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Neuroprotective role of estrogens: relationship with insulin/IGF-1 signaling

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1. ABSTRACT

Postmenopausal women have an elevated risk of developing a neurodegenerative disease. These clinical observation supported by basic research, suggest that estrogens are neuroprotective. Insulin resistance represents an independent factor in the etiology of age-associated disease and metabolic syndrome should be considered as a contributing factor to the higher post-menopausal vulnerability to neurological disorders. Elucidating the relationship between insulin resistance associated with aging in females, and the cross-talk between estradiol, insulin, and insulin-like growth factor (IGF-1) signaling pathways, will lead to a more complete understanding of the mechanism underlying estradiol-mediated neuroprotection. In past decades, estrogen replacement therapy (ERT) was commonly used as a palliative therapy during menopause, but the mid-term and long-term effects of estrogen as possible promoters of breast cancer and the increased risk of coronary illness or stroke, has limited current usage. A deeper understanding of the molecular mechanisms common to all forms of neurodegenerative diseases may hasten the development of protective strategies against chronic age-related deterioration and acute illness, ultimately providing a better quality of life for the elderly.

2. INTRODUCTION

Aging in both humans and rodents is strongly associated with a decline in insulin action through the development of insulin resistance. This contributes to progressive glucose intolerance and the development of type 2 diabetes during aging. Insulin resistance in aging is related to a metabolic syndrome associated with increased incidences of depression, neurodegenerative diseases, cognitive dysfunction, and memory loss. Many recent studies have focused on the impairment of insulin metabolism in the brain as a new pathogenic process for neurodegenerative diseases. The general conclusion is that diabetes-related cognitive dysfunction is a consequence of changes within the central nervous system (CNS) induced by chronic hyperglycemia and impairments in cerebral insulin signaling. Taken together, these findings support the hypothesis that insulin may have a role in the preservation of cognitive performance against multiple pathological processes during aging (1-3).

Basic experimental studies and clinical observations have also demonstrated the importance of estrogens in the preservation of cognitive function and protection against neuronal damage (4,5). This is evidenced

by the increased risk of developing neurodegenerative disorders during menopause, a state accompanied by a dramatic reduction in estrogen levels. There is no general mechanistic explanation, however, for the neuroprotective action of estrogens. Furthermore, the signaling pathways that allow these hormones to sustain or improve brain function during aging are unknown. Estrogen-mediated neuroprotection likely depends on both a "classical" signal pathway involving nuclear estrogen receptors (ERs) and on a "non-classical" pathway mediated by non-nuclear ERs (4,6). In the nervous system, these ERs have been found in extranuclear locations (cytosol and plasma membrane) in brain areas known to be altered during neurodegenerative processes. Moreover, the interaction of estrogens with non-nuclear ERs appears to activate distinct intracellular signaling pathways, such as PI3-K and Akt kinases (6-8), that may mediate the neuroprotective effects of these hormones.

In this review, we have summarized a large body of data demonstrating that maintenance of both estrogen and insulin signaling are vital for normal brain functioning and that loss of these signaling pathways in aging has deleterious effects on CNS function and may promote neurodegenerative disorders.

3. BRAIN INSULIN/IGF-1 SINGALING

The insulin family of peptides is involved in coupling metabolic rate and neural activity to nutrient availability in multicellular organisms. Indeed, a key function of these hormones in cell metabolism and growth has been firmly established. However, their significance in neuronal physiology is less well characterized, although progress in recent years on the neuroactive properties of insulin and insulin-like growth factor I supports an important role for these hormones in brain function (9,10).

Insulin is a trophic factor known to activate a variety of signaling pathways, such as ERK/MAPK, PI3-K/AKT/GSK3beta, BAD, FOXO, and TOR pathways that are essential for neuronal development and survival. Although multiple effects of insulin on single neurons and isolated brain structures have been demonstrated (11), very little is known about the role of insulin in the regulation of neuronal glucose uptake *in vivo*, or of the consequences of insulin signaling on human brain function.

