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Astrocyte-derived growth factors and estrogen neuroprotection: Role of transforming growth factor- α in estrogen-induced upregulation of glutamate transporters in astrocytes

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Abstract

Extensive studies from the past decade have completely revolutionized our understanding about the role of astrocytes in the brain from merely supportive cells to an active role in various physiological functions including synaptic transmission via cross-talk with neurons and neuroprotection via releasing neurotrophic factors. Particularly, numerous studies have reported that astrocytes mediate the neuroprotective effects of 17 β -estradiol (E2) and selective estrogen receptor modulators (SERMs) in various clinical and experimental models of neuronal injury. Astrocytes contain two main glutamate transporters, glutamate aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1), that play a key role in preventing excitotoxic neuronal death, a process associated with most neurodegenerative diseases. E2 has shown to increase expression of both GLAST and GLT-1 mRNA and protein and glutamate uptake in astrocytes. Growth factors such as transforming growth factor α (TGF- α) appear to mediate E2-induced enhancement of these transporters. These findings suggest that E2 exerts neuroprotection against excitotoxic neuronal injuries, at least in part, by enhancing astrocytic glutamate transporter levels and function. Therefore, the present review will discuss proposed mechanisms involved in astrocyte-mediated E2 neuroprotection, with a focus on glutamate transporters.

Keywords

Astrocytes; Neuroprotection; Estrogen; Growth factors; TGF- α ; GLT-1; GLAST; Glutamate transporters

1. Introduction

Astrocytes are the most abundant, non-neuronal glial cells in the brain and participate actively in normal physiology as well as in acute injury and the pathological process of chronic neurological disorders in the central nervous system (CNS) (Hamby, 2010; Ullian,

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2004). The traditional understanding of astrocyte function as merely supporting cells of the brain has completely changed since the discovery that astrocytes are involved in various important functions in the CNS; these include the promotion of glutamate clearance, K^+ buffering, antioxidant defense mechanisms and neuronal excitability by coupling with neurons (Allen, 2009; Clarke, 2013; Svendsen 2002; Zhang, 2010). Astrocytes are located in juxtaposition to neurons (which they outnumber 10:1 in some regions of the brain), and act as critical mediators of neuronal survival (Dhandapani, 2007). Besides maintaining neural tissue homeostasis in the brain, the multifunctional roles of astrocytes in the CNS have drawn significant attention to their potential as therapeutic targets for various neurological disorders (Barreto, 2011; Brann, 2007).

In addition to the importance of astrocytes, 17β -estradiol (E2) is now widely accepted as exerting a broad spectrum of actions in the CNS including neuroprotection (Lee, 2001; Wise 2002). This is true for disorders such as multiple sclerosis (MS), schizophrenia, depression, Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and acute ischemic stroke [reviewed in (Behl 2002; Garcia-Segura, 2001; Green, 2000)]. Moreover, a growing body of evidence suggests that astrocytes are a major cellular target for this E2-induced neuroprotection (Azcoitia, 2010; Dhandapani and Brann 2007; Sortino, 2005). Although the exact mechanism(s) involved in astrocyte-mediated E2 neuroprotection remain to be established, E2 increases the expression and function of the glutamate transporters in astrocytes, GLAST and GLT-1 (Lee, 2012b; Lee, 2009a). Since astrocytic glutamate transporters maintain optimal levels of glutamate in synaptic clefts, which prevents excitotoxic neuronal death, E2-induced enhancement of these transporters may be a crucial step leading to E2-induced neuroprotection. The fact that GLAST and GLT-1 do not contain an estrogen response element (ERE) in their promoters suggests that E2-induced upregulation of GLAST and GLT-1 is an indirect effect, mediated via activation of other cellular signaling pathways. Studies have shown that growth factors such as TGF- α and TGF- β are vital to this E2 action on glutamate transporters (Lee et al., 2012b; Lee et al., 2009a). Thus, the goal of this review is to shed light on the role of astrocyte-derived growth factors in E2 neuroprotection with a particular focus on mechanisms involved in E2-induced upregulation of astrocytic glutamate transporters, GLAST and GLT-1.

