ER-enhanced cell viability against oxidative stress may be linked to Trx expression

Viability against oxidative stress may be linked to Trx expression because the cytoprotective effect of 17 β -estradiol was significantly reduced by the Trx reductase inhibitor 5, 5'-dithio-bis(2-nitrobenzoic acid) (DTNB). Consistently, Trx (1 μ M) inhibited lipid peroxidation, proapoptotic caspase-3, and cell death during oxidative stress caused by serum deprivation. Pharmacological and molecular biological data indicate that cGMP-dependent expression of Trx may play a pivotal role in estrogen-induced neuroprotection. The present report is the first to link estrogen-induced neuroprotection to the observed chain of events following the activation of ICI 182,780-sensitive ER. The proposed neuroprotective pathway induced by estrogen at physiological concentrations includes induction of NOS1, activation of the cGMP-dependent protein kinase pathway, and sustained expression of Trx, a redox protein with potent antioxidative and antiapoptotic properties (**Fig. 1**).

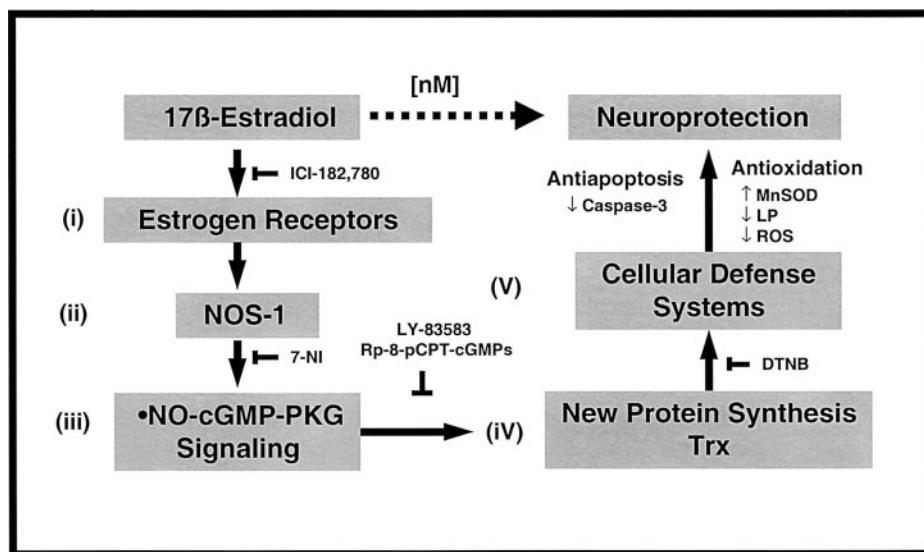
CONCLUSIONS

This original neuroprotection hypothesis of estrogen was based on gender differences in brain response to ischemia-reperfusion injury. 17 β -Estradiol has recently been shown to improve cognition in some postmenopausal women with senile dementia of the Alzheimer type. It was recently proposed that estrogen produces its neuroprotection through both receptor-independent and -dependent mechanisms. In ischemic animal models, estrogen may activate ER α and increase Bcl-2 for neuroprotection. Our unpublished RT-PCR results indicate that human brain-derived SH-SY5Y cells contain ER α and ER β (D. Huang, T. Andoh, and C. C. Chiueh, unpublished results). The present results reveal that low nanomolar concentrations of 17 β -estra-

