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Multifunctional Roles of Enolase in Alzheimer Disease Brain: Beyond Altered Glucose Metabolism

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Abstract

Enolase enzymes are abundantly expressed, cytosolic carbon-oxygen lyases known for their role in glucose metabolism. Recently, enolase has been shown to possess a variety of different regulatory functions, beyond glycolysis and gluconeogenesis, associated with hypoxia, ischemia, and Alzheimer disease (AD). AD is an age-associated neurodegenerative disorder characterized pathologically by elevated oxidative stress and subsequent damage to proteins, lipids, and nucleic acids, appearance of neurofibrillary tangles and senile plaques, and loss of synapse and neuronal cells. It is unclear if development of a hypometabolic environment is a consequence of or contributes to AD pathology, since there is not only a significant decline in brain glucose levels in AD, but also there is an increase in proteomics identified oxidatively modified glycolytic enzymes that are rendered inactive, including enolase. Previously, our laboratory identified α-enolase as one the most frequently up-regulated and oxidatively modified proteins in amnestic mild cognitive impairment (MCI), early-onset AD (EOAD), and AD. However, the glycolytic conversion of 2-phosphoglycerate to phosphoenolpyruvate catalyzed by enolase does not directly produce ATP or NADH; therefore it is surprising that, among all glycolytic enzymes, α-enolase was one of only two glycolytic enzymes consistently up-regulated from MCI to AD. These findings suggest enolase is involved with more than glucose metabolism in AD brain, but may possess other functions, normally necessary to preserve brain function. This review examines potential altered function(s) of brain enolase in MCI, EOAD, and AD, alterations that may contribute to the biochemical, pathological, clinical characteristics, and progression of this dementing disorder.

Keywords

Enolase; plasminogen (PGn); tissue-plasminogen activator (tPA); plasmin; MAPK/ERK1/2; amyloid β-peptide; *c-Myc*; hypoxia-inducible protein-1α (HIF-1α); Alzheimer disease (AD); amnestic mild cognitive impairment (MCI); early-onset AD (EOAD); excitotoxicity; hypoxia; hypometabolism

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1.0 Introduction

Enolase enzymes (EC 4.2.1.11) are a superfamily of abundantly expressed carbon-oxygen lyases known for their role in glycolysis and gluconeogenesis. Glycolytic enzymes, like enolase, are among some of the most well-characterized proteins to date; yet, enolase isoforms were previously believed to perform exclusively “house-keeping” functions for the cell. However, recent studies have demonstrated that enolase possesses a variety of different regulatory properties, in addition to their glycolytic functions in the brain (Pancholi 2001). In particular, enolase has been reported to be a neurotrophic factor, 14-3-2 (Hattori *et al.* 1994, Hattori *et al.* 1995, Takei *et al.* 1991), a hypoxic stress protein (Aaronson *et al.* 1995), *c-Myc* binding protein and transcription factor (Subramanian & Miller 2000, Ray & Miller 1991b), and a strong plasminogen (PGn) binding protein (Pancholi & Fischetti 1998, Nakajima *et al.* 1994). Furthermore, many of these non-glycolytic functions have been implicated in hypoxia and ischemia (Mizukami *et al.* 2004, Sousa *et al.* 2005), as well as Alzheimer disease (AD) (Dotti *et al.* 2004, Parnetti *et al.* 1995).

A largely sporadic, age-associated neurodegenerative disorder, AD typically affects populations over the age of 60. Pathologically, AD can be characterized by elevated oxidative stress and subsequent damage to brain proteins, lipids, and nucleic acids, the appearance of neurofibrillary tangles and senile plaques, and eventual loss of synapse and neuronal cells that result in a progressive decline in cognitive function. In rare instances, autosomal dominant mutations in the *amyloid-β precursor protein* (*AβPP)* or *presenilin* genes 1 and 2 (*PS-1/-2*) cause a familial form of AD (FAD) that produces the same clinical and pathological consequences as sporadic AD, but at a much earlier age (~30 years old) (Scheuner *et al.* 1996, Sturchler-Pierrat *et al.* 1997, Citron *et al.* 1992, Wisniewski *et al.* 1998). Recently, α-enolase has been identified as one of the most frequently identified differentially expressed brain proteins in human and animal tissues (Petrak *et al.* 2008). As described below, previous studies by our laboratory have found α-enolase to be the most consistently up-regulated and oxidatively modified proteins in brain of subjects with early-onset AD (EOAD), AD, and amnestic mild cognitive impairment (MCI) (Butterfield and Sultana, 2007; Butterfield et al., 2007) , arguably the earliest form of AD (Winblad *et al.* 2004, Petersen *et al.* 1999). These findings suggest that enolase may possess one or more ulterior functions, beyond simple glucose metabolism, that could be integral to both normal and pathological brain function. Therefore, the intent of this review is to examine potential function(s) of α- and γ-enolase isoforms in AD brain.

2.0 Enolase Functional Diversity

Beyond glucose metabolism (Fig. 1), enolase enzymes have been reported to have a number of other non-glycolytic functions, such as the ability to bind polynucleotides (al-Giery & Brewer 1992), being a τ-crystallin protein (Wistow *et al.* 1988), neurotrophic factor 14-3-2 (Hattori *et al.* 1994, 1995, Takei *et al.* 1991), heat-shock protein 48 (HSP48) (Iida & Yahara 1985), hypoxic-stress protein (Aaronson *et al.* 1995), *c-Myc* binding and transcription protein (Subramanian & Miller 2000, Ray & Miller 1991a), and a strong PGn binding protein (Pancholi & Fischetti 1998, Nakajima *et al.* 1994), among others (Table 1). This wide array of functions can be attributed to different DNA base sequences within enolase

genes. For example, the promoter region of *ENO1* contains a copy of the viral core consensus sequence (GTGG(A/T)(A/T)(A/T)G) (Jones *et al.* 1988), two copies of the octanucleotide sequence (ATTTGCAT) found in immunoglobulin (Ig) gene enhancers and promoters (Jones *et al.* 1988), a C2 binding site (CATGTG) present in Ig heavy chain enhancers (Peterson & Calame 1989), part of the liver-specific enhancer binding site sequence (TCNTACTC) (Grayson *et al.* 1988), a cAMP response element (CRE) sequence (position −298) (Grayson *et al.* 1988), and SP1 transcription factor binding site (Briggs *et al.* 1986). Although the functional significance of many of these elements is unknown, other sequences are better-characterized, particularly the carbohydrate response element (ChoRE) motif ($^{59-}$CACGTG^{-39}). Upon glucose stimulation, transcription factors, such as hypoxia-inducible factor-1α (HIF-1α) and *c-Myc*, bind the *ENO1* ChoRE motif and initiate transcription of the enolase enzyme (Thompson & Towle 1991, Towle 1995, Dang 1999).