2. Role of astrocytes in E2 neuroprotection

It is well-documented that E2 promotes neuronal survival {Sudo, 1997 #156} and offers neuroprotection against various stimuli including iron {Vedder, 1999 #157}, glutamate {Singer, 1996 #158}, kainate {Regan, 1997 #160} and H_2O_2 {Bonfont, 1998 #159} in neurons. However, it should be emphasized that astrocytes also play a critical role in mediating E2-induced neuroprotection as E2 is capable of exerting neuroprotection against a neuronal toxic insult in the presence of astrocytes under condition in which it is unable to protect neurons in the absence of astrocytes {Dhandapani, 2002 #10; Park, 2001 #11; Platania, 2005 #12}. The most remarkable evidence for the role of astrocytes in E2-induced neuroprotection is a study by the Sofroniew group, revealing that E2 was unable to protect neurons against neuronal injuries in a model of experimental autoimmune encephalomyelitis (EAE, an animal model of MS) when ER- α was genetically knocked out

in astrocytes, while it still exerted neuroprotection when neuronal ER- α was ablated {Spence, 2013 #115}. On the other hand, several studies have reported that neuronal ER- α mediated E2 neuroprotection against middle cerebral artery occlusion (MCAO) in mice {Elzer, 2010 #161}, glutamate neurotoxicity in hippocampal neurons {Gingerich, 2010 #44;Zhao, 2007 #39}. These results indicate that astrocytes might not be exclusively mediating E2-induced neuroprotection, but ample evidence reveals that astrocytes play a major role in this process (reviewed in {Dhandapani, 2007 #2; Mahesh, 2006 #234}.

Astrocytes express all estrogen receptor (ER) subtypes including classical ER- α and ER- β as well as the G protein-coupled ER, GPR30 (Garcia-Segura, 1999; Kuo, 2010; Pawlak, 2005b). Among several proposed mechanisms for E2 neuroprotection involving astrocytes, an anti-inflammatory action in astrocytes appears to be critical to achieve E2-induced neuroprotection (Spence, 2011). Thus, E2 exerts neuroprotection against EAE in astrocytes by decreasing chemokine CCL2 and CCL7 levels (Spence et al., 2013). Since neuroinflammation is associated with many neurodegenerative diseases including MS, an E2-induced anti-inflammatory effect in astrocytes may likely contribute to E2 effects on neuroprotection as a whole (Vegeto, 2008). It also appears that inflammation is involved in the impairment of astrocytic glutamate transporters in neuropathological processes since proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) also reduce the glutamate transporter GLT-1 in astrocytes (Sama, 2008; Sitcheran, 2005; Su, 2003). Even though the exact mechanism involved in E2-induced enhancement of glutamate transporters in astrocytes remains to be established, it is clear that this pathway needs to be vigorously pursued, as it may yield therapeutic strategies for neurological disorders associated with excitotoxic neuronal injuries.

3. Role of astrocyte-derived growth factors in E2 neuroprotection

It has been well documented that E2 action in astrocytes leads to the synthesis and release of various growth factors including nerve growth factor (NGF), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), transforming growth factors (TGF)- α and TGF- β , brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF); all of these have been shown to exert neuroprotection (Buchanan, 2000; Duenas, 1994; Flores, 1999).

E2 increases NGF mRNA and protein levels in primary astrocytes (Xu, 2013) and exerts synergistic neuroprotective effects with NGF against apoptosis (Gollapudi, 1999). E2 also increases BDNF mRNA and protein expression in astrocytes and exerts neuroprotection via BDNF (Sohrabji, 2006; Xu et al., 2013). Moreover, multiple *in vivo* studies have demonstrated that BDNF exerts neuroprotection against ischemic and traumatic brain injury (Beck, 1994; Kazanis, 2004; Yamashita, 1997). E2 also increases expression and secretion of GDNF in astrocytes (Xu et al., 2013), and GDNF protects NMDA-induced neuronal cell death by attenuating calcium influx and activation of the ERK pathway (Nicole, 2001). Another study has shown that E2 increases the production and release of GDNF in astrocytes and rescues spinal motoneurons from AMPA-induced excitotoxicity (Platanina et al., 2005). IGF-1 signaling also has been reported to play a critical role in mediating E2 neuroprotection via astrocytes. E2 and IGF-1 receptors are often co-localized in the same

cells and promote the survival of the same groups of neurons and stimulate adult neurogenesis (Mendez, 2005). E2 also exerts neuroprotective effect against ischemia by activation of GPR30, which is linked to transactivation of the IGF-1 receptor (Lebesgue, 2009). E2 increases expression of bFGF in astrocytes (Galbiati, 2002), and bFGF is known to induce neuroprotection against ischemia and glutamate-induced excitotoxic neuronal cell death (Kirschner, 1995; Nozaki, 1993).

