Role of Nuclear Estrogen Receptor Alpha in TrkB Signaling Following Neonatal Hypoxic Ischemic Encephalopathy

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BACKGROUND
There is increasing evidence that rapid non-genomic signaling via membrane localized extranuclear estrogen receptors may contribute to neuroprotection in the brain following ischemia. A link between estrogen receptor alpha (ERα) expression and tyrosine kinase B receptor (TrkB)-nerve growth factor receptor signaling is suggested by the finding that estradiol treatment induces phosphorylation of TrkB in the adult mouse hippocampi, and this response is absent in ERα null mutant mice. In this study, we hypothesized the TrkB signaling takes place through estrogen receptor localized in the membrane and not through nuclear ERα, in a neonatal mouse model of cerebral ischemia.

METHODS

Neonatal hypoxia ischemia (HI) was induced in P9 C57BLJ6/J male and female ERα wild type (WT) and “nuclear only” ERα mice (NOER mice) using Vannucci’s HI model. To assess TrkB signaling, mice treated either with TrkB agonist (7,8-dihydroxyflavone) or vehicle control (VC) starting 10 min post-HI, continued daily until sacrifice. Hippocampi were collected at 3 days post-HI. Western blot was performed to detect pTrkB(Y705) and total TrkB including full length (t-TrkB) and truncated TrkB (t-TrkB). All data were normalized to β-Actin using Image J. Multi-factorial ANCOVA that included genotype, sex, exposure type (HI or sham) and treatment (7,8-DHF vs VC) were conducted.

RESULTS

- Female neonates demonstrate greater phosphorylated TrkB expression compared to males after treatment with TrkB agonist, and this effect requires nuclear ERα.
- Membrane ERα is required for upregulation of the t-TrkB.
- TrkB agonist administration has no effect on the t-TrkB upregulation.
- HI might be inducing upregulation of different TrkB subtypes in male and female hippocampi through different mechanisms.

CONCLUSIONS

- p-TrkB expression in male hippocampi post-HI is increased compared to sham hippocampi.
- TrkB agonist therapy increased p-TrkB expression only in female hippocampi that lacks membrane ERα 3 days post-HI suggesting that the presence of nuclear ERα is required not membrane ERα.
- HI increased an increase in t-TrkB expression both in WT male and female hippocampi, TrkB agonist therapy did not have an effect on t-TrkB expression.
- HI failed to increase the t-TrkB expression in NOER mice hippocampi suggesting t-TrkB expression may require membrane ERα not the nuclear ERα.

ADDITIONAL KEY INFORMATION

Figure 1: Structures of TrkB isomers. There are three main domains which are extracellular domain (cysteine-rich, leucine-rich, cysteine-rich, and two immunoglobulin-like domains), transmembrane domain, and intracellular amino acid sequences. Truncated forms T1 and T2 possess 11 and 9 specific amino acid sequences, respectively.

Figure 2: Comparison of p-TrkB(Y705) protein expression levels between P9D3 sham, HI, and HI-T, male and female, WT (A) and NOER (B) mice after normalized to full-length TrkB and β-actin. Comparison of truncated TrkB protein expression levels between P9D3 sham, HI, and HI-T, male and female, WT (C) and NOER (D) mice after normalized to β-actin (n=2-8). (α compared to corresponding sham, # compared to corresponding HI, ● compared to corresponding HIT p<0.05)

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References: