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Estrogen-IGF-1 interactions in neuroprotection: Ischemic Stroke as a case study

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Abstract

The steroid hormone 17 β -estradiol and the peptide hormone insulin-like growth factor (IGF)-1 independently exert neuroprotective actions in neurologic diseases such as stroke. Only a few studies have directly addressed the interaction between the two hormone systems, however, there is a large literature that indicates potentially greater interactions between the 17 β -estradiol and IGF-1 systems. The present review focuses on key issues related to this interaction including IGF-1 and sex differences and common activation of second messenger systems. Using ischemic stroke as a case study, this review also focuses on independent and cooperative actions of estrogen and IGF-1 on neuroprotection, blood brain barrier integrity, angiogenesis, inflammation and post-stroke epilepsy. Finally, the review also focuses on the astrocyte, a key mediator of post stroke repair, as a local source of 17 β -estradiol and IGF-1. This review thus highlights areas where significant new research is needed to clarify the interactions between these two neuroprotectants.

Introduction

Estrogen and IGF-1 share common actions on many tissues and this first noted in tumor biology. More than 40 years ago, it was recognized that while 17 β -estradiol promoted tumor formation *in vivo*, the hormone did not show significant mitogenic activity in tumor cell cultures. A seminal study using tissue extracts from intact, ovariectomized and 17 β -estradiol-replaced ovariectomized animals showed that tissues from estrogen-replete animals stimulate mitogenic activity in tumor cell lines, while tissues from estrogen-deficient (ovariectomized) animals did not (Sirbasku, 1978). This led to the hypothesis that estrogens effects on target tissue may be mediated by locally-produced growth factors. Subsequent research has confirmed that estrogens effects dependent on interaction with specific growth factor families. In the case of tumor cells, it was later shown that 17 β -estradiol interacts with epidermal growth factor (Roos et al., 1986).

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In the brain, 17 β -estradiol has also been shown to interact with several growth factors during development and adulthood. BDNF (brain-derived neurotrophic factor), a member of the neurotrophin family of growth factors, is a good exemplar. Both estrogen and BDNF have overlapping actions in the forebrain especially in the regulation of basal forebrain cholinergic systems. BDNF-synthesizing forebrain neurons colocalize the estrogen receptors (Miranda et al., 1993), and 17 β -estradiol replacement in adult, ovariectomized female rats increases BDNF expression in various forebrain regions (Singh et al., 1995; Sohrabji et al., 1995; Gibbs, 1999; Jezierski and Sohrabji, 2000; Jezierski and Sohrabji, 2001). The BDNF gene contains non-canonical estrogen response elements capable of binding estrogen-ligand complexes (Sohrabji et al., 1995). Estrogen and the neurotrophins stimulate common second messengers, such as the MAP kinases ERK1 and ERK2 (Singh et al., 1999). Estrogen may employ this growth factor as a mechanism to regulate neural cell function, in much the same way as 17 β -estradiol interacts with epidermal growth factor (EGF) to regulate uterine growth and function and enhances DNA synthesis in mammary epithelial cells (Ignar-Trowbridge et al., 1992). The expanding role for estrogen in cerebrovascular disease and neurodegenerative disease focused attention on another growth factor, namely, IGF-1. Insulin-like growth factor (IGF)-1 is a potent growth-promoting factor, and its proliferative and survival actions have been reported for virtually all cell types. The main source of IGF-1 is the liver, and secreted IGF-1 affects diverse organ systems. However, deletions of hepatic IGF-1 do not affect growth and differentiation of body tissues, suggesting that locally available sources of IGF-1 are capable of exerting similar growth effects (Yakar et al., 1999). IGF-1 consists of a 70 aa polypeptide coded by the *igf1* gene. The *igf1* gene resides on the long arm of chromosome 12 and consists of 6 exons with long intronic sequences. In humans, two IGF-1 products are synthesized, IGF-1A which consists of exons 1,2,3,4,6 and IGF-1B which consists of exons 1,2,3,4,5. In rats, the gene consists of 6 exons, where exons 3 and 4 compose the mature IGF-1 protein (Daughaday and Rotwein 1989; Hoyt et al., 1992), also called IGF-1A. Alternate splices that contain exon 5 produce a peptide called IGF-1B. Moreover, alternate transcription start sites located in exon 1 and 2 and alternate polyadenylation sites results in several IGF-1 transcripts.

Modifications related to the N-terminal and C-terminal ends of the IGF-1 peptide produce significant biological effects. Post-translational modification of the IGF-1 peptide, where the N-terminal tripeptide is cleaved, results in a truncated IGF-1 molecule, first discovered in bovine colostrum (Francis et al., 1988). This molecule, called -3N-IGF-1, is believed to be more functionally active than the non-truncated form, possibly due to its low binding affinity for the IGF-binding protein (Szabo et al., 1988). Subsequently, the truncated tripeptide itself was demonstrated to have independent neuroactive functions. The N-terminal peptide or GPE (glycine-proline-glutamate) has been shown to inhibit glutamate binding to the NMDA receptor and potentiate the action of potassium on acetylcholine release in rat cortical slices (Sara et al., 1989). GPE has been shown to have neuroprotective properties in an experimental Parkinson's model (Guan et al., 2000) and in hypoxic injury (Svedin et al., 2007).

Formation of the mature IGF-1 peptide occurs with the cleavage of the C-terminal end of the IGF-1 propeptide. This C-terminal peptide, also called E-peptide, is coded on exon 4, 5 and

6. The E-peptide containing propeptide binds extracellular matrix with greater affinity, and this affinity is independent of the mature IGF-1 region (Hede et al., 2012). A muscle specific transgene of IGF-1E propeptide significantly enhances muscle regeneration after injury, while muscle specific mature IGF-1 does not enhance muscle regeneration but increases serum levels of IGF-1 (Rabinovsky et al., 2003). These and other studies support the idea that E-peptide containing propeptides may increase the bioavailability of IGF-1 at the site of synthesis via anchorage to the ECM.

While studies have focused closely on the role of the IGF-1 mature peptide in the CNS, the actions of GPE are poorly understood and the E-peptides are virtually unstudied. Furthermore, estrogen interactions with the propeptide, the E-peptide and GPE have not been explored. In view of their critical roles in injury and regeneration in other tissue, this remains an important area for future studies. This review will therefore focus on the mature IGF-1 peptide, which is far better studied.

I: Estrogen and IGF-1 interactions

A) Sex differences and the IGF-1 system—Sex differences in several somatic systems appear to be associated with IGF-1 and provide a rich literature for understanding the interaction of estrogen and IGF-1 in normal physiology. In the case of body composition, lean body mass (LBM) decreases with age in both men and women. However, lean body mass was positively correlated with IGF-1 levels in males but not in women. In fact, LBM was greater in women on hormone replacement therapy (HRT) while IGF-1 levels were higher in women who were not on HRT (Waters et al., 2003). Interestingly, the IGF-1 binding protein IGFBP3 also decreased with age, although the ratio of IGF-1 to IGFBP3 decreased faster in males than females (Waters et al., 2003). In a study of Thai children and adolescents, serum IGF-1 levels increased with age and peaked at 13-15 years in males, and a little earlier in females (11-13 years). After the peak concentrations, IGF-1 levels declines in males but remained high in females (Jaruratanasirikul et al., 1999). Similarly, a study of young children and adolescents in Turkey showed an earlier peak of IGF-1 in female adolescents as compared to males, and a significant correlation of sex steroids with IGF-1 levels (Kanbur-Oksuz et al., 2004). Both sex steroids and IGF-1 are important for longitudinal bone growth and represent another area of synergy between these two classes of hormones. In mouse models, however, skeletal dimorphisms were found to be independently regulated by both IGF-1 and sex steroids in a time dependent manner, suggesting that the cross talk between these two hormones is not the sole mechanism (Callewaert et al., 2010).

