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Brain Research

Research Report

Changes in the gene expression of estrogen receptors involved in the protective effect of estrogen in rat's trumatic brain injury

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ARTICLE INFO

Article history:
Accepted 12 May 2015
Available online 21 May 2015

Keywords:
Estrogen
Traumatic brain injury
ERα
ERβ
Gene expression
Female rats

ABSTRACT

It has been demonstrated that estradiol has neuroprotective effects after traumatic brain injury (TBI) in female rats. Since estrogen receptors have an important role in estradiol effects at the cellular level and the exact mechanism(s) of estradiol-induced neuroprotection has not yet been fully clarified, the present study was designed to determine the changes in the levels of estrogen receptors mRNAs and proteins involved in this phenomenon. All experiments were carried out on female Wistar rats. The brain edema and blood–brain-barrier (BBB) disruption were assessed. The TBI method was diffuse type and induced by the Marmarou method. Semiquantitative RT-PCR and immunoblotting were used to assess ERα and ERβ gene expression.

The data showed that the level of brain water content was significantly increased in TBI group. The increased water content was significantly attenuated in estradiol-treated (1 mg/kg) TBI rats. Disruption of BBB after TBI was significantly inhibited just by estradiol treatment. Estrogen-treated animals showed a significant increase in ERα mRNA (18%) and protein (35%) levels in the brain tissue. Furthermore, in the brain-injured rats the levels of ERβ mRNA were lower than those in control rats. Following estrogen treatment, the protein levels of ERβ were closed to those in control group.

In conclusion, the data demonstrate that estrogen treatment can protect brain against traumatic brain injury. Estrogen treatment increases ER mRNA and protein levels which

Abbreviations: TBI, traumatic brain injury; BBB, blood–brain-barrier; ER, estrogen receptors; OVX, ovariectomized; PBS, phosphate buffered saline; CNS, central nervous system; DMSO, Dimethyl sulfoxide; EB, Evans blue; TBS, tris-buffered saline; ANOVA, analysis of variance; GPER, G-protein coupled estrogen receptor; NO, nitric oxide

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http://dx.doi.org/10.1016/j.brainres.2015.05.017
0006-8993/

were coincident with its protective effects. It seems that such phenomenon participates in the induction of neuroprotective effects of estrogen.

This article is part of a Special Issue entitled 1618.

1. Introduction

Estrogen (E_2), an ovarian steroid hormone that serves as one of several acts, which not only regulates reproductive function but also growth, maturation, differentiation and other actions of body's tissues (Galea et al., 2006). E_2 is responsible for a variety of events that occur in the brain, such as the top acts of the brain mechanisms of pain, mood, sleep, Alzheimer's and Parkinson's disease, epilepsy and ready to ischemic injury (stroke) (Chakraborti et al., 2007; Wang et al., 2014). So, it has been reported that adult females compared to males have lower mortality and less neuronal damage following cerebral damage (Groswasser et al., 1998). Observations are increasing in that female sex hormones cause neuroprotection after trumatic brain injury (TBI), such as protecting the blood–brain-barrier and reduction of oxidative stress as well as edema after injury (Chen et al., 2005; Khaksari et al., 2011). Furthermore, it has been reported that E_2 has protective effects against brain injury and neurodegeneration. It has been reported that estrogen administration to ovariectomized rats reduces brain injury after focal ischemia (Jover et al., 2002). In addition, we previously found that estrogen can reduce brain edema and blood–brain barrier disruption as well as intracranial pressure after TBI (Shahrokhi et al., 2010).

E_2 can act through its intracellular receptors named receptor α, and β which exist in the brain (Merchenthaler et al., 2003). Involvement of these receptors in estrogen-induced neuroprotection has been suggested, so that the use of estrogen receptor antagonist can diminish the effect of estrogen on cerebral ischemia (Sawada et al., 2000). These receptors and their respective signaling pathways as well as their regulatory mechanisms are therefore subjects of intense research. It has been reported that estrogen receptor α (ERα) mediates estrogen neuroprotection (Dubal et al., 2001), while another study suggests that estrogen receptor β (ERβ) is also involved in this effect (Sampei et al., 2000). On the other hand, it has been shown that ERα and ERβ may act to modulate each other (Mazzucco et al., 2006)or act synergistically or antagonistic (Morales et al., 2006).

Estrogen receptors work synergistically in some tissues whereas act in opposite direction in other tissues (Tiwari-Woodruff et al., 2007). It has been shown that the neuroprotective effect of E_2 is mediated through ERα which accompanied with its anti-inflammatory effects (Morales et al., 2006; Suzuki et al., 2007). ERβ can modulate the activation of the ERα (Sinkevicius et al., 2008). ERα receptors have a dominant role in gene transcription in the cerebellum granule cells as compared to ER β (Gottfried-Blackmore et al., 2007). Changes in the expression of receptors α and β, after estrogen administration, or after cerebral ischemia have been reported so that 48 h after ischemia, a decrease in ERβ occurs in rats (Ezquer et al., 2008). ERα is also increased in female rats after cerebral ischemia (Westberry et al., 2008). Both estrogen and neuronal injury (global ischemia) can increase ERα expression, but have no effect on ERβ (Clipperton et al., 2008). In addition, it has been reported that estrogen reduces receptor α and β density in the mouse brain (Thakur and Sharma, 2007).

We have recently shown that the neuroprotective effects of estradiol on brain edema, BBB permeability, and neurological scores are mediated through both ERα and ER β (Asl et al., 2013). The present study was designed to determine whether estrogen has possible role in the regulation of ERα and ERβ expression in ovariectomized female rats after diffuse TBI?

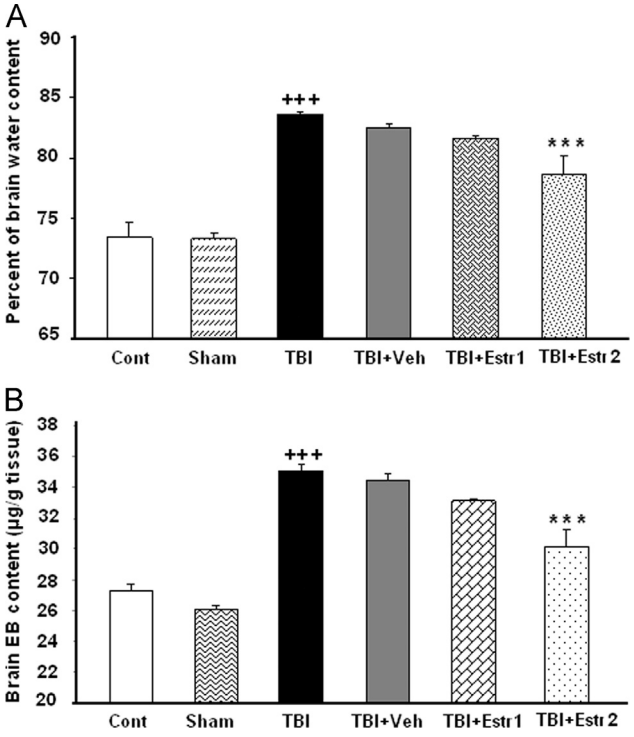

Fig. 1 – Effect of estrogen (E1=33.3 µg/kg and E2=1 mg/kg) on brain water (A) and Evans blue (B) contents. Data are expressed as mean±SEM ($n=7$). +++$P<0.001$ versus control group. ***$P<0.001$ versus TBI animals.