2.1 Enolase & *c-Myc*

Typically, functional diversity of proteins originates during transcriptional regulation. For example, Giallongo, *et al.* (Giallongo *et al.* 1990) discovered multiple transcription start sites in *ENO1* that are consistent with the lack of a canonical TATA box 19 to 27 base-pairs upstream of its CAP-site, which is primarily responsible for accurately positioning the correct mRNA start site. The significance of this finding is illustrated in its effects on translation initiation, which usually occurs at the first in-frame, 5'-AUG codon, representing the optimal context (Kozak 1999). However, reinitiation, direct internal initiation, and leaky scanning caused, in part, by the lack of a start site-directing TATA box, can produce more than one protein from a single mRNA, such as in the case of *ENO1* (Feo *et al.* 2000, Kozak 1999, Giallongo *et al.* 1990). Thus, the two *ENO1* gene products, α-enolase (48 kDa) and *c-Myc* binding protein-1 (MBP-1; ~37 kDa), share 97% sequence similarity (Giallongo *et al.* 1986). Two single-base insertions in MBP-1 result in a reading frame shift affecting its N-terminus as compared to the α-enolase coding region, while the C-terminal regions of both enzymes are identical (Ray & Miller 1991b, Onyango *et al.* 1998). Furthermore, α-enolase and MBP-1 have similar function, although quite different subcellular fates.

Normally, α-enolase is directed to the cytoplasm, in which it carries out its metabolic role, while MBP-1 is located in the nucleus where it is involved in transcriptional regulation of the *c-Myc* protooncogene. *c-Myc* is a DNA-binding phosphoprotein critical in the control of cell proliferation, differentiation, and apoptosis (Evan *et al.* 1992, Marcu *et al.* 1992, Spencer & Groudine 1991) that is commonly overexpressed in tumor cells. Like most "housekeeping" genes, *ENO1* mRNA translation is primarily under developmental control, significantly up-regulated during cellular growth and practically undetectable during quiescent phases (Holland *et al.* 1983, Giallongo *et al.* 1990). In transformed cells, the overexpression of *c-Myc* stimulates abnormal cell proliferation and up-regulation of several glycolytic enzymes, including α-enolase, in order to accommodate the mounting energy deficit (Hurlin & Dezfouli 2004, Osthus *et al.* 2000, Kim & Dang 2005). In turn, MBP-1 negatively regulates *c-Myc* transcription (Chaudhary & Miller 1995, Ray & Miller 1991b, Ray 1995), acting as a tumor suppressor and completing a regulatory "feedback-loop" of both *c-Myc* and glycolytic activity (Sedoris *et al.* 2007). Interestingly, although MBP-1 does not have enolase enzyme activity, both α-enolase and MBP-1 are able to act as tumor

supressors because *c-Myc* down-regulating activity lies within two hydrophobic N- and C-terminal regions present in both *ENO1* translation products (Subramanian & Miller 2000, Bentley & Groudine 1986).

2.2 Enolase & the Plasminogen System

The PGn system is best known for the pivotal role it plays in maintenance of vascular potency and thromolysis, by dissolving fibrin (Plow *et al.* 1991, Collen 1999). In order to elicit function, the glycoprotein PGn binds cell surface receptors via kringle domains that recognize exposed C-terminal lysine residues. Interestingly, PGn binds these receptors with low affinity, yet is more readily activated than free PGn (Plow *et al.* 1995), and subsequently produced plasmin has greater enzymatic activity and is protected from inactivation by inhibitors, such as α_2-antiplasmin (Plow *et al.* 1991). Therefore, virtually any surface protein exposing C-terminal lysines has the potential to bind and activate PGn processes, in addition to contributing to the high density and broad distribution of the many heterogenous PGn binding sites (Plow *et al.* 1995). Gangliosides (Miles *et al.* 1989), glycosaminoglycans (Andrade-Gordon & Strickland 1986), annexin II (Cesarman *et al.* 1994, Hajjar *et al.* 1994), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Lottenberg *et al.* 1992), and α-enolase (Redlitz *et al.* 1995, Pancholi & Fischetti 1998, Miles *et al.* 1991) are just a few examples of the different types of proteins reported to be PGn surface receptors. Most remarkable, however, is the discovery that glycolytic enzymes, GAPDH and α-enolase, are able to integrate into the cell membrane without possessing a signal sequence and retain enzymatic activity (Pancholi & Fischetti 1998). Some researchers speculate that a hydrophobic domain ($^{33-}$AAVPSGASTGIY^{-44}) within α-enolase might serve as an internal signal sequence, allowing its integration (von Heijne *et al.* 1991), while others suggest postranslational acylation (Bottalico *et al.* 1993) or phosphorylation (Cooper *et al.* 1984) may be a means of membrane association. Nevertheless, these two cytosolic enzymes are now part of a growing group of proteins that lack signal sequences, but are transported to the cell surface by an unknown mechanism.