Cardiovascular system: In cardiac biology, sex differences in cardiac contractile function may also be IGF-1 dependent, rather than sex hormone dependent. In female C57 mice, where IGF-1 levels are usually higher than males, cardiac myocytes exhibit reduced peak shortening, longer time-to-peak shortening and intracellular Ca⁺⁺ clearance as compared to males. However in a severe liver IGF-1 deficient mouse (LID), where males and females have similar levels of IGF-1, sex differences in cardiac myocytes are abolished. Sex differences in Ca⁺⁺ regulatory proteins were specifically attenuated in IGF-1 deficient animals (Ceylan-Isik et al., 2011). Age-related changes in the cardiovascular system are also strongly modulated by sex differences in the IGF-1 system. While IGF-1 and its receptor

decline with age in both males and females, there was an 84% decline in IGF-1 and IGFR in male myocytes, while in females this loss was 40% for IGF-1 and 28-40% for IGF-1 receptors. IGF-1 reduction with age was associated with tissue injury, such as fibrosis along the left ventricular wall. However, fibrosis was significantly less in females at all ages compared to males (Leri et al., 2000). Thus the favorable cardiac function in older females, where estrogen levels are low, as compared to males may be a function of IGF-1 availability.

Skeletal System: Sex specific differences in bone mineral density (BMD) may also be tied to IGF-1 levels. Bone mass in the tibia vertebra and femur occurs earlier in females than males and BMD is higher in females than males in adolescence (5-9 weeks). This corresponds to the peak IGF-1 levels in females (3-5 weeks). In males IGF-1 levels peak at 9 weeks, which also corresponds to peak BMD. Males experience increases in IGF-1 and IGFBP3 before increases in serum testosterone levels although IGF-1 and testosterone levels are well correlated. In females, however, the correlation between serum IGF-1 and estradiol was weak (Fukuda et al., 1998). Thus sex differences in BMD and bone growth may be better associated with IGF-1 levels rather than gonadal steroids.

Hepatic System: In the liver, hepatocyte regeneration also shows an important interaction of sex and the IGF-1 system. Partial hepatectomy in the liver-specific IGFR knock out mouse significantly decreased hepatocyte regeneration in male mice, but not in females (Desbois-Mouthon et al., 2006). In the musculoskeletal system, mass and total ATPase activity reduced with age and this reduction is greater in males than females. However, circulating IGF-1 was reduced by 61% in males and only 21% in females. Paradoxically, liver IGF-1 mRNA was elevated in aging males (Severgnini et al., 1999).

Central Nervous System: Sex differences have also been noted in the IGF-1 system in the brain. In the developing cerebellum, the IGF-1 receptor IGFR-1 is elevated in the 1st postnatal week in males (P0-P7) and falls thereafter. In females, however, IGFR1 expression reaches peak expression at 2 weeks postnatally (P14) (Haghir et al., 2013). While it is not clear how this impacts cerebellar development, it is worth noting that males have more Purkinje neurons than females (Wittman and McLennan, 2011). Similarly, IGFR expression is highest in the right hippocampus of males at P7, and in the left on P14. Females, on the other hand express peak levels of IGFR at P7 in both hemispheres. Interestingly, laterality differences were seen in males and females but at different time points. These time course changes suggest that gender and laterality differences in IGF-1 may influence function and development of the hippocampus (Hami et al., 2012).

In summary, sex differences in IGF-1 function provide an important body of information relevant to interactions between these two hormone systems. Furthermore, this section also underscores variation across organ systems, with a greater impact of IGF-1 in the cardiac system and skeletal systems, as compared to the hepatic system and the CNS.

B) Estrogen and IGF-1: common second messenger systems—Cell signaling pathways such as the MAP kinases, the PI3kinase/AKT amplify the actions of growth

factors and hormones, and serve as a point of synergy and interaction for the estrogen and IGF-1 systems.

Ligand-bound receptor tyrosine kinases, such as the IGF-1 receptor, recruit PI3 kinase when activated. The subsequent phosphorylation of PI3- kinase, via the Ras GTPase, activates AKT, a serine-threonine kinase, which exerts survival and proliferation via multiple pathways. The PI3K-Akt pathway is the prototypic mediator of IGF-1 effects on neurons. Initially shown to recruit glucose transporters (GLUT4) to the cell surface to internalize glucose, this pathway has also been shown to mediate IGF-1's effect on cell survival by inhibitory phosphorylation of the GSK3b, which promotes neuronal apoptosis (Hetman et al., 2000; Li et al., 2000). Similarly, 17b-estradiol has also been shown to modulate PI3K-Akt activation. One of the earliest systems where the non-nuclear effects of estrogen were recognized was the vascular system, specifically through interactions with the PI3K/Akt pathway. Estrogen receptor (ER)-alpha has been shown to bind the regulatory subunit of PI3K, and 17b-estradiol treatment increases this association resulting in Akt activation and elevation of downstream genes such as eNOS (Simoncini et al., 2000).

An important downstream mediator of the PI3K/AKT pathway is MTOR (mammalian target of Rapamycin). Activated AKT inhibits the formation of the tuberous sclerosis proteins TSC1 and TSC2, thus allowing activation of MTOR. Once activated, MTOR plays an important role in activating proteins that regulate translation initiation rates such as the eIFBP and ribosomal protein S6 kinases (Petroulakis et al., 2006). Hence MTOR signaling integrates extracellular signals from growth factors and hormones to control protein synthesis, cell-cell progression and cell metabolism. MTOR signaling can alter critical cell processes such as A-beta and tau protein homeostasis, which impact cell death in Alzheimer's disease. IGF-1 has been closely linked to this pathway in skeletal muscle hypertrophy, and has been shown to promote muscle hypertrophy through an activated PI3K/Akt/MTOR pathway (Latres et al., 2005). While this pathway is poorly studied in the brain, IGF-1 has been shown to regulate the glucose transporter GLUT-3 in PC12 cells through the PI3K/Akt/MTOR pathway via the transcription factor HIF-1a (Yu et al., 2012). Similarly, IGF-1 regulation of MCT2 the monocarboxylase transporter that facilitates lactate usage for energy is also dependent on this pathway. In cortical cells, IGF-1 increases MCT2 but not its mRNA, suggesting that its effects are at the translational, not transcriptional level. Rapamycin, which blocks mTOR abrogated IGF-1 action on MCT2 (Chenal et al., 2008). Several studies also link estrogen with this pathway, namely in peripheral estrogen sensitive tissues such as the uterus. The PI3k/AKT/mTOR pathway is critically involved with myometrial hyperplasia during pregnancy. Estradiol treatment, which is proliferative for uterine myocytes, induces IGF-1 in ovariectomized rats, which is then followed by an activation of the PI3K/AKT/mTOR pathway. Rapamycin blocks this hormone action on proliferation (Jaffer et al., 2009). Similarly, in the tumor cell line MCF-7, where both estrogen and IGF-1 are known to be critical mitogens, the PI3K-AKT-mTOR pathway appears to be the principal transducer of their actions. Estrogens proliferative actions on MCF-7 cells are mediated via IGF-1, and 17b-estradiol increases IRS-1 as well as p85 (the active subunit of the PI3K). Conversely, IGF-1 is proliferative only in steroid-treated cells (Bernard et al., 2006). Synthesis of IGF-1, which mediates tumor growth, is activated by IGF-1 and 17b-estradiol through this pathway and may be blocked by rapamycin (Martin

and Baxter, 2007). In fact IGF-1 may also employ this pathway to reduce or enhance the actions of ovarian hormones. IGF-1 decreases progesterone receptor in MCF-7 cells, via mTOR, while inducing ligand independent estrogen receptor function (Cui et al., 2003). This mechanism has not been explored in the brain and may represent a new level of interaction of and estrogen receptor systems.

A second common pathway for estrogen and IGF-1 is the MAP kinase signaling family. Mitogen-activated protein kinases (MAPKs) family consists of extracellular signal-regulated kinase (ERK), p38, and c-Jun NH₂-terminal kinase (JNK), and their several isoforms. MAP kinases are serine-threonine kinases that mediate intracellular signaling for a wide variety of cellular activities, including proliferation, differentiation, survival and death (Dhillon et al., 2007). Activated MAP kinase phosphorylates a large number of proteins including transcription factors such as the estrogen receptor (reviewed in Lu and Xu, 2006). Activation of ERK1/2 has been shown to inhibit apoptosis in hypoxia (Buckley et al., 1999), growth factor withdrawal (Erhardt et al., 1999), osmotic stress (Foncea et al., 2000) and exposure to tumor necrosis factor (TNF) and Fas ligand (Tran et al., 2000). Alternately, ERK activation is also linked to cell death, especially in neuronal populations. For example, persistent activation of ERK is associated with glutamate toxicity in neurons, which can be attenuated with ERK1/2 inhibitors (Stanciu et al., 2000). Inhibition of the upstream kinase MEK1/2 protects against cell death in a 6-OHDA injury model (Kulich and Chu 2001) and intravenous injection of the MEK1 inhibitor also reduces infarct volume in a stroke injury model (Nakamura et al., 2001).