No significant insulin synthesis has been detected in the CNS (12). However, the presence of insulin transcripts within specific neurons subtypes and extracellular secretion of the hormone have been demonstrated (13). Insulin is thought to cross the blood-brain barrier through a receptor-based saturable transport system that is operational at physiological levels of serum insulin. This transport is decreased in obesity and hyperglycemia (14,15), suggesting that at least part of brain insulin resistance may be caused by changes in insulin carriers that lead to reduced insulin within the brain.

The biochemical characterization of brain insulin receptors (IRs) indicates that they are similar to peripheral IRs, but exert different functions. Insulin receptors are widely expressed throughout the brain. It is also known that neurons express insulin-independent glucose transporters, suggesting that insulin and its receptors may have functions within the brain in addition to those related to the facilitation of glucose influx. Insulin receptors are expressed at particularly high densities in areas concerned with olfaction, appetite, and autonomic functions, including the striatum, cortex, choroid plexus, and olfactory bulb (16-18), and are particularly enriched in dendritic fields receiving rich synaptic input. In the brain, rather than mere regulators of glucose transport and metabolism, major functions of IRs are related to central regulation of body homeostasis, modulation of synaptic plasticity and cognition, and possibly in aging-related neurodegeneration (19-21).

On this way, neuronal insulin receptor knockout mice (NIRKO) allow us to study in depth the effects of insulin resistance in brain. Female NIRKO mice showed an increase on food intake, body weight, fat pad weight and serum triglycerides, suggesting insulin resistance syndrome. Moreover, the absence of brain insulin receptor is also related to a significative increase in phosphorylation of the microtubule-associated protein, Tau. Tau hyperphosphorylation is considered an early manifestation in Alzheimer´s disease, suggesting that the lack of brain insulin signaling could be promoting early neurodegenerative diseases (For Review 22).

The mechanistic and functional consequences of impaired central IR signaling remain to be elucidated, but the ubiquity of insulin, IGF-1(Insulin-like Growth Factor), and their receptors raise intriguing questions regarding the functional activities of the central IR system under physiological and pathophysiological conditions. The expression, regulation, and activity of brain glucose transporters are essential for neuronal function because glucose is the principle energy source for the brain. Insulin-independent glucose transporters such as GLUT-1 and GLUT-3 are widely expressed in the CNS, and appear to be responsible for most glucose uptake and utilization (23). However, these GLUT isoforms cannot account for all glucose utilization in the brain. The GLUT-4 isoform has also been found in low amounts in the CNS (24), where its translocation appears to be mediated by IRs (6), indicating a direct role for insulin receptor signaling in glucose transport. The data from our recent studies suggest that insulin activates signal transduction events in the brain to stimulate glucose transporter trafficking similar to effects in the periphery (6). Therefore, it is possible that these GLUTs may contribute to neuronal homeostasis by serving as metabolic sensors that signal interstitial glucose.

While the role of insulin is better known in relation to the control of food consumption and glucose metabolism, insulin-like growth factor 1 (IGF-1) is a pleiotropic signal involved in numerous processes required to maintain brain cell function. In the brain, IGF-1 was formerly considered a neurotrophic factor involved in brain growth, but other aspects of the neurobiology of IGF-1 are gradually emerging (9,10). It has been shown that IGF is abundantly expressed in many areas during development,

but that expression is restricted to select regions of the mature CNS, and at very low levels. In the adult brain, local IGF-1 expression is increased in response to injury, but the function significance is still uncertain. The known influence of peripheral IGF-1 on the brain necessitates a transport system from blood to brain, and this was actually described years ago (25). Intriguingly, the insulin and IGF-1 receptors are separate entities that activate almost identical pathways, but with highly distinct outcomes on their target cells. Both receptors occur as homodimers but can form functional heterodimers to form hybrid insulin/IGF-1 receptors with distinct functional properties and anatomical localization (26). Brain insulin and IGF-1 signaling could also depend on this hybrid receptor, but this possibility has not been demonstrated.