However, binding surface receptors, like α-enolase, alone cannot activate PGn conversion to plasmin; the PGn-proteolytic cascade must begin with cleavage by either tissue-PGn activator (tPA) or urokinase-PGn activator (uPA) (Bergman *et al.* 1997), both of which, can be found in human brain (Carmeliet *et al.* 1994, Sappino *et al.* 1993). tPAs and/or uPAs initiate fibrinolysis by binding fibrin aggregates, which leads to a conformational change that dramatically increases their affinity for PGn. As a result, PGn is cleaved by tPA/uPA into proteolytic plasmin (Tucker *et al.* 2000b). Of particular interest in human brain is tPA, a serine protease correlated with hippocampal late-phase long term potentiation (L-LTP) (Kandel 2001, Pang & Lu 2004). Transgenic mice overexpressing tPA exhibit enhanced L-LTP and improved spatial learning (Madani *et al.* 1999). Induction of L-LTP, in turn, enhances neuronal expression of tPA within the hippocampus (Qian *et al.* 1993), perpetuating a cycle of secretion and growth necessary for brain development. In addition, PGn has also been implicated in wound healing and inflammation through its involvement in cell proliferation and migration (Kalderon 1979, 1982, Tarui *et al.* 2002, Plow *et al.* 1991), as well as many intracellular signaling events by activation of proenzymes (Liotta *et al.* 1981, Blasi *et al.* 1987, Nagase *et al.* 1990), prohormones (Virji *et al.* 1980), progrowth

factors (Rifkin *et al.* 1990, De Sousa *et al.* 2005), and procytokines (Nakagawa *et al.* 1991, Konakova *et al.* 1998). Thus, in this review, the role enolase plays in conjunction with the tPA/PGn system will be analyzed with respect to subjects with AD.

3.0 Enolase & Alzheimer Disease

AD is an age-associated neurodegenerative disorder that typically affects the elderly, aged 60 and above. However, in rare instances it can affect younger populations, as early as 30 years old, in FAD (Scheuner *et al.* 1996, Sturchler-Pierrat *et al.* 1997, Citron *et al.* 1992, Wisniewski *et al.* 1998). Both AD and FAD can be characterized clinically by a progressive decline in cognitive function, and pathologically by synapse and neuronal cell loss, as well as the appearance of neurofibrillary tangles and senile plaques. Other hallmarks of AD and FAD pathology are oxidative stress and damage that induce protein and nucleic acid oxidation, lipid peroxidation, and apoptosis, which lead to declining brain function and loss of synapses and neurons (Butterfield & Lauderback 2002, Butterfield *et al.* 2001, 2002, Markesbery 1999, Aksenov *et al.* 2001, Butterfield 2002, Bader Lange *et al.* 2008). In the same way, MCI, considered a transition point between normal cognitive aging and probable AD (Petersen *et al.* 1999, Winblad *et al.* 2004), has also been reported to have elevated oxidative stress levels (Butterfield *et al.* 2006b, 2007, Butterfield & Sultana 2007, Keller *et al.* 2005, Markesbery *et al.* 2005, Markesbery & Lovell 2007, Lovell & Markesbery 2007). Oxidative modification of proteins during disease progression, in turn, results in diminished and/or complete loss of protein function, as indexed by levels of protein carbonyls, 3-nitrotyrosine, protein-/lipid-bound 4-hydroxy-2-nonenal (HNE), and S-glutathionylation (Butterfield & Stadtman 1997).

According to a recent report, α-enolase has been identified as differentially expressed in about 30% of all 2D-gel electrophoresis (2-DE)-based experiments in human and animal tissues published in recent issues of *Proteomics* (Petrak *et al.* 2008), rendering it one of the top 15 most frequently identified differentially expressed proteins. Our laboratory has reported that enolase is oxidatively modified in MCI, EOAD, and AD. In these studies compared to control brain, α- and γ-enolase were found to be excessively carbonylated (Sultana *et al.* 2006a, Butterfield *et al.* 2006a, 2006b, Castegna *et al.* 2002), nitrated (Reed *et al.* 2008b, Sultana *et al.* 2006b, Castegna *et al.* 2003), HNE-modified (Reed *et al.* 2008a, Perluigi *et al.* 2009), and *S*-glutathionylated (Newman *et al.* 2007) in brain areas such as the inferior parietal lobule (IPL), hippocampus, and frontal cortex, but not in cerebellum, a brain region essentially devoid of pathology in AD. Whether the extensive oxidative modification of enolase is simply due to its proximity to the many redox reactions occurring throughout the cell or a result of structural susceptibility to oxidation is unknown. However, both possibilities are conceivable since enolase can be found in numerous regions of the cell and possesses many active-site Lys and His residues that are extremely susceptible to Michael addition by compounds such as HNE (Butterfield & Stadtman 1997). For example, γ-enolase has been identified as a component of the NADH-dichlorophenol-indophenol (DCIP) reductase complex, one of several *trans*-plasma membrane oxidoreductases (PMOs) located within synaptic plasma membranes and recycling vesicles (Bulliard *et al.* 1997). PMOs function as antioxidant enzymes and extracellular redox sensors that regulate cell proliferation and axonal guidance in response to external pro- or anti-oxidants (Toole-

Simms *et al.* 1991, Fuhrmann *et al.* 1989), thereby placing enolase in direct contact with reactive oxygen species (ROS) that could readily modify its activity and structure. Furthermore, our laboratory has also reported that enolase is present within mitochondria, one of the largest ROS producers within the cell, and contributes this organelle's function (Poon *et al.* 2005).

Another predominant feature in MCI, EOAD, and AD is the manifestation of glucose hypometabolism (Mielke *et al.* 1996), associated with the oxidative-inactivation of several glycolytic enzymes, including enolase (Sultana *et al.* 2007, Butterfield *et al.* 2006b, Castegna *et al.* 2002) (Fig. 2). Because the brain is one of the greatest consumers of glucose, hypometabolism can cause the up-regulation of glycolytic enzymes in an effort to combat the mounting energy deficit and hypoxic environment (Mielke *et al.* 1996). Moreover, previous studies have shown that cells resistant to Aβ toxicity had a greater flux of glucose through glycolysis and the hexose monophosphate shunt (Soucek *et al.* 2003). Interestingly, although the glycolytic function of enolase does not directly produce ATP or the reduced energy carrier NADH (Fig. 1), in all studies of MCI, EOAD, and AD brain from our laboratory, enolase levels are increased (Castegna *et al.* 2002, Sultana *et al.* 2007), while levels of pyruvate kinase, phosphoglycerate kinase, and GAPDH vary throughout disease progression (Fig. 2). Furthermore, our laboratory has shown that α-enolase was one of only four proteins, glycolytic or otherwise, consistently up-regulated and oxidatively modified in the progression from MCI to AD (Fig. 2). These remarkable findings suggest that enolase may well be involved in more than just metabolic processing of glucose, but perhaps possesses other critical functions vital to preserving brain function, which are discussed subsequently in this review.