17 β -estradiol treatment in vivo results in a dose-dependent activation of ERK and Akt and has a synergistic effect on IGF-1 mediated activation of the pAkt/PKB pathway (Cardona-Gomez et al., 2002). In addition to increasing ER-IGFR dimers, 17 β -estradiol also promotes the association between the regulatory subunit of PI3K and the insulin receptor substrate (IRS)-1, thus increasing the possibility of activating the IGFR. The interplay between the estrogen and IGF-1 pathways may be maturation dependent. In postpubertal animals, ovariectomy and subsequent replacement with estradiol resulted in sustained activation of Akt and GSK3 β , however this effect is not seen in animals ovariectomized before puberty (Sanz et al., 2008).

Estrogen and IGF-1 are both potent regulators of cellular proliferation and survival. *In vivo*, 17 β -estradiol transiently activates the IGF-1 receptor through tyrosine phosphorylation, leading to an interaction of ER- α with IGFR-1 and a greater interaction with components of the PI3K pathway (Mendez et al., 2003). In a medial forebrain bundle injury that models Parkinson's disease, IGF-1 attenuates lesion effects and also mediates the neuroprotective effects of estrogen (Quesada et al., 2004). IGF-1 and estrogen both signal through MAPK and Akt pathways and Akt inhibitors blocked the survival effects of both 17 β -estradiol and IGF-1 (Quesada et al., 2008), implicating the Akt survival pathway in estrogen and IGF-1 mediated neuroprotection. Interestingly, in the same study, a MAPK inhibitor had no effect on estrogen and IGF-1 actions. In fact the actions of estrogen and IGF-1 on ERK activation and neuroprotection appear to be controversial. For example, hippocampal cell death due to ischemia is reduced by 17 β -estradiol pretreatment, and hormone treatment is shown to activate IGF-1 receptors and stimulate MAPK. Similarly, 17 β -estradiols effects on memory,

which are blocked by the JB-1 antagonist, is accompanied by a ERK activation as well as choline acetyltransferase, the acetylcholine-synthesizing enzyme (Witty et al., 2013), indicating that the MAP kinase pathway may be involved in the co-activation of 17 β -estradiol and IGF-1 systems. In tumor cells, IGF-1 activation of the MAP kinase pathway renders the cells insensitive to antiestrogen's such as tamoxifen (Zhang et al., 2011). However, other reports have shown that interaction between these two trophic agents, while neuroprotective, appears to suppress the ERK pathway. In the damaged substantia nigra, for example, co- treatment of 17 β -estradiol and IGF-1 reduces cell loss and suppresses ERK activation (Quesada et al., 2008). Similarly in an ischemic stroke model, infarct volume is reduced by estrogen and IGF-1 and is preceded by a suppression of ERK activation in the cortex (Selvamani and Sohrabji, 2010). One possible explanation to reconcile these disparate findings is that there may be a time dependent modulation of ERK; enhancement in the short term and suppression in the long term. Alternately, heterodimers of the estrogen and IGF-1 receptor, unlike ER/IGFR homodimers, may inhibiting ERK activation, which in turn has been shown to promote PI3K/AKT activation (Zhuang et al., 2007) (Figure 1).

II: Estrogen and IGF- neuroprotection

In experimental models of neurodegenerative diseases (Garcia-Segura et al., 2000; 2006), estrogen and IGF-1 also have overlapping neuroprotective actions. Both prevent excitotoxin-induced cell death in hilar neurons (Azcoita et al., 1999), and estrogen receptor antagonists can inhibit the actions of the IGF receptor. Similarly, recovery from excitotoxic lesions of the nigrostriatal dopaminergic pathway is improved by either estrogen or IGF-1, and the IGF-1 inhibitor JB-1 attenuates the protective actions of both estrogen and IGF-1 (Quesada and Micevych, 2004). In aging ovariectomized animals where estrogen replacement has been shown to improve memory function, estrogens effects can be abrogated by treatment with JB-1 (IGFR inhibitor), indicating that estrogen may exert part of its effects through the IGFR signaling pathway (Witty et al., 2013). Similarly, in an ischemic stroke model, JB-1 treatment abolishes the neuroprotective actions of estrogen (Selvamani and Sohrabji, 2010). In fact, IGF-1 can activate estrogen receptors in the absence of estradiol (Ma et al., 1994). Both *in vivo* (El Bakri et al., 2004) and *in vitro* studies show that estrogen can regulate the IGF-1 receptor (Cardona-Gomez et al., 2002). Estrogen treatments can also transiently promote dimerization of the estrogen receptor and the IGFR (Mendes et al., 2003), providing a substrate for synergistic interactions.

A) Ischemic Stroke: Effects of Estrogen—Cerebrovascular diseases such as stroke offer an instructive example of 17 β -estradiol and IGF-1 cooperative action. Stroke is one of the leading causes of adult disability and the fourth leading cause of mortality in the US. Stroke occurs disproportionately among the elderly, and among this group, more often in women than men. Stroke-related disability and institutionalization is also likely to affect women more than men (Lai *et al.* 2005). Newer studies suggest that the risk for stroke in middle aged women doubles that of men (Towfighi *et al.* 2007), and that women account for 60% of stroke-related deaths (Lloyd-Jones *et al.* 2010), even after normalization for age. The 5-yr stroke recurrence is disproportionately higher in females (20%) as compared to males (10%) in the 45-64 age range (Roger *et al.* 2011). A Canadian stroke registry study reported that 10% of women stroke patients were discharged to long term care as compared

to 5% men (Kapral *et al.* 2005), while the Danish National Registry study reported that women have more severe strokes than men although they exhibited a survival advantage compared to men, especially at advanced ages (Olsen & Andersen 2010).

Sex differences in stroke outcome in clinical studies are also reflected in experimental data. Female rats have a smaller infarct and better cerebral blood flow than age-matched males (Alkayed *et al.* 1998). However, although female mice sustain a much smaller infarct as compared to age matched males (Manwani *et al.* 2011), aged females showed significantly more mortality and poorer stroke outcomes as compared to older males. The female advantage seen in these suggests that estrogen may be protective for stroke. Using natural variations in circulating estrogen levels across the estrous cycle, Liao and colleagues (2001) showed that the extent of ischemic damage was inversely related to circulating levels of estrogen. In fact, replacement with 17 β estradiol and its inactive stereoisomer 17 α estradiol (Simpkins *et al.* 1997) as well as the conjugate equine estrogen preparation (McCullough *et al.* 2001) all reduce infarct volume in female animals. Exogenous 17 β -estradiol replacement is neuroprotective when given prior (Dubal *et al.* 1998); Selvamani and Sohrabji, 2010) or subsequent to the injury (Liu *et al.* 2005, Yang *et al.* 2003).

However, hormone effects on stroke outcomes are more complicated in the context of aging. Our studies have specifically addressed this reproductive transition phase by studying acyclic, middle-aged female animals (Johnson *et al.* 2006, Selvamani & Sohrabji 2010a, Selvamani & Sohrabji 2010b). Middle cerebral artery occlusion (MCAo) in this middle aged female results in a significantly larger infarct as compared to young females (Selvamani & Sohrabji 2010a), suggesting that the loss of ovarian hormones may accelerate age-related stroke severity. Paradoxically, 17 β -estradiol treatment to ovariectomized reproductive senescent females increases infarct volume, indicating that the aging brain may be refractory to estrogen-dependent neuroprotection (Selvamani & Sohrabji 2010a, Selvamani & Sohrabji 2010b). This was confirmed by another study where long term 17 β -estradiol replacement to aged females also failed to improve stroke (Leon *et al.* 2012). In aging females, the loss of estrogens is also accompanied by decreasing levels of other hormones, including IGF-1. In fact, post-stroke IGF-1 replacement to middle-aged females pretreated with 17 β -estradiol abrogates the neurotoxic effects of 17 β -estradiol in this group (Selvamani and Sohrabji, 2010b), indicating that collaborative actions of 17 β -estradiol and IGF-1 are critical for the aging brain.