3.1. Insulin resistance and neurodegenerative disease

The peripheral consequences of diabetes are well known, but the true extent of the diabetes-induced neurological complications are still under investigation. Diabetes produces a variety of neurochemical, neuroanatomical, and behavioral changes indicative of accelerated brain aging that can be reversed by insulin replacement, supporting a role for insulin in the improvement of cognitive performance during normal brain aging and under conditions of age-related neuropathology (3,27,28). Although the relationship between brain glucose, insulin signaling, and cognitive function has been well established, the mechanisms that allow glucose-induced and insulin-induced cognitive enhancement are still unclear.

Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by hyperinsulinemia, hyperglycemia, and hypo-responsiveness of the insulin receptor. Interestingly, some of the consequences of insulin resistance in NIDDM associated with hyperinsulinemia and hyperglycemia, including glucose intolerance, adiposity, atherosclerosis, and hypertension, are also risk factors for Alzheimer's disease (AD). Clinical studies have revealed that patients suffering NIDDM have a two- to three-fold greater risk for developing AD (29-33). Any disturbance in the metabolism of insulin in the CNS may have deleterious consequences on normal brain functioning. For example, individuals suffering from AD and PD show reduced insulin receptor expression in the brain, but it is uncertain whether this is a cause or consequence of neurodegeneration (34,35). Overall, these data indicate that diabetes mellitus may accelerate the brain aging process. Furthermore, cerebral atrophy and diabetes may interfere with cerebral amyloid and Tau metabolism. Although the molecular origin has not been fully elucidated, alterations in insulin and glucose homoeostasis in the periphery may affect brain insulin and IR functions, promoting oligomerization of beta-amyloid (Abeta) and inducing Tau hyperphosphorylation, two hallmarks of AD (36,37).

The relationship between insulin and the metabolism of Aβand Tau has received increased attention in recent years (36,38). It is now believed that increased Abeta production is a central pathogenic event in familial AD, while decreased Abeta clearance is dominant in sporadic AD (39). The insulin degrading enzyme (IDE) is the only known protease involved in Abeta degeneration. This enzyme likely prevents formation of brain amyloid deposits by cleaving the component peptides. Interestingly, among the main substrates of IDE are Abeta peptide, insulin, and amylin, which are degraded by IDE with similar efficiency. On the other hand, insulin has a regulatory effect on IDE levels in the CNS; excessively high insulin levels compete with Abeta for degradation, while low insulin levels in the brain, followed by insulin resistance, may reduce brain IDE levels and thereby impair Abeta clearance (40). It has been demonstrated that peripheral infusion of insulin in healthy older humans increased the Abeta concentration in the brain within 120 minutes, and was correlated with memory impairment (41). This phenomenon can be explained by the insulin effect on the degradation of Abeta transported outside the brain. High plasma insulin levels may interfere with the degradation of plasma Abeta, thereby obstructing a peripheral Abeta-clearing sink. This peripheral pathway contributes to Aβclearance, so obstruction may result in a high accumulation of Abeta in the brain, ultimately favoring AD development. These findings indicate that, together with glucose homeostasis, optimal insulin levels promote Abeta clearance by maintaining IDE expression in the brain. Consequently, insulin may exert protective roles against AD. In contrast, either pathologically low or high insulin concentrations in the brain may contribute to AD pathology.

4. BRAIN ESTROGEN SIGNALING AND NEURODEGENERATION

Numerous clinical studies have demonstrated that the incidence of neurodegenerative diseases increases after menopause, indicating that reductions in estrogens confer increased susceptibility or reduce endogenous neuroprotective efficacy. Indeed, AD, PD, and ischemic brain injury dramatically increase when ovarian functioning declines. These observations have encouraged the development of estrogen replacement therapy (ERT) in order to decrease the risk and/or severity of neurodegenerative processes. Some, but not all, clinical studies have reported improved memory processing and cognition in postmenopausal women following ERT (42,43), and numerous studies of ovariectomized animal models have confirmed more severe consequences following ischemia that are partially ameliorated by estrogen replacement (44-49). Estrogen is a well established neuroprotective agent in many animal models of brain injury, including stroke (50-54). Pretreatment with a physiological dose of estradiol protects the cortex against delayed cell death induced by middle cerebral artery occlusion (MCAO) by reducing both caspase activity and DNA fragmentation in the ischemic penumbra (55). One potential mechanism for estradiol-induced neuroprotection may be through the modulation of genes involved in the control of cell death and apoptosis, including anti-apoptotic bcl-2 family proteins (56-59).