3.1 Enolase, the Plasminogen System, & Glutamate Excitotoxicity

Glutamate excitotoxicity, a well-known phenomenon in AD brain, is characterized by the increased release and impaired uptake of glutamate, which mediates the toxic build-up of extracellular glutamate, leading to overstimulation of glutamate receptors, N-methyl-D-asparatate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainite receptors. Collapsing cellular ATP reserves and Na^+ ion gradients exacerbate this process, and eventually lead to rising intracellular Ca^{2+} levels, due to the opening of voltage-gated Ca^{2+} channels and release of Ca^{2+} from the endoplasmic reticulum (ER) (Fig. 3). Increased cytoplasmic Ca^{2+}, in turn, further depolarizes the cell membrane and activates cytotoxic intracellular pathways that lead to neuronal death, such as inducing the Ca^{2+}-dependent secretion of the serine protease, tPA (Gualandris *et al.* 1996, Fernandez-Monreal *et al.* 2004b, Baranes *et al.* 1998). During glutamate excitoxicity, excessive neuronal activity and extracellular secretion of tPA can cause neuronal death by augmenting microglial activation (Rogove & Tsirka 1998), increasing plasmin activation and degradation of laminin (Tsirka *et al.* 1995, Chen & Strickland 1997), and potentiating NMDA receptor signaling processes (Nicole *et al.* 2001, Tsirka *et al.* 1995, 1997).

Apoptotic cells often secrete and/or exhibit signal molecules within the membrane that allow activated microglia to scavenge, recognize, and bind damaged cells that require clearance, in order to prevent further injury to surrounding areas. In a study by Siao *et al.* (Siao & Tsirka

2002), glutamate-injured neurons were shown to release sufficient tPA to activate $tPA^{-/-}$ microglia in a cytokine-like manner (Rogove & Tsirka 1998). Furthermore, activated microglia were shown to secrete tPA in a proteolytic-independent manner, activating neighboring microglia, thereby, effectively amplifying the signal for microglial activation. Ultimately, this effect can lead to recruitment of microglia to the site of injury and can promote a timely resolution of cellular injury, an overly sensitive inflammatory response, or both (Siao & Tsirka 2002). For example, after Ca^{2+}-induced neuronal secretion, tPA cleaves an N-terminal residue on the NR1 subunit of the NMDA receptor, exacerbating NMDA receptor-evoked Ca^{2+} influx during excitotoxic processes (Fernandez-Monreal *et al.* 2004a, Nicole *et al.* 2001), and consequently, propagating an overly sensitive inflammatory response. Alternatively, activated microglia and apoptotic neuronal cells also synthesize PGn, in addition to tPA (Tsirka *et al.* 1997, Nakajima *et al.* 1992a, 1992b, 1992c). In general, tPA secretion alone is not sufficient to cause neuronal degeneration during excitotoxic insult (Tsirka *et al.* 1996, 1997); therefore, presenting PGn on the membrane surface could either provide a way to further localize activated microglia to areas in which neurons have been injured, or suggests that neuronal secretion of tPA is not necessarily intended to be detrimental.

As described previously, PGn has been implicated in inflammation, as well as many intracellular signaling events by activation of proenzymes, prohormones, progrowth factors, and procytokines (Section 2.2). Studies by Nagata *et al.* (Nagata *et al.* 1992, 1993) have shown that microglia-derived PGn has a neurotrophic effect on neurons, since purified rat PGn enhanced neurite outgrowth and dopamine uptake of mesencephalic neurons. The effects elicited by PGn were found to be mediated by α-enolase on the neuronal surface (Nagata *et al.* 1993, Nakajima *et al.* 1994). Enolase has frequently been reported as a strong PGn binding protein within the brain (Section 2.2), due to its extracellular C-terminal Lys residues, and is known to be up-regulated in MCI, EOAD, and AD brain (Section 3.0). When microglial and/or neuronal PGn binds membrane-integrated enolase, PGn is rapidly activated through tPA proteolytic cleavage (Section 2.2). Consequent production of plasmin endows neurons with the catalytic amplification of tPA/PGn signaling, due to the broad spectrum of substrates affected by proteolytic plasmin (Redlitz *et al.* 1995). Moreover, binding enolase protects plasmin from inactivation from inhibitors, like α_2-antiplasmin (Bergman *et al.* 1997, Plow *et al.* 1991). Therefore, it can be speculated that the up-regulation of enolase, in addition to enhanced membrane-resident PGn and extracellular secretion of tPA, by excitotoxic neurons and/or activated microglia during AD progression may initially be an attempt to propagate neuronal-preservation pathways, that ultimately go awry (Fig. 4).

3.2 Enolase, the Plasminogen System, & MAPK/MEK/ERK1/2 Signaling

As previously mentioned, a predominant feature in MCI, EOAD, and AD is the manifestation of glucose hypometabolism, which generally causes the up-regulation of glycolytic enzymes, including enolase, to combat the mounting energy deficit (Section 3.0). Thus, the lack of aerobic metabolism in AD brain is indicative of a widespread hypoxic environment. Many studies have found glycolytic ATP to be of utmost importance to the maintanence of ER Ca^{2+} stores, by acting as the chief ATP fuel-source for plasma

membrane ion pumps, such as the Na^+/K^+-ATPase and Ca^{2+}-ATPase, rather than ATP produced by mitochondrial oxidative phosphorylation (Kahlert & Reiser 2000, Kauppinen *et al.* 1988, Brorson *et al.* 1999, Silver & Erecinska 1997, Xu *et al.* 1995). However, neither glycolysis nor oxidative phosphorylation alone is capable of sustaining power to these pumps; therefore, a constant ATP supply must be provided by both pathways in order to prevent membrane depolarization (Kauppinen *et al.* 1988). In addition, collapsing cellular ATP reserves and Na^+ ion gradients can exacerbate glutamate excitotoxicity, lead to rising intracellular Ca^{2+} levels, and activate intracellular signal pathways that either lead to neuronal death or survival (Fig. 3).