B) Ischemic Stroke: Effects of IGF-1—Independently of estrogen, IGF-1 is also positively associated with stroke outcomes. IGF-1 and IGF-BP3 levels are reduced in stroke patients as compared to those reported in the literature for healthy individuals. Within the stroke population, plasma IGF-1 levels were lower in individuals with a large infarct (Schwab *et al.*, 1997), and stroke-associated mortality was lower in patients with higher IGF-1 levels (Denti-*et al.*, 2004). Conversely, higher IGF-1 levels as associated with improved stroke outcomes. In a study of 255 stroke patients, higher IGF-1 levels or a higher IGF-1/IGFBP3 ratio was associated with better functional outcome at 3 months. Interestingly, baseline severity was not different between high and low IGF-1 groups, suggesting that IGF-1 modulates stroke sequelae and not the immediate phase (De Smedt *et al.*, 2011). In a case cohort of older individuals in the Cardiovascular Health Study, neither

IGF-1 nor IGFBP-1 predicted MI or stroke, but low IGFBP-3 levels were associated with higher levels of coronary events. Analyzing non-fatal MI only, suggested that low IGF-1 and IGFBP-3 levels elevated the risk of these events (Kaplan et al., 2007).

In experimental models, also, exogenous IGF-1 treatment is neuroprotective and has been shown to reduce ischemic stroke injury in many species including rats, mice and sheep (Gluckman et al., 1992; Lee and Bondy, 1993; Johnston et al., 1996; Guan et al., 2001). IGF-1 promotes post stroke neuronal survival, myelination and angiogenesis (Smith, 2005; Wang et al., 2000), as well as stroke-induced neurogenesis (Yan et al., 2006) and progenitor cell proliferation (Dempsey et al., 2003). The peptide hormone is effective for stroke when delivered intravenously (Rizk et al., 2007), intracerebroventricularly or intranasally (Lin et al., 2009), as also peripheral administration of the N-terminal tripeptide of IGF-1 (GPE) (Shapira et al., 2009). Additionally, treatment with IGF-1, IGF-2 and IGF binding protein (IGFBP) ligand inhibitors, which displace and therefore increase bioactive IGFs from their binding protein, all improved infarct volume in MCAo models (Mackay et al., 2003).

Cerebral injury upregulates IGF-1 and its receptors, and this is believed to be a protective response. In the Mongolian gerbil, IGF-1 positive cells were detected in GABAergic interneurons in CA1 and in pyramidal and non-pyramidal cells of CA2/3, 12h and 24 h after ischemia. Four days later, this neuronal IGF-1 disappears and is replaced in astrocytes and microglia. The temporal pattern suggests that these support cells may act to prevent delayed cell loss mechanisms (Hwang et al., 2004). In a head contusion model, IGF-1 mRNA levels increased at the site of the injury during the first 72h after trauma (Sandberg-Nordqvist et al., 1996), underscoring the fact that IGF-1 systems are mobilized during cerebral injury. In asphyxia, which is a transient neural injury, IGF-1 and IGFBP-2 and -3 are elevated in glial cells (Gluckman et al., 1998), while in a hypo-ischemic model where animals were placed in 10% oxygen for 4 days, IGF-1 and IGFR mRNA was significantly reduced (Gaddipati et al., 1999). In the MCAo suture model, IGF-1 was increased in brain and plasma, whereas IGFBP-2 decreased in the brain. In brain microvessel endothelial cells, IGF-1 and IGFBP-2 were both elevated by OGD. However, exposing neurons to conditioned media from OGD exposed BMEC increased neuronal apoptosis, while conditioned media from untreated (normal) BMEC increased neuronal survival (Wang et al., 2013). Thus increased expression of IGF-1 per se may not always be protective. In rats, cerebral ischemia using a 2-vessel occlusion model increased IGFR in the CA1 CA3 and dentate gyrus of the hippocampus, however IGF-1 treatment, pre or post stroke was not protective in this model (Bergstedt and Wieloch, 1993).

C) Ischemic Stroke: Estrogen and IGF-1 interactions—Evidence supports the hypothesis that IGF-1 may mediate estrogen's neuroprotective effects. In a Parkinson's disease model, inhibiting IGFR abrogated the protective response to 17 β -estradiol, suggesting that steroid-mediated neuroprotection involves the IGF-1 signaling pathway (Quesada et al., 2004). In an experimental stroke model, JB-1 induced blockade of the IGF receptor in young females completely abolished the protective effect of 17 β -estradiol (Selvamani and Sohrabji, 2010b). In vitro studies have also confirmed that estrogen mediated neuroprotection is mediated by IGFR, such that the addition of JB-1 to the media

of human fetal neuroblast cells abolished the protective effects of 17 β -estradiol on peroxide and amyloid-induced toxicity (Luciani et al., 2012).

Interestingly, in a bilateral carotid artery occlusion model of stroke, loss of hippocampal cells in middle-aged females was reduced by either 17 β -estradiol or IGF-1, with no additional benefit from combining the two hormones (Traub et al., 2009). In the case of the sodium calcium exchanger in cortical neurons, however, combining the two compounds enhanced the independent action of 17 β -estradiol and IGF-1. Furthermore, this action was independent of the classical receptors, since neither estrogen nor IGF-1 receptor antagonists could prevent these effects (Sanchez et al., 2011). Similarly, both estrogen and IGF-1 enhance neurogenesis in the dentate gyrus of the hippocampus and the hormones appear to have a synergistic effect. The ER antagonist, ICI 182,780 blocked the actions of IGF-1 in the presence or absence of 17 β -estradiol implicating the estrogen receptor in IGF-1 mediated effects (Perez Martin et al., 2003). Conversely, in a kainate acid injury model, where both 17 β -estradiol and IGF-1 protected hippocampal hilar cells, an antagonist to either the estrogen receptor or the IGF receptor blocked estrogen's effects. Reciprocally, IGF-1's protective actions were inhibited by the ICI 182,780 compound, suggesting that IGF-1 acts through the estrogen receptor (Azcoitia et al., 1999).

Overall, the published studies support the conclusion that estrogen and IGF-1 are neuroprotective independently and collaboratively. An important area in need to greater study is the interaction of estrogen and IGF-1 during aging, where the cells that synthesize these compounds and respond to these compounds undergo metabolic and functional changes.

III: Estrogen and IGF-1 interactions: Post-stroke repair processes

Thus far, most studies examining the interactions of estrogen and IGF-1 have focused directly on neurons, however, in stroke, as in other neurovascular and neurodegenerative diseases, neuronal survival is directly impacted by changes in the vascular and immune systems. Within minutes of ischemic stroke, neurons deprived of oxygen and glucose undergo anoxic depolarization and succumb to cell death. Excitotoxicity caused by hypoperfusion also results in microvascular damage and damage to the blood brain barrier, initiating post stroke inflammation. The role of estrogen and IGF-1 has been investigated on some of these post stroke processes, although the interaction between the two systems is poorly understood.

A: Post-stroke repair processes: Blood brain barrier—The blood brain barrier consists of closely aligned endothelial cells, encased in basement membrane and supported by astrocytic endfeet and pericytes (Risau & Wolburg 1990, Abbott *et al.* 2006, Kulik *et al.* 2008, Haseloff *et al.* 2005) (Figure 2). The principal role of the blood brain barrier is to regulate the influx and efflux of cells, molecules and ions thus maintaining the homeostatic environment of the brain. Alterations in the blood brain barrier are thus likely to elevate the risk for stroke and severity, by increasing the influx of cytotoxic cells and proteins. Age increases blood brain barrier permeability (Kalaria 1999; Abbott 2000; Bake and Sohrabji, 2004; Farrall and Wardlaw 2007; Grammas et al., 2011), and is further increased with

neurologic disease such as vascular dementia and Alzheimer's disease (Farrall & Wardlaw 2007). Blood brain barrier disruption enhances infiltration of serum proteins into brain parenchyma (Loftspring *et al.* 2006) and triggers an inflammatory response. The presence of tight junctions between adjacent endothelial cells that act as a gatekeeper to blood borne toxins (Brightman & Reese 1969). Following stroke, blood brain permeability is enhanced in older animals and tPA, the thrombolytic compound approved for stroke treatment, further increases barrier permeability (Kaur *et al.* 2011).