Most actions of estrogen are induced through its interaction with intracellular estrogen receptors (ERs) that act as transcription factors. These classical or "genomic" mechanisms typically occur over the course of an hour,

whereas "nongenomic" mechanisms occur in minutes through extranuclear estrogen receptors or other non-ER plasma membrane-associated estrogen-binding proteins that activate different second messengers and kinase pathways. Two mammalian ERs have been characterized to date, ERalpha and ERbeta, that are widely, but not uniformly, distributed in different areas of the CNS involved in cognitive function, including the hypothalamus, hippocampus, cerebral cortex, midbrain, brainstem, and forebrain. Estrogen receptor expression and distribution appear to be modified during development and aging. In particular, ERalpha has been implicated in neuroprotection in different cell and animal models (8,60-62). Estradiol treatment in wild type and ERbeta-null ovariectomized mice prevented brain injury, whereas this protection was abolished in ERalpha-null animals (63). Also, in ovariectomized rats treated with estradiol, an increase in angiogenic factor angiopoietin-1 was detected in the brain that was abolished in alpha-ERKO mice (64). However, other beneficial effects of estrogen on the brain appear to be independent of ERs. In a study of stroke induction by reversible MCAO, protection against tissue damage was observed in ERalpha-null females following estradiol exposure (65).

Data from a wide range of cell types have demonstrated the existence of numerous intracellular mechanisms that could underlie estrogen-inducible promotion of neuronal survival and cognitive function (66-68). As mentioned above, estrogen activates a plethora of signaling cascades in neurons, including mitogen-activated protein kinase (MAPK), phosphoinositol 3-kinase (PI3K), protein kinase C (PKC), and Ca2+ influx, and downstream effectors like the immediate early gene c-Fos (67,69-75). Each of these signaling pathways has been associated with estrogen regulation of neuronal function and survival. Collectively, the complex signaling cascades activated by 17beta-estradiol in healthy neurons enhance the biochemical, genomic, and morphological mechanisms for preservation of cognitive functions, and may proactively induce mechanisms of protection against neurodegenerative insults.

In the last years, a new point of view of the neuroprotective role of the estrogens has been related to aromatase. This enzyme is able to convert testosterone and other C19 steroids to estradiol and it is found in various tissues, including the brain. It has been proposed that estrogen aromatization of androgens plays a pivotal role in the control of genomic and non-genomic actions of estrogens. In addition to the physiological regulation of its activity in association with modifications in synaptic function and brain plasticity, the expression and activity of aromatase is also altered after brain injury. In this sense, the induction of aromatase expression in astrocytes after brain damage is accompanied by a significant increase in aromatase brain activity and increased levels of estradiol within the brain (for Review 76, 77).

On the other hand, clinical studies such as The Women's Health Initiative Memory Study (WHIMS), found that conjugated equine estrogens (CEE) with or without medroxyprogesterone acetate (MPA) somewhat increased the risk of dementia and cognitive decline in postmenopausal women (78). This study is now a widely cited example of the harm that can be caused by certain regimens of hormone replacement, especially when begun ten or more years after menopause. In this sense, it has been proposed "the critical window hypothesis". This hypothesis states that estrogen supplementation initiated early in menopause may have beneficial effects on brain function; however, estrogen therapy that is initiated several years post-menopause may be ineffective or even detrimental. This provides insight into a therapeutic window of opportunity and suggests that the beneficial effects of estradiol treatment diminish once this window of efficacy has closed. Although the reason for this therapeutical window remains partially unknown, the hormonal status can strongly influence cognitive status, and properly-timed hormone therapy seems to be critical to maintaining healthy cognitive function (79).