TGF- β is also one of the key growth factors that is induced by E2 and released from astrocytes to exert neuroprotection against various neuronal toxic insults (Dhandapani, 2003a; Dhandapani and Brann 2002; Dhandapani and Brann 2007; Sortino, 2004). Activation of the PI3K/Akt pathway is required for E2-induced TGF- β release from astrocytes (Dhandapani, 2005), while c-Jun-AP-1 signaling is involved in TGF- β -induced neuroprotection (Dhandapani, 2003b). We have reported that E2 and tamoxifen significantly increase the expression of TGF- β 1 mRNA in rat primary astrocytes (Lee et al., 2009a). It appears that TGF- β 1 mediates E2-induced upregulation of GLAST mRNA and protein levels and attenuates the manganese (Mn)-induced reduction of GLAST expression. TGF- β appears to exert multiple neuroprotection mechanisms including anti-apoptotic and anti-inflammatory actions that protect against excitotoxicity and neuronal regeneration (Dobolyi, 2012). Moreover, the levels of TGF- β are increased following brain ischemia, traumatic injury, MS, AD, PD and viral encephalomyelitis in order to induce neuroprotection [reviewed in (Dobolyi et al., 2012)].

E2 has been shown to increase TGF- α mRNA and protein levels in hypothalamic astrocytes (Ma, 1994), and astrocytes are considered to be the main neural cell type to mediate TGF- α -induced neuroprotection (Junier 2000; White, 2011). We have reported that both E2 and tamoxifen, a SERM, upregulated TGF- α mRNA and protein levels in rat primary astrocytes (Lee et al., 2012b). While tamoxifen exerts an antagonistic effect in breast tissue (Jordan 2006), multiple studies have reported its agonist actions in brain tissue (Kimmelberg, 2000; Osuka, 2001). As an example, we found that tamoxifen exerts an agonist effect on glutamate transporters in astrocytes, by increasing TGF- α and GLT-1 expression (Lee et al., 2012b). Since long-term treatment with E2 can induce adverse peripheral effects (such as uterine and breast cancer), development of neuroSERMs that exert brain-specific agonist effects, while exerting antagonistic activities in peripheral tissues, would be ideal to treat neurodegenerative diseases (Littleton-Kearney, 2002).

4. Molecular mechanisms of E2/SERMs neuroprotection

The ER-dependent molecular mechanisms for E2/SERMs-induced neuroprotection could be common in all neural cell types and may be broadly categorized into two different groups; (i) genomic pathways mediated by the activation of nuclear estrogen receptors (ERs), and (ii) non-genomic pathways involving activation of cellular signaling pathways.

4.1. Genomic pathways mediated by ER- α and β

ER- α and - β are widely expressed throughout the brain where they are localized to neurons and glial cells. ER-mediated gene regulation involves either direct binding of ER dimers to ERE sequences in the target gene DNA, or indirect binding of ER with other transcription

factors through protein-protein interactions (Marino, 2006). The neuroprotective action of E2 via a genomic pathway was demonstrated by showing that *in vivo*, a 24 h pre-treatment period was required to achieve protection of E2 against MCAO (Dubal, 1998). In support of this notion, treatment with the nonselective ER antagonist, ICI 162,780, increased the infarct size in female mice following MCAO (Sawada, 2000). To date the majority of studies from ER subtype-specific knockout mouse models have found that ER- α is more critical than ER- β in inducing neuroprotection (Simpkins, 2012; Spence et al., 2013; Zhang, 2011). Therefore, E2 was unable to reduce MCAO-induced neuronal injury in ER- α knockout mice, but effectively reduced neuronal injury in ER- β knockout mice, indicating that ER- α plays a more important role than ER- β does in E2 neuroprotection (Dubal, 2001). The importance of ER- α in E2-induced neuroprotection was further bolstered by the observation that only an ER- α agonist, but not an ER- β agonist, was able to reduce infarct size in rat MCAO models; further there was significant increase of ER- α mRNA expression in the ischemic region in the early development of infarct following MCAO (Dubal, 2006; Farr, 2007). A recent study using an EAE animal model also reported that ER- α , but not ER- β , mediated E2 neuroprotection by anti-inflammatory effects in astrocytes (Spence et al., 2013).

