Regular Article

Age and 17β-estradiol effects on blood–brain barrier tight junction and estrogen receptor proteins in ovariectomized rats

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A B S T R A C T

Age and estrogen levels alter blood–brain barrier (BBB) tight junction (TJ) regulation, impacting brain homeostasis and pathological outcomes. This examination evaluated BBB TJ and estrogen receptor (ER) protein expression changes in young (8–10 week) and middle-aged (10–12 month) ovariectomized female Fisher-344 rats with chronic 17β-estradiol or placebo treatment. Middle-aged rats showed decreased protein expression of occludin with 17β-estradiol (55 kDa band) or placebo (45, 55, 60 kDa bands) treatment compared to respective young. In young animals, 17β-estradiol treatment increased expression of the occludin 55 kDa band over placebo; however, this effect was lost in the middle-aged animals. In both young and middle-aged animals, expression of claudin-5 (23, 32 kDa bands) and ERα (66 kDa) was significantly reduced in the middle-aged animals compared to young placebo treated animals. Measurement of BBB TJ permeability via in situ perfusion of 14C-sucrose showed no change with age or treatment. Our results show that increasing age and 17β-estradiol treatment alters the expression of ERα and distinct BBB TJ protein isoforms without altering functional paracellular permeability.

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Introduction

The blood–brain barrier (BBB) exists as a physical and metabolic barrier between the peripheral circulation and the central nervous system to maintain brain homeostasis. A primary component of BBB is the existence of highly specialized tight junctions (TJs). These TJs regulate the paracellular flux of hydrophilic molecules across the BBB. In aging populations, significant structural and functional changes in the BBB can contribute to negative pathological outcomes (Zeevi et al., 2010). Shifts in estrogen levels affiliated with the onset of menopause, as well as with hormone therapies, may further contribute to alterations in BBB function. To date, little is known as to the impact of aging and hormone treatment with regards to BBB TJ protein alterations.

The integrity of the BBB TJs is maintained by three key transmembrane proteins: occludin, claudins, and junction adhesion molecules (JAMs). Occludin is a tetraspanning membrane protein capable of regulating the TJs to changes in acute vascular dynamics (Sandoval and Witt, 2008). Claudins are a family of proteins, with claudins-5 identified as a primary regulator of TJ permeability (Piontek et al., 2008). Increased claudin-5 expression has been shown to decrease BBB paracellular permeability (Honda et al., 2006). In contrast, claudin-5-deficient mice show increased small molecule paracellular permeability (Nitta et al., 2003). Lastly, the JAMs are a family of immunoglobulin proteins. JAMs are involved in maintaining TJ integrity, signaling of cytoskeletal-associated proteins, and are also involved in leukocyte diapedesis (Mandell and Parkos, 2005; Weber et al., 2007). Homophilic JAM-A (a.k.a. JAM-1, JAM) interactions have been shown to stabilize cellular junctions (Mandell et al., 2004), while decreased JAM-A expression has been shown to correlate with loss of BBB TJ integrity (Yeung et al., 2008). It has become increasingly evident that changes/shifts in function and regulation of these respective TJ proteins contribute to BBB paracellular regulation and altered microvascular function.

There is strong evidence that the processes of aging and hormonal changes alter BBB TJ integrity, which may predispose individuals to enhanced negative outcomes subsequent to pathological stress (Bake et al., 2009; Bake and Sohrabji, 2004; Chi et al., 2006; DiNapoli et al., 2008; Selvamani and Sohrabji, 2010). In this regard, age dependent alterations in BBB integrity and use of estrogen based treatments have significant implications with regards to stroke outcomes. Stroke modeling has typically been evaluated in young ovariectomized (OVX) females, where estrogen treatments have been shown to reduce infarct volumes (Dubal et al., 1998; Simpkins et al., 1997). Yet, the impact of estrogen replacement in aging animals remains controversial, with some studies identifying reductions in infarct volumes (Dubal and Wise, 2001; Toung et al., 2004) and others identifying increased infarct volumes (Selvamani and Sohrabji, 2010). Thus, understanding alterations in BBB TJ proteins is critical in...