While there is clear evidence of age-associated disruption of blood brain barrier, alterations in barrier function in estrogen-deficient conditions such as menopause or reproductive senescence are relatively understudied. Low estrogen levels associated with menopause or reproductive senescence will negatively impact barrier and endothelial function due to the known role of estrogen on this cell type. In most cases, estrogen's effects on blood brain barrier permeability appear to be protective. Specifically, 17 β -estradiol reduces barrier permeability following injury in young female rodents (Liu *et al.* 2005), reduces ischemic- (Chi *et al.* 2002, Chi *et al.* 2005) and VEGF-induced (Chi *et al.* 2004) leakiness of the blood-brain barrier in the cortex (Chi *et al.* 2002), and attenuates VEGF-mediated blood-retina barrier (Chen *et al.*, 2011) and tPA mediated barrier breakdown and hemorrhagic transformation in male rats (Li *et al.*, 2011). 17 β -estradiol treatment also increases glucose uptake and transport (Bishop & Simpkins 1995, Shi *et al.* 1997), reduces edema in an experimental stroke model by reducing the activity (O'Donnell *et al.* 2006) and abundance (Chang *et al.* 2008) of the Na-K-Cl cotransporter. Oral conjugate estrogens also reduce vascular lesions and leukocyte permeability caused by A-beta 40 and A beta-42 infusions (Rhodin *et al.* 2003). The ER-beta agonist DPN has been shown to reduce surrogate markers for blood brain barrier permeability in a stroke injury such as vasogenic edema and extravasation of IgG, although tight junction proteins and the water channel aquaporin 4 was not affected (Shin *et al.*, 2013). However, the synthetic estrogen ethinyl estradiol is shown to increase the endothelial permeability to albumin (Gammal & Zuk 1980) and 17 β -estradiol acts synergistically with myelin basic protein to cause mast cell infiltration into the brain parenchyma (Theoharides *et al.* 1993). Moreover, in estrogen-deficient middle aged female rats (reproductive senescence), there is a constitutive increase in the permeability of the blood brain barrier in the forebrain as compared to estrogen-sufficient young females (Bake & Sohrabji 2004), accompanied by increased perivascular IgG expression in hippocampus, a commonly used marker to assess barrier integrity in aging and disease dysregulation of junctional proteins in cerebral (Bake *et al.* 2009). In fact, cerebral microvessels from a small sample of pre and post-menopausal women also confirmed this reproductive age-related loss of junctional localization (Bake *et al.* 2009).

The effect of IGF-1 on blood brain barrier permeability is less studied and more controversial. In the spinal cord, topical applications of high doses of IGF-1 reduced the breakdown of the blood-spinal cord barrier and spinal edema and improve axonal repair in a traumatic injury to the T10-T11 segments (Sharma, 2005), while low dose IGF-1 was ineffective. In an acute demyelinating EAE model of multiple sclerosis, intravenous IGF-1 given over 8 days reduced permeability of the blood-spinal cord barrier and improved motor outcomes. Higher doses of IGF-1 were more effective than lower doses (Liu *et al.*, 1995).

However, other evidence indicates that IGF-1 treatment in an acute inflammatory may actually increase blood brain barrier permeability. Intraventricular injections of LPS in a neonate rat, which is an experimental model of periventricular leukomalacia, causes rapid dysregulation of the blood brain barrier, activation of microglia and astrocytes and recruitment of leukocytes to the brain. Low dose IGF-1 treatment in this model prevented cell death associated with this injury, however, IGF-1 also increased permeability of the blood brain barrier, recruitment of leukocytes and caused intracerebral hemorrhage (Pang et al., 2010). High doses of IGF-1 in conjunction with LPS increased mortality, indicating that the peptide hormone may anomalous effects in acute inflammation.

Cerebral edema due to cirrhotic liver disease and portal vein occlusion was not improved by IGF-1 treatment (Odena et al., 2012). In fact, in peripheral tissues, IGF-1 treatment is known to cause mild generalized and reversible edema. In a small study with 8 health participants, IGF-1 treatment resulted in greater vascular permeability in skin and retina as compared to controls (Hussain et al., 1995). In view of the limited number of studies examining IGF-1's actions at the blood brain barrier, it is not surprising that there is virtually no data on the interactions of estrogen and IGF-1 on this unique structure. This remains an important and understudied area of investigation for stroke neuroprotection. Since both estrogen and IGF-1 promote survival and proliferation of target cells, it is likely that dual treatment may improve survival of endothelial cells in microvessels, thus preserving barrier function.

B: Post-stroke repair processes: Angiogenesis—The formation of new vessels is an adaptative response to ischemic injury (del Zoppo & Milner 2006, Sonntag *et al.* 2007), usually initiated by hypoxia-induced upregulation of HIF-1 and VEGF which promote endothelial cell growth (Kanaan et al., 2006; Pugh and Ratcliffe, 2003). While usually devoid of blood, new vessels formed post-stroke provide an angiogenic niche that promotes neurogenesis (Palmer et al., 2000; Zhang et al., 2004; Chopp et al., 2007; Arai et al., 2011). Neurons and astrocytes within the neurovascular unit also secrete trophic factors such as IGF-1 (Li et al. 2010), VEGF (Ferrara *et al.* 2003), and PDGF-b (Marti *et al.* 2000, Jin *et al.* 2002, Beck & Plate 2009) to induce angiogenesis, which in turn enhances proliferation and differentiation of neuronal precursor cells to promote neurogenesis (Sun *et al.* 2003).

Estrogen promotes angiogenesis in many vascular beds (Rubanyi et al., 2002), and has also been shown to decrease free radical production, increase cell survival, and stimulate angiogenesis in cerebral endothelial cells (Krause *et al.* 2006). However much less is known about the effects of estrogen on stroke-induced angiogenesis. In a study examining the effect of estrogen on angiogenesis and stroke, 17 β -estradiol treatment increased Ang-1 and microvessel density prior to stroke (Ardelt *et al.* 2005), suggesting that the hormone may enhance angiogenic potential. In the peri-infarct area, 17 β -estradiol increased microvessel density at 10 days post stroke in ovariectomized female rats, although this was not associated with functional recovery (Ardelt *et al.* 2007). Subsequent studies also showed that this effect was dose dependent, in that post-stroke vessel density was greater with a 0.72mg estradiol pellet versus a low dose (0.18mg) or control pellet. The higher dose was also associated with functional recovery (Ardelt et al., 2012). Interestingly, in a study using male rats, 17 β -estradiol treatment failed to increase microvessel density in the peri-infarct

area, but preserved motor function in this group (Ulbrich et al., 2012). Thus estrogen-mediated angiogenesis may be dose and sex restricted.

The effect of IGF-1 on angiogenesis is well known although its role in injury-induced angiogenesis is limited. More than 20 years ago, it was shown that IGF receptors are present on vascular endothelial cells (Bar et al., 1988) and that IGF-1 increased endothelial cell migration and tube formation in collagen matrices (Nakao-Hayashi et al., 1992; Nicosia et al., 1994). IGFR is poorly expressed in peripheral tissue beds in the adult as compared to the neonate, however, tissue damage increased IGFR in endothelial cells (Hansson et al., 1989) and in microembolic injury in the heart (Kluge et al., 1995). Evidence shows that IGF-1 increases VEGF levels, the prototypic angiogenic growth factor (Bermont et al., 2000; Smith et al., 1999). In aging, angiogenesis is impaired and sprout formation of microvessels grown in collagen gels was impaired in aged mice as compared to young animals. Treatment with minimal serum media and IGF-1 improved sprout formation in aged microvessels, obliterating the difference between young and aged microvessels (Arthur et al., 1998). Among the earliest studies to examine the effect of IGF-1 on angiogenesis focused on the ischemic retina. IGF-1 injected intravitreally, in the ischemic eye, resulted in proliferation of blood vessels (Grant et al., 1993). In the IGF-1 homozygous knockout, the number of capillaries per muscle fiber in the diaphragm is reduced (Fournier and Lewis, 2000), while blockade of the IGFR reduced angiogenesis in a colon cancer model (Reinmuth et al., 2002). IGF-1 induces migration of endothelial cells and promotes tubular formation in combination with high glucose levels (Shigematsu et al., 1999). This latter finding is particularly worrisome since ocular neovascularization in diabetic retinopathy is a major public health problem and also cautions the wide application of IGF-1 as a therapeutic in disease such as stroke, where diabetes or metabolic disease is a frequent comorbidity.