4.1. MAP-Kinase pathway and neurodegeneration

The rapid effects of estrogen have been linked to the activation of the MAPK signal transduction pathway (80). This activation may be important in many physiological effects of estrogen that cannot be explained by classic ER-mediated transcription. Activation of MAPK by estrogen receptors has been implicated in estrogen mediated neuroprotection (81) and cell cycle regulation (82). *In vitro* studies demonstrated that estrogen elicited a rapid and sustained activation of both ERK1 and ERK2 in neurons, an effect which requires activation of MEK (83), the signaling protein immediately upstream of ERK. Moreover, estrogen is capable of increasing B-Raf kinase activity (83). Activation of ERK is much more prolonged in the CNS (83) than in non-neural cells (80). It has been proposed that the prolonged activation of ERK may distinguish the pro-differentiation effects of growth factors such as nerve growth factor (NGF) from the proliferative effect of epidermal growth factor (EGF) and other growth factors that trigger a much more transient activation of ERK (84).Moreover, estradiol elicits neuroprotection against Aβ-induced toxicity in neuronal cells. This protection is also related to the activation of Raf-1/MEK/ERK1/2 pathway, via an ER. (85)

Although most studies examining estradiol (E2)-initiated rapid signaling utilized cell lines or tissue explant preparations, there is accumulating evidence that low doses rapidly (within 30 min) increase MAPK phosphorylation *in vivo*. Bryant et al. (86), in an elegant study demonstrated that the intraperitoneal administration of 15 μg/kg of 17-β estradiol, but not the inactive isomer 17-alfa estradiol, rapidly elevated phospho-ERK2 levels in the rat brain in a region specific manner. The rostral nucleus accumbens, diagonal band of Broca, paraventricular nucleus (PVN), arcuate nucleus, and the anteromedial visual cortex exhibited statistically significant ERK phosphorylation in response to E2.

On the other hand, the molecular machinery for activation of the MAP kinase cascade appears to be preorganized at the cell surface of quiescent cells, and it is

tempting to speculate that association of the estrogen receptor with multimeric caveolar-like complexes of signaling kinases could function as a plasma membrane estrogen receptor transduction complex that mediates the rapid effects of estrogen (87-90). However, the physiological relevance of caveolin proteins in membrane ERs signaling in the nervous system is still unknown.

4.2. PI3-kinase and estrogen signaling

Interaction of E2 with a plasma membrane-associated ER is capable of activating PI3K, which in turn activates Ca^{2+} independent PKC that phosphorylates L-type Ca^{2+} channels and promotes Ca^{2+} influx. The resulting rise in intracellular Ca^{2+} activates Ca^{2+} dependent PKCs that then phosphorylate Src kinase. In parallel, PI3K also activates the Akt kinase, which can phosphorylate and inactivate the pro-apoptotic protein BAD (91). In addition, E2 increases mitochondrial sequestration of Ca^{2+}, protecting neurons against dysregulation of Ca^{2+} homeostasis and promoting normal mitochondrial activity. In addition, E2 may preserve mitochondrial respiration in part through its ability to increase either cytochrome c oxidase levels or enzymatic activity (92, 93).

In our previous work, we demonstrated the expression of two ERalpha isoforms in cortex and diencephalon (6), named ERalpha67 and ERalpha46, that had been previously described in other studies (94-98). ERalpha67 was observed to interact with insulin receptor substrate-1 (IRS-1), although this interaction was reduced in aged animals, as well as in ovariectomized rats in the presence or absence of estradiol, suggesting that the hormone did not take part in ERalpha67-IRS-1 interactions. We have also studied the relationship between ERalpha67, ERalpha46, and the PI3K regulatory subunit (p85alpha), detecting an estrogen independent ERalpha67-p85alpha interaction only in untreated rats that was reduced with aging, whereas ERalpha46-p85alpha interaction was maintained during aging in the presence of estradiol (6). Therefore, together with Cardona-Gomez et al. (99) and Znamensky et al. (100), these results show that the activation of PI3K signaling pathways by estradiol appears to play an important role in estrogen-mediated neuroprotection, mainly through the control of ERalpha46-p85alpha interactions. ERalpha46 may act as an activation function-1 (AF-1) competitive inhibitor of ERalpha67, due, in part, to its ability to out-compete ERalpha67 for ER binding (95).