This view (of the sole importance of ER- α in E2 neuroprotection) has been contradicted by the report that there was no additional damage in ER- α knockout mice following MCAO (Sampei, 2000). The importance of ER- β in neuronal survival was also confirmed in ER- β knockout mice by demonstrating morphological abnormalities of neurons as well as severe neuronal deficits in the cortex in these mice (Wang, 2001). In addition, both ER- α and ER- β were able to mediate E2-induced neuroprotection against amyloid β -induced toxicity in the hippocampal-derived neuronal cell line HT22 (Fitzpatrick, 2002). Similarly, selective agonists for either ER- α or ER- β were able to rescue glutamate-induced excitotoxic cell death in hippocampal neurons (Zhao and Brinton 2007; Zhao, 2004). Although further studies are warranted to determine the precise role and individual contributions of ER subtypes to E2-induced neuroprotection, the genomic pathway of the ER certainly plays an important role in E2-mediated neuroprotection.

4.2. Non-genomic signaling pathways

The neuroprotective actions of E2 in the brain also involve activation of intracellular signaling pathways via GPR30 (Gingerich et al., 2010; Liu, 2012). Although GPR30 might be primarily responsible for activation of intracellular signaling pathways, membrane-associated ER- α and ER- β can also mediate these effects (Bao, 2011; Kelly, 2009; Kuo et al., 2010; Marin, 2009). It has been shown that E2-induced activation of ERK pathway is required for E2 neuroprotection against glutamate-induced excitotoxicity in primary cortical neurons (Singer, 1999) and in hippocampal CA1 neurons during global ischemia (Jover-Mengual, 2007). Similarly, activation of PI3K/Akt pathway is crucial in E2-induced neuroprotection against ischemia (Choi, 2004; Jover-Mengual, 2010). The direct interaction of ER- α with the p85 α subunit of PI3K may play a part in the non-nuclear E2 signaling mechanism (Simoncini, 2000). Furthermore, E2 activates the ERK-Akt-CREB-BDNF signaling pathway via a non-genomic ER mechanism in hippocampal CA1 region leading to the neuroprotection from ischemic injury and preserving cognitive function following global

cerebral ischemia (Yang, 2010). A study from our laboratory has shown that E2 protects against Mn cytotoxicity in cultured astrocytes as well as in neurons via the ERK and PI3K/Akt pathways (Lee, 2009b). SERMs also induce neuroprotection against oxygen-glucose deprivation-induced cell death via GPR30 (Abdelhamid, 2011). Thus, E2 exerts neuroprotection via GPR30 against various toxic insults, including ischemia, glutamate- and oxidative stress-induced cell death in hippocampal and cortical neurons (Gingerich et al., 2010; Lebesgue, 2010; Liu, 2011).

5. Role of astrocytic glutamate transporters in E2 neuroprotection

5.1. Role of glutamate transporters in neuroprotection

Two astrocytic glutamate transporters, GLAST and GLT-1, play an essential role in removing excess glutamate from the synaptic cleft, thereby maintaining glutamate homeostasis and preventing excitotoxic neuronal injury in the CNS (Rosenberg, 1989). An impairment of GLT-1 expression and function has been also observed in ALS (Rothstein, 1995), and indeed, down-regulation of both GLAST and GLT-1 has been associated with various neurodegenerative diseases (Kim, 2011). Since dysfunction of these astrocytic glutamate transporters is associated with various neurological disorders, targeting these transporters for the development of therapeutic strategies to treat various neurodegenerative diseases could be both important and ideal (Kim et al., 2011; Lin, 2012). The beta-lactam antibiotic ceftriaxone and E2 have been reported to increase the expression and function of GLT-1 and GLAST (Lee et al., 2009a; Pawlak, 2005a). The clinical uses of E2 are hampered due to adverse effects from its long-term usage. The results showing that tamoxifen, a SERM, exerts agonist effects in the brain where it enhances glutamate transporter expression and function, could be a promising gateway to the development of optimal neuroSERMs. To achieve this goal, it requires further understanding on the molecular mechanisms involved in tamoxifen-induced enhancement of astrocytic glutamate transporters and neuroprotection. We will briefly update the findings from our laboratory on the mechanisms of E2/tamoxifen action toward enhancing GLAST and GLT-1 expression and function.