The problem of post stroke angiogenesis is complicated by the fact that stroke occurs most often in older populations, where both steroid hormone levels and IGF-1 levels are deficient. The mechanics of angiogenesis in this group are poorly studied. In aging, there is a constitutive decline in the number/density of vessels in the cerebral cortex, and this is partly attributed to the decline of growth hormone and IGF-1 in this group (Sonntag et al., 1997). Moreover, ischemia-induced capillary angiogenesis is impaired in older animals (Ingraham et al., 2008). Interestingly, IGF-1 mRNA levels are not shown to decrease with aging, however IGF-1 protein levels do decrease (Sonntag et al., 1999) suggesting that there may be translational repression of IGF-1. In elderly ischemic patients, well established angiogenesis is positively correlated with higher IGF-1 levels (Schwab et al., 1997). In animal models, systemic IGF-1 increases vessel density, while exercise, which is known to promote angiogenesis, does not do so if animals have low serum IGF-1 levels. Similarly, brain injury stimulated angiogenesis is abrogated in the presence of an IGF-1 antagonist (Lopez-Lopez et al., 2004).

Thus while estrogen and IGF-1 both influence angiogenesis, there are very few studies that have directly examined this interaction either in vivo or in vitro. An example of such an interaction is seen in hormone-induced prostate cancer, where 17 β -estradiol or testosterone pellets to the tumor-prone Noble rat causes prostatic cancer. This treatment also induces VEGF and IGF-1 receptor expression in epithelial and endothelial cells (Wang and Wong

1998). Similarly, in breast cancer tissue, IGF-1 elevates urokinase plasminogen activator (uPA-1), which increases angiogenesis (Dunn et al., 2000).

C: Post-stroke repair processes: Inflammation—Neuroinflammation is a critical aspect of stroke and responsible for secondary cascades of cell death. Anti-inflammatory compounds such as minocycline, for example, have been shown to improve infarct volume in experimental stroke (Koistinho et al., 2007). Activation of microglial and astrocytes locally mediate the early inflammatory events in stroke, followed by recruitment of activated splenocytes to the ischemic brain. Subsequent to this early phase of immune activation is a later immunosuppressive phase that affects somatic health and recovery. Both estrogen and IGF-1 have direct and indirect actions on inflammatory cells, which are reviewed here, although the combined actions of both hormones have not been studied in the context of neuroinflammation.

Estrogen has pleiotropic effects on the inflammatory response. In specific tissues such as uveitis of the anterior chamber of the eye, (Miyamoto et al., 1999), carrageenan-induced pleurisy in the lungs (Cuzzocrea et al., 2000; 2001) and adjuvant-induced arthritis (Badger et al., 1999), estrogen appears to decrease inflammation. On the other hand, estrogen promotes prostatitis (Naslund et al., 1988), stimulates edema (Tchernitchin and Galand, 1983) and increases vascular permeability and influx of macrophages in the uterus (De and Wood, 1990; Kaushic et al., 1998). In *in vitro* models, 17 β -estradiol pretreatment attenuates LPS-induced superoxide release, phagocytic activity and an increase in iNOS in microglia (Bruce-Keller et al., 2000, Drew and Chavis, 2000; Vegeto et al., 2000; Vegeto et al., 2001). 17 β -estradiol pretreatment *in vivo* reduces activation of local microglia and peripheral monocyte expression following intraventricular injections of the bacterial pathogen, lipopolysaccharide (LPS; Vegeto et al., 2003) and subcutaneous injections of proteolipid protein in an experimental autoimmune encephalomyelitis model (Subramanian et al., 2003) in young adult female mice, and suppresses the inflammatory response in influenza A pathogenesis (Robinson et al., 2011). Similarly, estrogen receptor specific ligands such as tamoxifen and raloxifen are also able to suppress LPS-activation of microglial cultures and LPS activation of microglia *in vivo* in both males and females (Arevalo et al., 2012).

Both these compounds, as well as other selective estrogen receptor modulators (SERMS) also suppress LPS-induced cytokine release in astrocytes (Arevalo et al., 2012). Similarly, pre-treatment with 17 β -estradiol, HPTE (ER-a agonist/ER-b antagonist), PPT (ERa agonist) and DPN (ER-b agonist) suppressed IL-1 β and TNF α in astrocyte cultures derived from young adult (3-4 mos.) and middle aged females (9-11 mos., acyclic) (Lewis et al., 2008), suggesting that activation of either estrogen receptor in this cell type was anti-inflammatory.

However, estrogens action in *ex vivo* microglial cultures derived from either young adult or middle aged females are not uniformly immunoprotective. LPS treatment increased nitric oxide production in microglia derived from young females, as well as cytokines. However 17 β -estradiol treatment attenuated NO production, but failed to attenuate cytokine expression and MMP-9 activity. In contrast, 17 β -estradiol failed to suppress cytokine expression and NO in microglia from middle aged females, and paradoxically, increased

MMP-9 lytic activity in these cultures (Johnson and Sohrabji, 2004). These studies indicate that estrogen effect on the inflammatory response is modulated by other aging-related events in microglia. One possibility is the loss of IGF-1 stimulation with aging, which may alter the actions of estrogen.

IGF-1 also exhibits anti-inflammatory actions in non-neuronal cells. In muscle regeneration, IGF-1 mediated repair is associated with suppression of the anti-inflammatory response. In a transgenic mouse where local IGF-1 expression was restricted to muscle, IGF-1 reduced key components of the inflammatory pathway including high mobility protein group -1, the transcription factor NF κ B, and cytokines such as macrophage migration inhibitory factor (MIF), TNF- α and IL-1 β (Pelosi et al., 2007). In the brain, IGF-1 is produced by microglial cells and activation of human brain-derived microglia by LPS, poly I:C or interferon suppressed IGF-1 synthesis. Th2 cytokines did not effect IGF-1 production and a cAMP analog increased expression of this growth factor (Suh et al., 2013). Similar to estrogen treatment, IGF-1 treatment to astrocytes also suppresses the response to LPS. In vitro, IGF-1 gene therapy or exogenous IGF-1 decreased TLR4 receptor expression on astrocytes and attenuated nuclear translocation of the p65 NF κ B protein (Bellini et al., 2011). *In vivo* administration of IGF-1 also downregulates glial activation. In an experimental model of depression caused by ICV injections of LPS, animals experience sickness behaviors, loss of weight and feed intake and reduced social exploration, as well as elevation of prototypic inflammatory mediators IL-1 β and TNF- α . Pretreatment with IGF-1 however, reduced measures of depression and decreased inflammatory cytokines, as well as GFAP (Park et al., 2011). These data support the idea that IGF-1 exerts an anti-inflammatory effect in the brain and improves behavioral outcomes, such as depression, associated with neural inflammation. Compelling evidence of the protective role of microglial IGF-1 in stroke comes from a mouse model where microglial cells were destroyed using a mutant HSV and the antiviral ganciclovir. Ablation of proliferating microglia cells after induction of ischemic stroke increased infarct volume, decreased stroke-induced IGF-1 and elevated inflammatory cytokines in the infarcted hemisphere. Stimulating microglial cells with macrophage colony stimulating factor, on the other hand, increased IGF-1 synthesis (Lalancette-Hebert et al., 2007). Similarly, antagonists to microRNA, a class of translational repressors, that target the IGF-1 gene also increases IGF-1 in microglia (Selvamani et al., 2012). Besides its direct actions on local inflammatory cells, IGF-1 also reduces the inflammatory cascade by preventing the binding of monocytes to endothelial cells (Motani et al., 1996), which precedes their entry through the endothelial barrier.

Individually, IGF-1 and estrogen both appear to promote an anti-inflammatory phenotype acting on both microglia and astrocytes. Thus it could be assumed that both hormones would promote synergistic effects, however, little direct evidence of this interaction is available for inflammation. In skin injury model, investigators were able to dissociate the contribution of each hormone by examining the effects of IGF-1 replacement in ovariectomized WT and ER- α KO mice. IGF-1 replacement repaired skin wounds by suppressing inflammation and promoting reepithelialization. However, in the ER- α KO mouse, IGF-1 continued to promote reepithelialization, but did not promote healing and increased local inflammation

(Emmerson et al., 2012), suggesting that the inflammatory response may require the combined action of IGF-1 and ovarian steroids.