5. CROSS-TALK BETWEEN ESTROGEN AND INTRACELLULAR INSULIN/IGF-1 SIGNALING

Insulin resistance in the elderly is associated with increased rates of atherosclerotic vascular disease, due in part to metabolic disorders, such as hyperinsulinemia, dyslipidemia, and hypertension, leading to metabolic syndrome. In this sense, some neurodegenerative diseases may also be considered, in some cases, the result of metabolic syndrome (43,101). This fact suggests that insulin and estrogen signaling mechanisms may be interacting to modulate neuronal responses to injury. Insulin-like growth factor-1 appears to play an important role in neuroprotection; it can reverse age-related effects (102) and attenuate the age-related decrease in cerebral glucose utilization (103). Moreover, gonadal hormones have been shown to regulate the expression of IGF-1 receptor mRNA and IGF-1-binding protein mRNA in adult female rat brains (104). Several studies have shown that low doses of E2 increase IGF-1 binding in the brain by significantly increasing IGF-1 receptors (IGF-1R) expression (105). In addition, the interaction between estrogen and IGF-1 systems appears to be reciprocal, since the intracerebroventricular administration of IFG-1 also increased the association between ER and IGF-1R. The link between this two signal pathways suggest complementary or mutually dependent functions in the prevention of age-related neuronal dysfunction (106,107).

One of the main signaling pathways known to participate in both estradiol- and insulin-mediated intracellular signaling is the PI3K-Akt pathway, suggesting it as a possible point of convergence. This pathway may participate in estradiol-mediated neuroprotection, as exposure to a PI3K inhibitor reversed the neuroprotective effect (108). It is reasonable to propose that estrogen given acutely after an ischemic insult might mediate neuroprotectionthrough PI3K/Akt signaling (51). Indeed, PI3K acts by phosphorylation and activation of the serine-threonine kinase Akt, and it is well known that Akt promotes cell survival by suppressing genes implicated in apoptotic cell death. In each case, Akt phosphorylates and thereby inactivates its target. Targets of Akt known to play a role in neuronal apoptosis following brain ischemia include pro-apoptotic proteins of the Bcl-2 family, pro-caspase-9 (the precursor of the initiator of the caspase death cascade caspase 9), members of the Forkhead family of transcription factors, which promote transcription of pro-death genes, and glycogen synthase kinase 3beta (GSK3β) (106,107,109,110). The ability of neurotrophins such as BDNF to promote neuronal survival requires functional PI3K/Akt signaling (111). Therefore, one possible mechanism for acute estrogen protection involves a synergistic activation of both the ERK/MAPK and PI3K/Akt pathway by estrogens. The activation of the ERK/MAPK signaling pathway could up-regulate BDNF, which in turn would stimulate the PI3K/Akt pathway through the activation of its target receptor trkB. Moreover, because trkB receptors can promote neuron survival through the activation of both the MAPK and PI3K/Akt pathways (112-114), such a mechanism could create a powerful positive feedback loop to suppress proapoptotic protein transcription by sustained activation of Akt.