Specifically, intracellular pathways involving activation of the mitogen-activated protein kinase (MAPK) cascade are of particular interest on account of one downstream target, enolase. Among many different proteins that MAPKs can activate by phosphorylation, the extracellular signal-regulated kinase 1/2 (ERK1/2) is known to function in cell survival responses by translocating to the nucleus and inducing rapid gene expression (Davis 1993, Karin 1995, 1998, Chang & Karin 2001), often in response to ROS (Jimenez *et al.* 1997, Chuang *et al.* 2000, Kishida *et al.* 2005, Conde de la Rosa *et al.* 2006, Kulich *et al.* 2007), a well-known initiator and/or consequence of AD pathology (Section 3.0). In a study by Mizukami, *et al.* (Mizukami *et al.* 2004), ERK1/2 was shown to be involved in the maintenance of intracellular ATP through induction of α-enolase expression, resulting in cardiomyocyte survival in ischemic hypoxia and re-oxygenation (Mizukami *et al.* 2004). Sousa, *et al.* (Sousa *et al.* 2005) provided additional insight into ERK1/2-induced expression of *ENO1* mRNA, revealing that active PGn on the cell surface activates MAPK and ERK1/2 in fibroblasts, through the proteolytic action of plasmin, which leads directly to the transcriptional regulation of *ENO1*. Their data also demonstrate that PGn-regulated *ENO1* expression is not only restricted to fibroblast cells, suggesting that this signaling cascade, involving up-regulation of α-enolase via MAPK and ERK1/2, probably exists in the brain, as well (Sousa *et al.* 2005). Further evidence to support this notion is that all MAPK pathways, including ERK1/2, are known to be activated in AD brain, as ERK 1/2 immunoreactivity can be found in tangle-bearing and non-tangle-bearing neurons (Hyman *et al.* 1994), as well as in dystrophic neurites of senile plaques (Trojanowski *et al.* 1993).

Because over 25 proteins have been identified to be downstream targets of ERK1/2 signaling (Lewis *et al.* 2000), the exact intracellular mechanism by which the MAPK/ERK1/2 survival pathway induces *ENO1* expression has yet to be established. However, two possible mechanisms suggested by Mizukami, *et al.* (Mizukami *et al.* 2004) in the heart, involving *c-Myc* and HIF-1α (Sections 2.0-2.1), may provide insight into α-enolase regulation in the brain. In their 2004 study, Mizukami, *et al.* (Mizukami *et al.* 2004) found that ischemic hypoxia and re-oxygenation induced α-enolase expression in cells transfected with *c-Myc* cDNA, suggesting that ERK1/2 may induce α-enolase expression through *c-Myc*. Induction of *c-Myc* mRNA expression is dependent upon Ets transcription factor binding sites located within the *c-Myc* promoter region that are activated by ERK1/2 phosphorylation (Brunner *et al.* 1994, McCarthy *et al.* 1997, Cheng *et al.* 1999, Gupta *et al.* 1993). Furthermore, like MAPK and ERK1/2, strong, active *c-Myc* immunoreactivity has been noted in a subpopulation of reactive astrocytes, dystrophic neurites of senile plaques,

and neurons with neurofibrillary degeneration in AD (Ferrer & Blanco 2000), implicating a role for MAPK/ERK1/2 activation of *c-Myc*, that would, in turn, induce expression of α-enolase in AD. Since the *ENO1* promoter region contains two *c-Myc* binding motifs within the ChoRE sequence (Section 2.0), *c-Myc* can directly transactivate production of α-enolase. Interestingly, activation of *c-Myc* by the ERK1/2 pathway selectively up-regulates α-enolase production over MBP-1, as MBP-1 would inhibit *c-Myc* by binding its promoter (Section 2.1).

Alternatively, the MAPK/ERK1/2 pathway can also induce α-enolase transcription through the hypoxia-inducible factor, HIF-1α, which up-regulates glycolytic gene expression during hypoxia, a common phenomenon in AD pathology (Semenza *et al.* 1996, Wang *et al.* 1995, Aaronson *et al.* 1995) (Section 3.0). Cells adapt to hypoxic conditions by inducing activation of transcription factors such as HIF-1, a key regulator of oxygen homeostasis that accumulates in response to low cellular oxygen levels (Wang & Semenza 1993a, 1993b, Wang *et al.* 1995). Previous studies indicate that embryonic stem cells deficient in HIF-1α expressed decreased levels of mRNAs encoding over ten different glucose transporters and glycolytic enzymes, including α-enolase (Iyer *et al.* 1998), insinuating HIF-1α protective effects are largely attributable to increased metabolic flow. Yang *et al.* (Yang *et al.* 2005) suggest that HIF-1α up-regulation of glucose transporters and glycolytic enzymes during hypoxia may favor glycolytic ATP over ATP produced by oxidative phosphorylation, thereby compensating for diminished ATP supplies resulting from oxygen-deprived mitochondria. This explanation is also consistent with studies that suggest the chief ATP-fuel-source for many cellular functions comes directly from glycolysis, rather than mitochondrial-produced ATP, as mentioned above (Kahlert & Reiser 2000, Kauppinen *et al.* 1988, Brorson *et al.* 1999, Silver & Erecinska 1997, Xu *et al.* 1995).

