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

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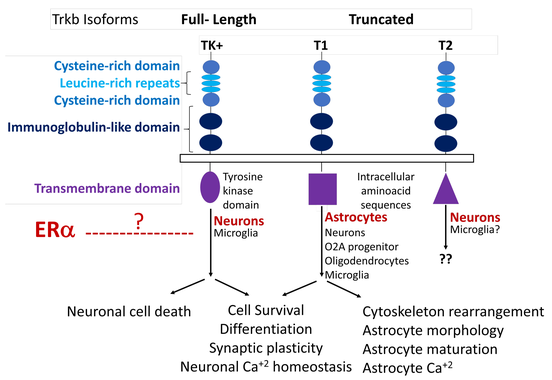
BACKGROUND

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[1]. 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.

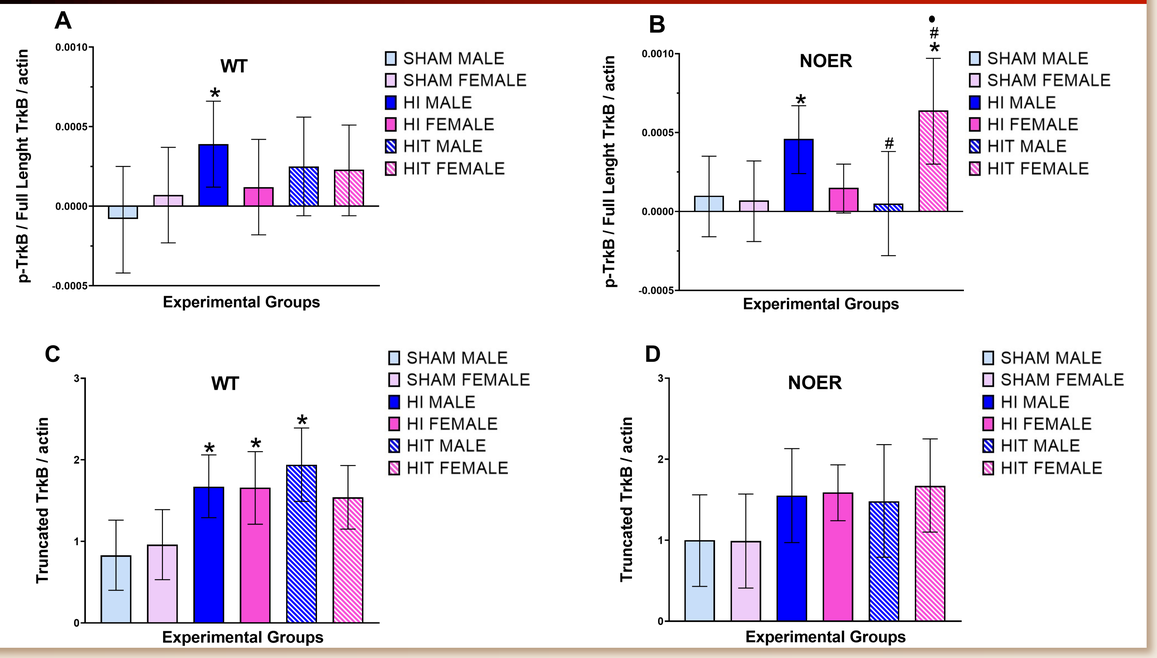
METHODS



Neonatal hypoxia ischemia (HI) was induced in P9 C57BL/6J male and female ERα wild type (WT) and "nuclear only" ERα mice (NOER mice) using Vannucci's HI model[2]. 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 (f-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.

- 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.

RESULTS



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 induced an increased 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 isoforms. 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).(∗ compared to corresponding sham, # compared to corresponding HI, ● compared to corresponding HIT $p<0.05$)

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References:
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2. Vannucci, R. C. A model of Perinatal Hypoxic-Ischemic Brain Damage. *Ann N Y Acad Sci.* (1997).