The abundant coexpression of estrogen receptors with IGF-1 receptors in the brain and immnunoprecipitation studies (61) suggest that interactions between intracellular signaling pathways engaged by IGF-1R and estrogen receptors are possible (115,116). For example, the PI3K/Akt pathway is also triggered by IGF-1R, which is also known to be activated by ERalpha (117). Thus, in adult ovariectomized rats, systemic estradiol administration produced a transient increase in tyrosine phosphorylation of the brain IGF-1R, as well as transient interaction of IGF-1R with ERalpha and p85alpha (61). The cooperative effects of

IGF-1 and estradiol related to neuroprotection have also been assessed in ovariectomized rats exposed to systemic administration of kainic acid to induce degeneration of hippocampal hilar neurons (118), an experimental model of excitotoxic cell death. Both the systemic administration of estradiol and the intracerebroventricular infusion of IGF-1 appeared to prevent hilar neuronal loss induced. Furthermore, the protective effects of estrogen were blocked by intracerebroventricular infusion of the IGF-1R antagonist JB-1. In addition, neuroprotection by IGF-1 was blocked by the ER antagonist ICI 182-780 (118). Inhibition of ERs also blocked the neuroprotective effects of IGF-1 in the rat hippocampus. Finally, IGF-1R activation is essential for several actions of estradiol in the brain, including hormonal regulation of cell survival.

In the brain, there is abundant coexpression of nuclear estrogen receptors and IGF-1 receptor in the same cells. Both factors cooperated in neuroprotection in an animal model of brain injury and in an experimental model of Parkinson disease (119). Insulin-like growth factor 1 activated ERKs, leading to the phosphorylation of estrogen receptors, and estrogen receptors can physically interact with IGF-1 receptors and with the downstream proteins IRS-1 and PI3K, enhancing IGF-1 signaling in the brain (119). The neuroprotective actions of estradiol may be mediated, at least in part, through the IGF-1 receptor signaling cascade and the anti-apoptotic kinase Akt. In addition, estradiol induces a transient activation of GSK3beta in the adult female rat brain, followed by a more sustained inhibition. In fact, GSK3beta can act downstream of Akt and could be a point of interaction between estrogen and IGF-1 signaling. However, under pathological conditions, GSK3beta may be responsible for the hyperphosphorylation of Tau in Alzheimer's disease (120) and its inhibition is associated with the activation of survival pathways in neurons (121). Interestingly, estradiol regulates the activity of GSK3beta and decreases the phosphorylation of Tau in the rat hippocampus *in vivo* (99). Furthermore, estradiol increases the association of Tau with phosphorylated GSK3beta, and the association of PI3K-p85 with beta-catenin, another substrate of GSK3 (94). Therefore, the interaction of ERalpha with brain IGF-1 receptor signaling pathways may explain the interdependence of estradiol and IGF-1 in the regulation of different neural events. The ERK and Akt signaling cascades may mediate the interaction of IGF-1 and estradiol in the regulation of neuronal differentiation, synaptic function, synaptic remodeling, neuroprotection, and sexual behavior. The synergistic interaction of IGF-1 and estradiol in the phosphorylation of Akt may be critical for neuroprotection. The Akt kinase regulates several transcription factors that may be involved in the control of neuronal survival, such as cAMP-response-element-binding protein (CREB), nuclear factor kappa (NF-kappaB) and several members of the Forkhead family (61,122-124). In addition, activation of Akt results in the phosphorylation of the Bcl-2 family member Bad, and this may suppress Bad-induced cell death (91,125). Furthermore, Akt activation enhances Bcl-2 promoter activity and both IGF-1 and estrogen induce Bcl-2 expression in neurons. Interestingly, IGF-1 receptor activation is necessary for the induction of Bcl-2 by estradiol in the adult brain. Downstream of Akt, IGF-1 and estradiol may also interact on the regulation of microtubule dynamics, neuritic growth, synaptogenesis, synaptic plasticity, and neuronal survival by acting on GSK3beta and its substrates beta catenin and Tau (99). Overall, these findings suggest that ERalpha/IGF-1R interactions are functionally relevant to novel neuroprotective signaling pathways, and that clearly warrant further investigation.