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Figure 1. Proposed cGMP-mediated neuroprotective pathway of physiological concentrations of 17 β -estradiol in the human neuroblastoma SH-SY5Y cell model. Binding of nanomolar levels of 17 β -estradiol with ER α and ER β , which are sensitive to blockade by a selective receptor antagonist ICI 182,780 (i). 17 β -estradiol induces NOS1 in brain neurons and other cells, including SH-SY5Y cells (ii). Estrogen-induced expression of NOS1 leads to the formation of nitric oxide (\bullet NO) and activation of the cGMP and PKG-mediated signaling pathway (iii), which can induce synthesis of cytoprotective proteins such as the redox protein Trx (iv), thereby enhancing cellular defense systems (v). In addition to inhibition of lipid peroxidation (LP) and caspase-induced apoptosis, Trx induced MnSOD to suppress the generation of reactive oxygen species (ROS) in mitochondria. These results suggest that neuroprotection induced by physiological concentrations of estrogen (<10 nM) is mediated by both antioxidative and antiapoptotic actions produced by Trx and MnSOD. Consistently, the observed cytoprotective effects of 17 β -estradiol were blocked by selective inhibitors against ER (ICI 182,780), NOS1 (7-nitroindazole or 7-NI), guanylyl cyclase (LY-83583), PKG (Rp-8-pCPT-cGMPs), and Trx reductase (DTNB), respectively. Trx-overexpressing animals are less vulnerable in ischemia-induced brain injury. Trx also protects SH-SY5Y and PC-12 cells against severe oxidative stress and damage caused by 1-methyl-4-phenylpyridinium. cGMP-dependent induction of cytoprotective proteins such as Trx and MnSOD may play a pivotal role in enhancing cell viability to promote neuroprotection after treatment of human SH-SY5Y neurotrophic cells with 17 β -estradiol at nanomolar concentrations.



diol activated ICI 182,780-sensitive ER in SH-SY5Y cells, leading to protection against serum deprivation-induced oxidative stress and neurotoxicity. Binding of 17 β -estradiol to ER may promote a high affinity binding of ER–DNA binding domain homodimers to estrogen response elements for regulating genes in target cells. ER α and ER β play different roles in gene regulation. ER β rather than ER α mediates transcriptional activation of NOS1 by 17 β -estradiol. There is a severe degeneration of neuronal cell bodies throughout the brain, especially in the substantia nigra of 2-year-old ER β knockout mice. It is highly likely that ER β gene, protein, and related signaling transduction pathways may be necessary not only for up-regulating NOS1 but also for neuronal survival, thereby playing an important role in the pathogenesis of neurodegenerative diseases in the brain.

It is known that nitric oxide (i.e., S-nitrosoglutathione), 8-bromo-cGMP, and Trx each protect cells and brain neurons against oxidative stress. This study is the first to link the estrogen-induced neuroprotection to a nitric oxide-mediated chain of molecular events after the induction of NOS1 by 17 β -estradiol, such as activation of the cGMP-dependent protein kinase pathway and the delayed, sustained expression of Trx. In addition to suppressing lipid peroxidation, membrane permeable Trx-(S)₂ (1 μ M) blocked the catalytic activity of the proapoptotic caspase-3 induced by serum deprivation in SH-SY5Y cells. Moreover, the present results revealed that estrogen-mediated cytoprotection can be

prevented by the blockade of redox cycling of Trx from the inactive oxidized Trx-(S)₂ to active reduced Trx-(SH)₂ by a potent Trx reductase inhibitor, DTNB. Owing to its redox-active cysteinyl sites (-Trp-Cys³²-Gly-Pro-Cys³⁵-Lys-), reduced Trx-(SH)₂ profoundly modulates thiol-containing transcription factors (i.e., AP-1, NF- κ B) and enzymes such as caspases, NOS, Ref-1, ASK1, p53, and p21.

The present results further demonstrate that after induction of Trx, estrogen increased MnSOD expression in SH-SY5Y cells. MnSOD expression in mitochondria would augment antioxidative effects of Trx. This Trx-dependent neuroprotective mechanism of estrogen could foster research and development of selective ER modulators for neuroprotection devoid of female hormone activity and associated carcinogenesis. The present results thus infer that 17 β -estradiol-enhanced expression of Trx may be the missing link between NOS1 expression and neuronal survival after treatment with estrogen. The present results are consistent with our prior proposal that cGMP-dependent neuroprotection is mediated by Trx because it can be blocked by Trx mRNA antisense. It is concluded that the proposed ICI 182,780-sensitive ER mediate induction of NOS1, activation of the signaling pathway of \bullet NO-cGMP-PKG, thereby leading to the cGMP-dependent induction of cytoprotective proteins such as Trx and MnSOD and enhancing cell viability with the promotion of neuroprotection. FJ