During MAPK/ERK1 signaling, ERK1 is reported to phosphorylate the C-terminal transactivation domains of HIF-1α in hypoxic HMEC-1 endothelial cells, stimulating HIF-1α transcriptional activity (Richard *et al.* 1999, Minet *et al.* 2000), thus, demonstrating that up-regulation of enolase by HIF-1α can be controlled by MAPK/ERK1/2 signaling (Semenza *et al.* 1996). Moreover, a study by Soucek *et al.* (Soucek *et al.* 2003) noted that overexpression of a non-degradeable form of HIF-1α prevents Aβ(1-42)-induced neurotoxicity. Considering all MAPK pathways, including ERK1/2 and HIF-1α, are activated in AD brain, it is possible that up-regulation of enolase through MAPK/ERK1/2 signaling serves a direct neuroprotective function in AD. However, it should be noted that although there are multiple neuroprotective benefits to MAPK/ERK1/2 activation, the *over*-activation of these kinases, especially with respect to cell type, varying apoptotic signals, and diverse downstream targets, can increase sensitivity to neurodegeneration, especially during oxidative insult (Chu *et al.* 2004, Slevin *et al.* 2000, Zhu *et al.* 2002a, 2002b).

Because the ChoRE sequence of many glycolytic genes, including *ENO1*, is analogous to the binding site for *c-Myc* and HIF-1α (Section 2.0), both of these transcription factors are able to up-regulate glucose metabolism via ERK1/2 signaling under hypoxic/hypometabolic conditions (Semenza *et al.* 1996). For that reason, it is quite possible that both mechanisms are utilized under hypometabolic/hypoxic conditions, either separately or simultaneously, in MCI, EOAD, and AD brain. Therefore, we speculate that the increased levels of enolase

found in MCI, EOAD, and AD brain are attributable to neuronal and/or glial intracellular survival pathways induced by excitotoxic, hypoxic, and/or oxidative stress. Extracellular tPA cleavage of PGn bound to membrane-resident enolase stimulates plasmin activation of the MAPK ERK1/2 pro-survival pathway, that, in turn, up-regulates transcription of glycolytic enzymes, like enolase, in an effort to counteract the hypometabolic imbalance of ATP and critical ion gradients, and perhaps saving the cell from an apoptotic death (Fig. 4). Enhancing the translation of α-enolase, in turn, would allow for additional tPA/PGn binding, thus, perpetuating catalytic amplification of not only MAPK/ERK1/2 survival signaling, but perhaps other self-preservation pathways as well.

3.3 Enolase, the Plasminogen System, & Aβ

AD is pathologically characterized by increased levels of oxidative stress and damage, as well as the accumulation of neurofibrillary tangles and amyloid plaques, that ultimately lead to synapse and neuronal cell loss (Section 3.0). Amyloid plaques are the result of an over-accumulation of the amyloid-β (1-40) and/or (1-42) peptides (Aβ), derived from β- and γ-secretase cleavage of the AβPP. As AD pathology progresses, the more toxic Aβ(1-42), in particular, rapidly aggregates into fibrils in a β-sheet conformation, similar to the cross-β-structure that fibrin peptides adopt during fibrinolysis (Kranenburg *et al.* 2002). Interestingly, it is this β-sheet conformation that endows fibrin the ability to bind and activate tPA in the PNS (Kranenburg *et al.* 2002); yet, thus far, fibrin has not been found in the brain (Dotti *et al.* 2004). Although fibrin and Aβ(1-42) have no relative sequence similarity, Aβ(1-42) is able to bind and activate tPA through its aggregated β-sheet structure, thereby substituting for fibrin in PGn activation by tPA, but not uPA, in the brain (Wnendt *et al.* 1997, Kingston *et al.* 1995).

Studies by Tucker *et al.* (Tucker *et al.* 2000a, 2000b), suggest that Aβ accumulation ultimately leads to the activation of the tPA/PGn system by inducing tPA expression *in vitro* and *in vivo*, in a positive feedback-loop manner. Through tPA cleavage of PGn, activated plasmin can degrade oligomeric and fibrillar Aβ, effectively blocking Aβ neuronal toxicity. Van Nostrand *et al.* (Van Nostrand & Porter 1999) further demonstrated that plasmin cleavage yields an N-terminal truncated form of Aβ with altered β-sheet properties that enhanced stimulation of tPA activity in a positive feedback-loop manner. Interestingly, plasmin has been noted to preferentially increase α-cleavage of AβPP (forming neurotrophic sAβPPα), either by cleaving AβPP directly, or by activating other proteases (Ledesma *et al.* 2000). Considering that plasmin is known to have an affinity for Lys residues (Weinstein & Doolittle 1972), and can activate metalloproteinases (Kleiner & Stetler-Stevenson 1993), such as candidate α-secretases, ADAM 10 and TACE (Buxbaum *et al.* 1998, Lammich *et al.* 1999), plasmin could contribute to Aβ degradation in two ways. First, plasmin may enhance AβPP α-cleavage, increasing the production the non-toxic sAβPPα over the more toxic Aβ(1-42), and/or, secondly, by directly degrading all forms of Aβ produced, including sAβPPα, p3, Aβ(1-40), and Aβ (1-42) in the form of oligomers and fibrils (Ledesma *et al.* 2000). In contrast, Melchor *et al.* (Melchor *et al.* 2003) demonstrated a significant decrease in tPA activity in the hippocampus and amygdala of AD patients, implying that diminished plasmin levels are not a consequence of Aβ deposition, but, rather, a cause. These researchers also reported that Aβ accumulation exacerbates diminishing tPA activity by

inducing expression of PAI-1 (Melchor *et al.* 2003), a potent tPA inhibitor (Gils & Declerck 1997).