IV: Estrogen and IGF-1 interactions: Post stroke neurologic disease

Stroke can result in other neurologic diseases such as post-stroke depression and epilepsy. Stroke is a leading cause of epilepsy and factors that affect stroke severity may also contribute to epilepsy. In an animal model of temporal lobe epilepsy, IGF-1 treatment has been shown to attenuate seizure severity induced by kainic acid injections. IGF-1 also increased hippocampal neurogenesis and protected against kainic acid induced neurodegeneration (Miltiadous et al., 2011). In late gestation fetal sheep subject to cerebral ischemia by occlusion of the carotid artery, IGF-1 attenuated neuronal loss and also delayed the onset of seizures and reduced their frequency (Johnston et al., 1996).

In kindling, which is widely used as a model for epilepsy, neuronal excitability is linked to structural changes and synaptic reorganization within the hippocampus. In situ binding assays for the insulin and IGF receptors showed that I125 IGF-1 binding was significantly reduced in animals that received 100 kindling stimuli (Kalynchuk et al., 2002). The authors propose that this reduction in binding sites may be due to receptor occupation by increased availability of IGF-1 from local or peripheral sources, as has been shown in the case of other peptides (Roder et al., 1996). Interestingly, there appears to be no sex difference in the emotional response to kindling (Wintink et al., 2003), although sex differences have been noted in seizure models. Males display greater susceptibility to convulsants such as pilocarpine and kainic acid as compared females. Furthermore, gonadectomized males replaced with testosterone had increased seizure susceptibility, stronger and more frequent seizures as compared to those not replaced with testosterone. Interestingly, while testosterone enhances seizure occurrences, kainic acid-induced seizures decrease plasma testosterone, while elevating plasma corticosterone levels (Mejias-Aponte et al., 2002). Sex differences in epileptogenesis may also be linked to developmental/early stressors such as cortical developmental abnormalities, neonatal hypoxia, etc. Male and female rat pups subject to a two-hit model consisting of a freeze-induced cortical lesion at P1, and a prolonged hyperthermic seizure at P10 revealed striking sex differences. All males subject to this protocol developed mesial temporal lobe epilepsy when measured 3-4 months of age, while none of the females showed epilepsy (Desgent et al., 2012). While IGF-1 and IGFR expression was not reported in this study, peak IGFR expression in the hippocampus occurs earlier in females than males (Hami et al., 2012), indicating the availability of an early protective substrate in females. Similarly, sex differences are also evident in seizure behavior following ethanol withdrawal. Male and female mice subject to 4 cycles of ethanol vapor and subsequent withdrawal were tested for handling induced seizures. At the initial withdrawal, males and females showed a similar degree of seizure behavior, however, on subsequent withdrawal bouts, males had robust increases in seizure severity while females did not. Interestingly, this was not associated with estrogen levels or with sex differences in blood alcohol levels Veatch et al., 2007), although sex differences in blood IGF-1 levels have been reported (Chaler et al., 2009; Gupta et al., 2011).

Overall, the evidence supports the hypothesis that both estrogen and IGF-1 affect seizures and underscore their role in post stroke therapies for specific subsets of patients, although direct evidence for such actions is lacking. Besides epilepsy, other neurological consequences of stroke include depression and dementia, and neural targets for these diseases are also sensitive to estrogens and IGF-1. However, the interaction effects of IGF-1 and 17 β -estradiol on these post-stroke diseases are also poorly understood.

V: Estrogen and IGF-1: Astrocytes as critical cellular mediator

Although the largest amount of estrogen and IGF-1 is synthesized by peripheral sources such as the ovary and the liver respectively, local production of both hormones has been reported for several tissues, including the brain. The astrocyte is known to synthesize both 17 β -estradiol and IGF-1, and in view of its critical role in ischemic injury, and its proximity to ischemic neurons and the blood brain barrier (Figure 2), astrocytic secretions may be crucial for brain repair and recovery.

In the case of 17 β -estradiol, production of aromatase is central to synthesis of local hormone. Early studies established that aromatase is expressed in neurons in the rat and quail hypothalamus (Canick et al., 1986; Schlinger and Callard, 1989), areas essential for neuroendocrine function. However, the discrepancy between high aromatase activity and low neuronal expression of aromatase in non-hypothalamic areas pointed to another source for this enzyme, namely glia. Aromatase was detected in astroglial cells of the zebra finch forebrain (Schlinger et al., 1994), as well as radial glia in the rainbow trout (Menuet et al., 2003) and glioblastomas (Yague et al., 2004). Subsequently, aromatase expression as well as estradiol synthesis was confirmed in cortical astrocytes from neonatal rats (Zwain et al., 1997). In the hippocampus, no aromatase expression was detected in astrocytes in the uninjured animal, however, systemic injections of kainic acid or stab wound injury in the brain caused an injury-induced upregulation of aromatase in hippocampal astrocytes (Garcia-Segura et al., 1999). Similarly, cerebral ischemia results in rapid upregulation of aromatase in astrocytes in the rat (Carswell et al., 2005) and nitrosative stress upregulates aromatase in cultured fetal sheep astrocytes (Lepore et al., 2011). Pharmacological inhibition of aromatase exacerbates neuronal death following mild excitotoxic stimuli, suggesting that the induction of aromatase and consequent estradiol synthesis is neuroprotective (Azcoitia et al., 2003). Greater expression of aromatase in astrocytes from females is also associated with neuroprotective advantage in the case of ischemic stress or anti-oxidant induced cell death, although not when combined with inflammatory mediators (Liu et al., 2007). Glial aromatase is also thought to promote injury-induced neurogenesis in the zebra finch brain (Peterson et al., 2007).

Similarly, IGF-1 is also synthesized by astrocytes. In late embryonic development, IGF-1 is synthesized neurons, astrocytes and oligodendroglial precursors (Baron-Van Evercooren et al., 1991; Ballotti et al., 1987), underscoring the role of this growth factor in brain development. In a transgenic mouse where IGF-1 is overexpressed in astrocytes, there is marked overgrowth in the brain, including increased brain weight, DNA and protein concentrations, and neuron number (Ye et al., 2004). In glial tumors, IGF-1 expression appears to be correlated with tumor malignancy related to aberrant angiogenesis (Hirano et

al., 1999). In a cuprizone demyelinating model, IGF-1 is induced in astrocytes located in degenerating white matter (Komoly et al., 1992) and in the spinal cord in experimental autoimmune encephalitis (Liu et al., 1994). A similar increase in astrocytic IGF-1 is seen 3 days after ischemic injury in neonatal rats (Gluckman et al., 1992). In adult male rats, IGF-1 expression is seen in astrocytes in the areas surrounding the infarcted tissue, and in microglia within the infarcted region (O'Donnell et al., 2001). Since astrocytes are capable of synthesizing both 17 β -estradiol and IGF-1 and are positioned at critical targets such as the blood brain barrier and in proximity with ischemic neurons, cell-specific therapies actively targeting this cell can be exploited for new stroke pharmacotherapies. Specifically, epigenetic modifiers to increase transcription of the aromatase and IGF-1 gene and microRNA technologies to enhance translational of these proteins would be critical for development of the next generation of stroke therapies.

In summary—While the cooperative actions of 17 β -estradiol and IGF-1 have been demonstrated in vivo in stroke models, the cellular and molecular site of action and the second messengers required for these actions remain poorly understood. While evidence shows that both compounds promote neuronal survival by acting directly on neurons, there is no direct evidence for such actions in vivo. No doubt neuronal survival is enhanced by this process, however, it is likely that estrogen and IGF-1 actions converge on non-neuronal cells to promote neuronal survival. As this review shows, the endothelium may be one such site of convergence, promoting blood barrier integrity, angiogenesis and reduced inflammation.