6. PERSPECTIVES IN NEURODEGENERATIVE DISEASES AND ESTROGEN TREATMENT

The aging process affects all tissues and organs, including the brain. Hormones are involved in the aging process since the level of many changes with age. Several hormones, such as growth hormone, IGF-1, dehydroepiandrosterone, and sex hormones decrease with aging in mammals (126). In humans, these changes are associated in time with the elevated incidence or progression of neurodegenerative disorders, increased depressive disorders, and other psychological disturbances (127, 128). The decrease in the levels of neuroprotective hormones in the aged may result in a reduced endogenous protective capacity against both environmental and genetic factors that promote neurodegeneration. In particular, IGF-1 and estradiol appear to be particularly important in the process of neuroprotection and co-activation of downstream signaling can reverse the age-related effects (103). It has been established that the estradiol and IGF-1 systems interact to prevent age-induce neural dysfunction (106, 107) through a complex interaction between their intracellular signaling pathways, particularly PI3K/Akt.

Currently, ERT is accepted only in cases of climacteric symptoms that alter the quality of life, and is usually set at the lowest effective dose for the shortest time possible. In contrast, the use of estrogen therapies for palliate menopausal symptoms have been limited since prolonged hormone treatments appear to increase the risk of breast cancer, coronary illness, epilepsy, stroke, or pulmonary embolism. Moreover, the use of estrogen therapy for neuroprotection in older postmenopausal women is controversial because it can precipitate neurovascular and cardiovascular accidents (129, 130). Thus, IGF1 therapy is a potential alternative. Animal studies showed that chronic IGF-1 infusion in the brain restores the diminished spatial learning capacity of aged rats that were stressed during prenatal life. In addition, IGF-1 also up-regulates neurogenesis in the hippocampus of these animals and reduces their HPA axis dysfunction. Interestingly, IGF-1 increased estradiol levels in the plasma of aged rats that were subjected to prenatal stress (131). Moreover, in a recent study in middle-aged female rats subjected to global ischemia, Traub et al. demonstrated that significantly more neurons survived in animals treated with either estradiol or IGF1, but that simultaneous treatment produced no additive effect (132).

Moreover, clinical studies also support neuroprotective effects of IGF-1. Higher levels of endogenous IGF-1 after ischemic stroke in humans are

associated with improved functional outcome (133). In fact, among hospitalized patients, IGF1 levels are decreased in stroke victims and inversely correlated with mortality at 3 and 6 months (134). This suggests that exogenous supplementation of IGF-1 is a biologically plausible avenue for clinical trials. Human data indicate that IGF-1 is safe and well tolerated. There is some precedent for large scale human trials with IGF-1 therapy for cognitive deficits even though some early results were inconsistent (135, 136). This discord may be related to the systemic route of administration; intranasal delivery of IGF-1 may better deliver active drug to the central nervous system and recently, it has been showed that intranasal IGF-1 delivery may be more practical than systemic administration for protection against brain injury in humans. (137)

However, at present, the intricate relationship between IGF-1 and estrogen signaling in brain is not fully understood. Therefore, in order to design safe neuroprotective hormonal therapies we first need to determine how aging affects the signaling of estradiol and IGF-1 in the brain.

7. SUMMARY

A large number of clinical and experimental studies have demonstrated that estradiol and insulin contribute to the functional preservation of the brain during aging. Although we have only begun to identify potential molecular mechanism of this neuroprotective role, further investigation on the relationship between insulin resistance associated with aging in females, and the cross-talk between estradiol and insulin mechanisms, including activation of IRS-1/PI3-k/Akt and IGF-1-IR signaling pathways, may lead to a more complete understanding of the precise mechanisms underlying beneficial effects in the nervous system.

It is generally accepted that aging evokes profound changes in insulin signaling in the periphery that are important for initiating or exacerbating neurodegenerative diseases. However, it is still unclear whether aging per se causes progressive brain deterioration or whether progressive impairment of glucose homeostasis may ultimately induce the brain aging process.

Reported data indicates that the estrogen decline occurring in menopause accounts for the increased risk of progressive dysfunction in the central nervous system. Therefore, a better understanding of the molecular mechanisms underlying brain deterioration related to both hypoestrogenic and hyperinsulinemic processes may contribute to new approaches for effective replacement therapies that may enhance quality of life in the elderly.

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