However, these papers do not discuss the effects of alternatively spliced AβPP derivatives on PGn processing in AD brain, specifically isoforms containing the kunitz-type serine protease inhibitor (KPI) domains. Alternative splicing of the gene encoding AβPP on chromosome 21 yields three AβPP isoforms of 695 (KPI(−)AβPP), 751, and 770 amino acids; of which, the 751 and 770 amino acid species (KPI(+)AβPP) contain a 56 amino acid KPI domain (Ponte *et al.* 1988, Tanzi *et al.* 1988). KPI domains are highly analogous to the proteinase inhibitor, protease-nexin II and are known to potently inhibit serine proteases, such as prothrombic enzymes and plasmin, but not uPA or tPA (Shimokawa *et al.* 1993, Xu *et al.* 2005, 2009, Van Nostrand *et al.* 1989, 1990, Smith *et al.* 1990, Schmaier *et al.* 1993, Mahdi *et al.* 1995, Konduri *et al.* 2001). In AD subjects, KPI(+)AβPP mRNA and protein levels are significantly elevated in many areas of the brain and cerebral spinal fluid (CSF), found in senile plaques, and are associated with increased production of Aβ, while KPI(−)AβPP levels are significantly reduced (Palmert *et al.* 1989a, 1989b, Saito *et al.* 1993, Kitaguchi *et al.* 1990, Hyman *et al.* 1992, Moir *et al.* 1998, Zhan *et al.* 1995, Willoughby *et al.* 1995, Preece *et al.* 2004).

Therefore, the above-mentioned models of tPA and plasmin regulation (Shimokawa *et al.* 1993, Van Nostrand & Porter 1999, Melchor *et al.* 2003, Tucker *et al.* 2000a, 2000b, Konduri *et al.* 2001, Van Nostrand *et al.* 1990, Menendez-Gonzalez *et al.* 2005) are not necessarily incompatible, since there might be a negative feedback-loop mechanism between tPA and/or plasmin activity and Aβ deposition. For example, plaque formation may, indeed, trigger the up-regulation of PGn, but the loss of tPA activity (i.e., by PAI-1) prior to AD onset might render a positive feedback-loop (as mentioned above) ineffective (Cacquevel *et al.* 2007). Moreover, increased levels of KPI(+)AβPP in AD brain would serve to exacerbate this negative feedback-loop, wherein both tPA and plasmin activity are inhibited by AD pathology. Thus, oxidative modification of α-enolase and/or other proteins results in the up-regulation of α-enolase during AD progression and may be an integral part of a system in which neurons attempt to degrade accumulating Aβ. However, the oxidative modification of α-enolase in MCI, EOAD, and AD may render this enzyme either incapable of binding PGn/plasmin while membrane-integrated, or completely unable to integrate into the plasma membrane in order to initiate a PGn/plasmin proteolytic survival cascade. In either case, plasmin would be unable to effectively degrade Aβ or initiate the MAPK/ERK1/2 survival pathway. Consequently, free plasmin proteolytic activity could be inhibited by α_2-antiplasmin (Section 2.2; Fig. 4).

Lastly, Aβ accumulation in MCI, EOAD, and AD brain can arise from overproduction, decreased degradation (i.e., by plasmin, neprolysin, or other proteases), and/or by a third mechanism: decreased efflux from the brain. Efflux of brain-resident Aβ is primarily facilitated by the low-density lipoprotein-related receptor-1 (LRP-1), which mediates endocytic processing of both secreted and transmembrane forms of AβPP through the blood-brain barrier (BBB) (Kounnas *et al.* 1995, Knauer *et al.* 1996). Interestingly, studies demonstrate that plasmin interacts with α_2-macroglobulin (α2M), a “pan-protease inhibitor” (Kovacs 2000, Bu *et al.* 1992) that is bound and internalized by LRP when complexed with

proteases, such as plasmin (Qiu *et al.* 1996, Rebeck *et al.* 1995). α2M is an atypical protease inhibitor, in that cleavage of its "bait region" traps proteases, but does not block or alter the protease active-site or proteolytic ability (Kovacs 2000, Borth 1992). Studies by Qiu *et al.* (Qiu et al. 1996) demonstrate that a 700 kDa α2M-serine protease complex is responsible for significant Aβ(1-40) and Aβ(1-42) degradation and clearance from the brain. Although, the identity of this particular serine protease is unknown, it is conceivable that plasmin, a serine protease known to bind and degrade Aβ proteins, may be a likely candidate.

Hence, oxidative dysfunction and consequent altered binding of membrane-integrated enolase by PGn in MCI, EOAD, and AD brain could conceivably lead to decreased efflux of brain-resident Aβ. Altered binding of PGn to enolase at the cell surface could significantly reduce or completely inhibit production of the tPA/PGn cleavage product, plasmin, precluding the potential association of α2M with plasmin, and, therefore, Aβ clearance via LRP. Furthermore, since LRP is also a receptor for free and/or α2M-complexed KPI(+)AβPP, which competitively inhibits clearance of Aβ when bound to LRP (Kounnas *et al.* 1995, Ulery *et al.* 2000, Moir & Tanzi 2005, Conboy *et al.* 2005), elevated KPI(+)AβPP levels in AD brain, in addition to altered PGn-enolase binding, may exacerbate decreased efflux of Aβ. Studies to test these notions are now underway in our laboratory.

4.0 Enolase in Other Neurodegenerative Diseases

AD is just one of many age-related neurodegenerative disorders exhibiting a progressive decline in cognitive function, as well as extensive synapse and neuronal cell loss. Shared clinical characteristics between diseases such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), Huntington's disease (HD), amyolateral sclerosis (ALS), and Niemann-Pick disease (PiD) involve common pathologies including oxidative stress and damage, mitochondrial dysfunction, hindered protein degradation, abnormal intracellular signaling, and cell-cycle arrest (Beal 1995, Duda *et al.* 2000, Jenner & Olanow 1998, McNaught & Jenner 2001, Cassarino *et al.* 2000). Unfortunately, not much research to date has focused on the expression, function, and/or oxidation state of the enzyme enolase in these other neurodegenerative diseases, although many studies have utilized NSE as a marker for global neuronal and glial cell loss. Studies conducted by our laboratory, among a few others, however, have shown decreased activity, as well as increased expression and oxidative modification of α- and γ-enolase in mouse models of HD (Perluigi *et al.* 2005b, Sorolla *et al.* 2008), PD (Stauber *et al.* 2008, Poon *et al.* 2005, Gomez & Ferrer 2009, De Iuliis *et al.* 2005), DLB (Gomez & Ferrer 2009), and fALS (Perluigi *et al.* 2005a, Casoni *et al.* 2005), in addition to AD (Section 3.0). Conversely, it is interesting to note that α-enolase expression is down-regulated in the striatum of maneb- and paraquat-induced PD mouse models (Patel *et al.* 2007), revealing that the specific route of pathologic cell signaling, oxidative modification, and protein expression may depend upon which factors, genetic and/or environmental, ultimately induce onset of disease pathology.