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Highlights

- Estrogen and IGF-1 interactions are critical for normal development and response to neural injury
- Estrogen/IGF-1 co-treatment reduces infarct volume and improves recovery in experimental stroke
- Estrogen and IGF-1 separately influence post-stroke angiogenesis and inflammation
- New research should focus on estrogen-IGF-1 interaction on critical post stroke events
- Astrocytes may serve as a critical cellular source of local IGF-1 and estrogen in the ischemic brain

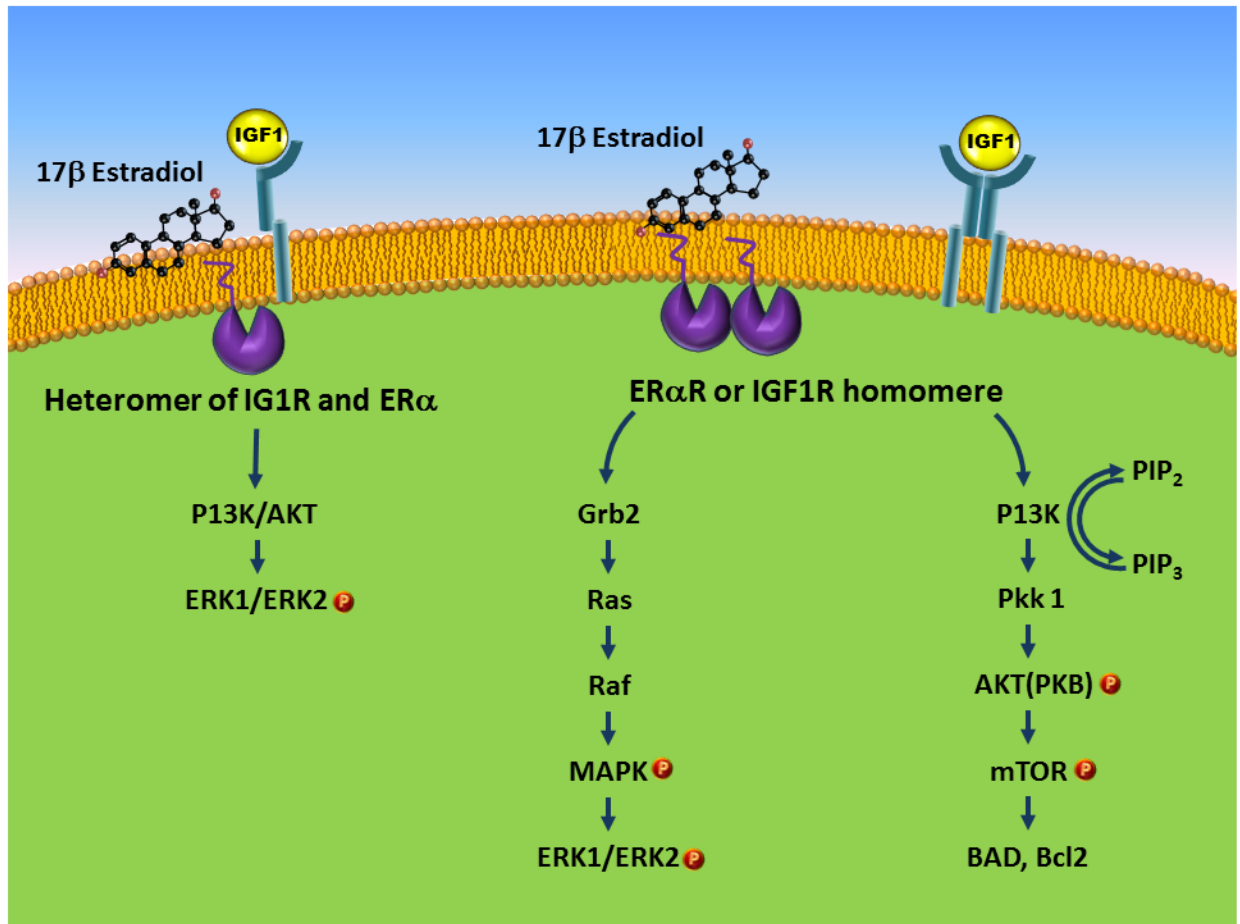


Figure 1. Schematic representation of the estrogen and IGF-1 receptor interaction with second messenger systems. Homodimers of ER- α and IGF1R can activate the MAPK pathway and the PI3K/AKT pathway. Co-treatment with estrogen and IGF-1, which can result in ER- α /IGFR heterodimers, have been shown to inhibit ERK activation.

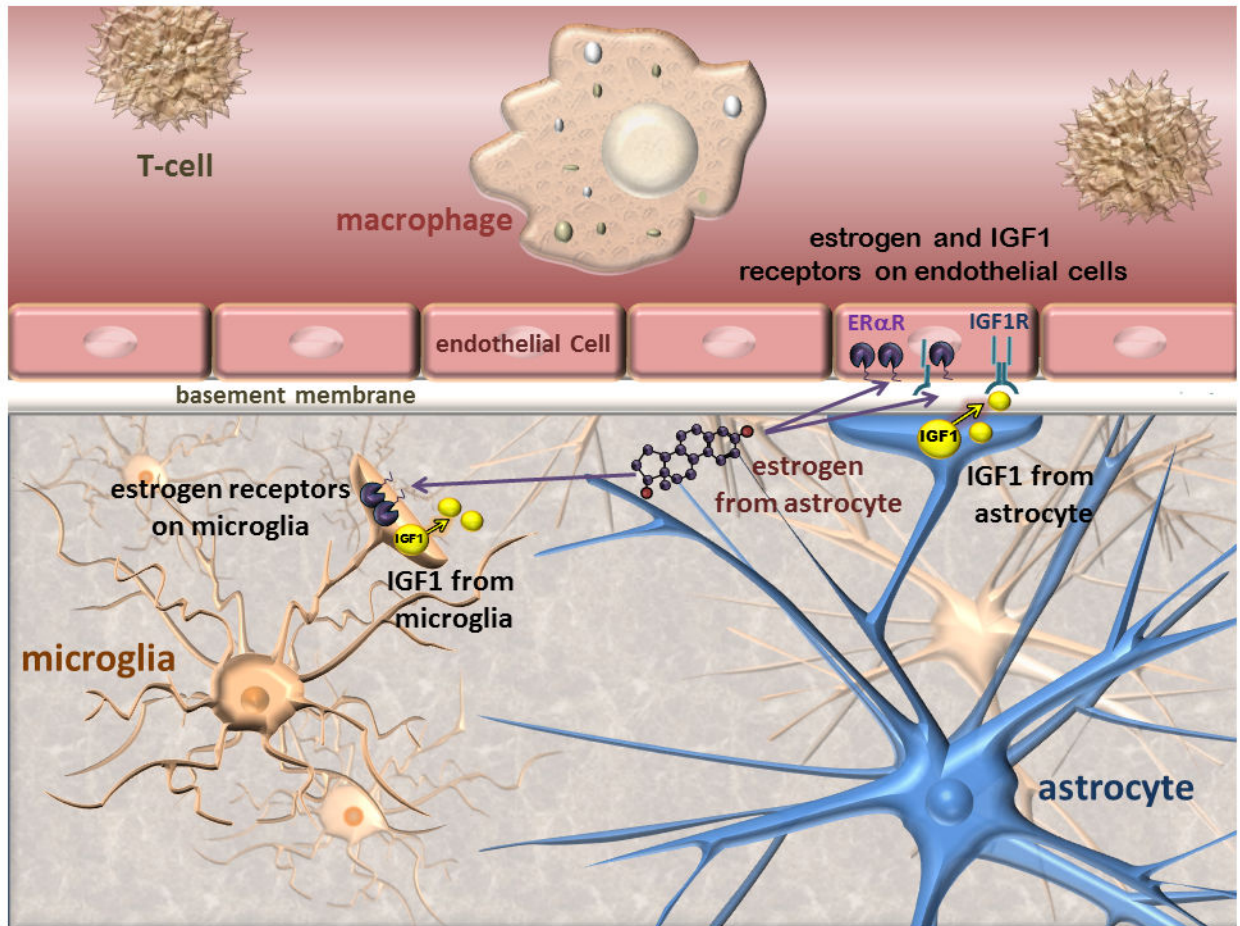


Figure 2.

Schematic representation of the blood brain barrier: The blood brain barrier consists of closely arranged endothelial cells surrounded by basement membrane. Circulating immune cells are shown in the lumen of microvessels, while microglia are located in the brain parenchyma. Astrocytic endfeet contact the endothelial layer and provide physical and growth factor-mediated support to the barrier. Estrogen receptors and the IGF-1 receptor are located on endothelial cells, astrocytes and microglia. Astrocytes also synthesize IGF-1 and neurosteroids including estrogen. Activated microglia can also synthesize IGF-1. In cerebral ischemia, this morphological arrangement ensures steroid and growth factor support for endothelial cells.