5.2. E2 and tamoxifen attenuate Mn-induced impairment of glutamate transporters in astrocytes

Cultured astrocytes from the cortex of AD patients showed reduced glutamate uptake consistent with down-regulation of GLAST and GLT-1 expression; E2 treatment *in vitro* reversed glutamate uptake along with expression of both GLAST and GLT-1 (Liang, 2002). To study the mechanisms underlying E2-induced attenuation of impaired astrocytic glutamate transporters, we used Mn, an environmental toxin which in high doses induces PD-like pathological features referred to as manganism (Dobson, 2004; Pal, 1999). Thus, it is an excellent model to study mechanisms for neurodegeneration associated with impairment of astrocytic glutamate transporters. Mn has been shown to decrease glutamate uptake along with promoter activity, mRNA and protein levels of GLAST and GLT-1 in astrocytes; E2 or tamoxifen completely block these deleterious effects of Mn (Lee et al., 2012b; Lee et al., 2009a). In addition to PD, Mn neurotoxicity is also associated with several other neurodegenerative disorders including AD, HD and ALS (Bowman, 2011; Lee et al.,

2012b), and therefore, the ability of E2 or tamoxifen to afford protection against Mn-impaired glutamate transporters may lead to the development of neuroSERMs to treat multiple neurodegenerative diseases.

5.3. E2 and tamoxifen upregulate GLT-1 via nuclear ER- α and ER- β , as well as G protein-coupled ER GPR30

Astrocytes express ER- α , ER- β and GPR30 (Lee et al., 2012b; Lee et al., 2009b); selective agonists for these ER subtypes (PPT, DPN and G1, respectively) increase GLT-1 protein expression and glutamate uptake activity (Lee et al., 2012b). These results suggest the all three ERs play a role in E2-induced upregulation of GLT-1. Moreover, intracellular signaling pathways such as ERK/MAPK and PI3K/Akt pathways are involved in E2/tamoxifen-induced upregulation of GLT-1 (Lee et al., 2012b). Further study is required to understand, in more detail, the molecular mechanisms by which E2 or tamoxifen increases GLT-1 expression via ER- α and ER- β associated signaling pathways. On the other hand, GPR30 plays a critical role in GLT-1 regulation, as shown by the fact that inhibition of GPR30 with its specific antagonist G15, or siRNA knockdown, abrogates the stimulatory effects of G1, a selective agonist of GPR30, on GLT-1 expression (Lee, 2012a). G1-induced upregulation of GLT-1 is mediated by multiple signaling pathways, including ERK/MAPK, PI3K/Akt, protein kinase A and Src.

5.4. E2 and tamoxifen upregulate GLT-1 via the NF- κ B and CREB pathways at the transcriptional level

Extensive studies from our laboratory in recent years have led to the identification of transcription factors that are involved in E2/tamoxifen-induced upregulation of GLT-1 (Karki, 2013). The GLT-1 promoter lacks an ERE, but it contains three NF- κ B and one CRE consensus sites. This indicates that the E2 action on enhancing GLT-1 promoter activity is indirect, rather than due to the direct binding of nuclear ER- α / β to the GLT-1 promoter. Therefore, it appears that these two transcription factors, NF- κ B and CREB, play a critical role in E2/tamoxifen-induced enhancement of GLT-1 expression. Previous studies have shown that NF- κ B mediates the effects of epidermal growth factor (EGF) and dibutyryc cyclic AMP (dbcAMP) on GLT-1 upregulation (Sitcheran et al., 2005; Su et al., 2003). We found that the NF- κ B pathway also plays a critical role in E2/tamoxifen-induced enhancement of GLT-1 expression and function (Karki et al., 2013; Lee et al., 2012a). G1 or E2/tamoxifen induced the direct binding of p50 and p65 subunits of NF- κ B to the GLT-1 promoter, indicating their critical roles in E2/tamoxifen-induced upregulation of GLT-1. Pharmacological inhibitors of NF- κ B abrogated these effects. G1 and tamoxifen also activated the CREB pathway as evidenced by the fact that they induced phosphorylation of CREB and increased CRE reporter activities. G1 and E2/tamoxifen also induced CREB binding to the GLT-1 promoter. In addition, an inhibitor of protein kinase A (upstream activator of CREB) abrogated the G1/tamoxifen-induced GLT-1 expression, supporting the crucial role of these transcription factors in GLT-1 regulation (Karki et al., 2013; Lee et al., 2012a).

5.5. TGF- α as a mediator in E2/tamoxifen-induced upregulation of GLT-1

The synthesis and release of growth factors from astrocytes following E2/tamoxifen treatment may represent a major mechanism by which E2 or tamoxifen upregulates GLT-1 and promotes neuroprotection. Our findings that knockdown of TGF- α abrogated G1 and E2/tamoxifen-induced increases in GLT-1 expression in astrocytes, further confirmed the role of TGF- α in E2-induced GLT-1 upregulation (Lee et al., 2012b). Moreover, G1 and E2 increased the phosphorylation epidermal growth factor receptor (EGFR), the receptor for TGF- α , while inhibition of the EGFR with the pharmacological inhibitor AG-1478 abolished G1 and E2/tamoxifen-induced upregulation of GLT-1 (Karki et al., 2013; Lee et al., 2012a). E2/tamoxifen also increased TGF- α promoter activity, mRNA and protein levels, suggesting a role of TGF- α as a mediator in E2/tamoxifen-induced upregulation of GLT-1 (Karki et al., 2013). The neuroprotective role of TGF- α in neuronal injury was further evidenced by showing that treatment with TGF- α in spinal cord injury exerted neuroprotection by activating EGFR in astrocytes (White et al., 2011). Based on all of our findings, we proposed a model indicating the pathways responsible for E2/tamoxifen-induced upregulation of GLT-1 (Fig. 1). The effects of E2 or tamoxifen are mediated by nuclear ER- α , ER- β and GPR30, which collectively lead to the increased expression of TGF- α which in turn, enhances the GLT-1 expression in astrocytes.

6. Summary

Numerous *in vitro* studies using cell cultures as well as *in vivo* animal models have documented the neuroprotective effects of E2 via astrocytes. However, the adverse effects associated with long term use of E2 has hampered its clinical utility, while SERMs has drawn significant attention as possible alternatives for E2 due to their unique pharmacological properties, i.e., exerting tissue-specific agonist or antagonist properties. Moreover, the findings that astrocytes play critical roles in E2/SERMs-induced neuroprotection have placed these non-neuronal cells into an important position for future research related to strategies to treat neurological diseases. The impairment of astrocytic glutamate transporters in various neurodegenerative diseases and the capability of E2/SERMs to enhance the expression and function of these transporters have further bolstered the importance of developing neuroSERMs. Taken together, understanding the molecular mechanisms of E2-induced neuroprotection by enhancing astrocytic glutamate transporters will definitively attribute to development of novel therapeutics to treat multiple neurological disorders.

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Highlights

1. Estrogen upregulates glutamate transporter GLT-1 expression in astrocytes
2. Estrogen increases GLT-1 expression via GPR30 using the NF- κ B and CREB pathways
3. TGF- α mediates estrogen-induced upregulation of GLT-1 and GLAST in astrocytes
4. Tamoxifen, a selective estrogen receptor modulator, enhances GLT-1 expression via NF- κ B and CREB
5. Estrogen and tamoxifen attenuate manganese-induced reduction of GLT-1 expression

Fig. 1. Mechanisms of E2/tamoxifen-induced upregulation of GLT-1. E2 and TX activate ER-α, ER-β and GPR30 leading to upregulation of TGF-α. This upregulated TGF-α is released into the extracellular compartment as autocrine mode and activates its receptor EGFR, ultimately resulting in enhancement of GLT-1 expression and function via the NF-κB and CREB pathways. E2, TX and G1 also attenuated Mn-induced down-regulation of GLT-1.