Considering that hypometabolism/hypoxia, excitotoxicity, the plasminogen system, MAPK/ERK1/2 signaling, HIF-1α, and *c-Myc* have all been implicated as being both protective and deleterious in the pathologies of HD, PD, ALS, DLB, and PiD (Demestre *et al.* 2006, Varma *et al.* 2007, Zhu *et al.* 2002a, 2003, Berding *et al.* 2001, Kulich *et al.* 2007, Ferrer *et al.*

2001a, 2001b, 2001c, Apostol *et al.* 2006, Beal 2008, Glas *et al.* 2007, Yang *et al.* 2005, Ferrer & Blanco 2000), it is quite likely that enolase dysfunction, dysregulation, and oxidative modification reported by our laboratory in the aforementioned tauopathies and Lewy body variants is a result of similar, if not identical, protective pathways suggested for MCI, EOAD, and AD above. However, more studies are needed to investigate this notion/theory since the plasminogen system, MAPK/ERK1/2, HIF-1a, and *c-Myc* signaling may all act differently in each neurodegenerative disease, in response/accordance to different cell types, apoptotic stimuli, and available downstream targets.

5.0 Conclusion

Although the main cause(s) of AD remain unknown, it is evident that up-regulation of glycolytic enzymes, like enolase, is significant to disease progression. Results from our laboratory support the view that enolase is more than just a glycolytic enzyme, but possesses other functions critical to brain cell survival. In this review we propose an expanded role for enolase that occurs concurrently with up-regulation of this enzyme in AD brain, a role to promote neuronal protection from Aβ accumulation and possibly glutamate excitotoxicity via action of the PGn and MAPK/ERK1/2 systems. However, given that oxidative modification of enzymes generally leads to dysfunction (Butterfield *et al.* 2007), these putative roles of enolase to protect against neuronal death in AD brain fail. Moreover, noting that both harmful and neuroprotective effects of both the MAPK/ERK1/2 and PGn systems in the brain are known, it is evident that the ability of tPA/PGn and plasmin to modulate neuronal death or survival via MAPK/ERK1/2 and enolase in MCI and/or AD would depend upon the cell type and apoptotic stimulus (Tucker *et al.* 2000b). In addition, since our laboratory has identified enolase to be one of the most oxidatively modified proteins in MCI, EOAD, and AD (Sultana *et al.* 2006a, 2006b, Butterfield *et al.* 2006a, 2006b, Castegna *et al.* 2002, 2003, Reed *et al.* 2008a, 2008b, Perluigi *et al.* 2009, Newman *et al.* 2007), as well as in response to Aβ(1-42) (Boyd-Kimball *et al.* 2005), it is possible that this enzyme becomes either completely unable to integrate into the cell membrane in order to bind PGn, or the oxidative dysfunction of enolase renders this enzyme incapable of binding to PGn/plasmin while membrane-integrated. These scenarios are consistent with the reported decreased levels of plasmin in AD brain (Ledesma *et al.* 2000). Taken together, whether or not the up-regulation of enolase in AD brain stretches beyond the basic need for metabolic ATP remains unknown, but it is highly likely that oxidative dysfunction of multifunctional enolase extends beyond altered glucose metabolism in ways that contribute to biochemical, pathological, and clinical characteristics of AD. If sustained by ongoing studies, this hypothesis would suggest that enolase is a promising therapeutic target of this devastating dementing disorder.

Supplementary Material

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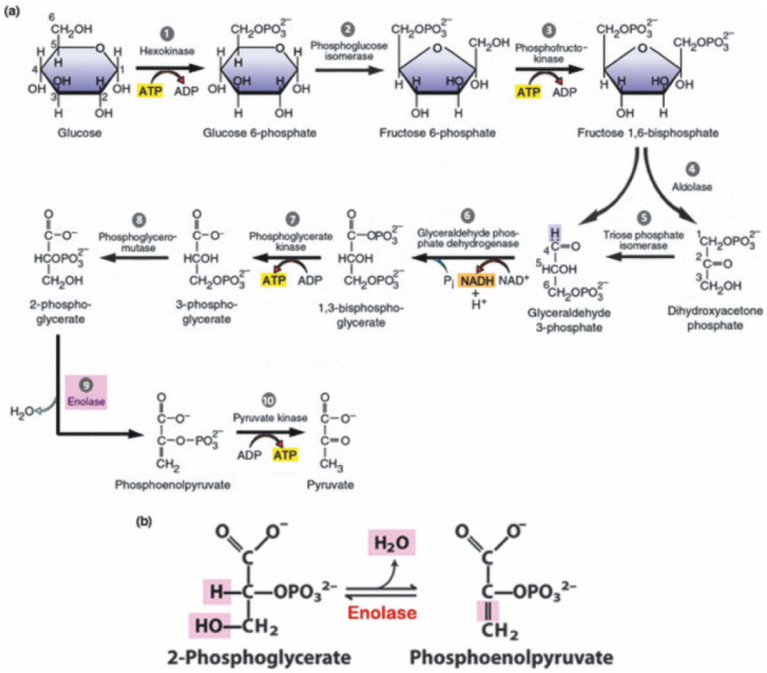

Figure 1.

a) *Glycolysis.* Schematic representation of aerobic glycolysis. Because two 3-carbon triose chains are produced from the reaction between aldolase and fructose-1,6-bisphosphate, steps 5-10 are completed twice (not shown). ATP production in steps 7 and 10 is thought to be the chief fuel-source for plasma membrane ion pumps, including the Na^+/K^+-ATPase and Ca^{2+}-ATPase, rather than ATP produced by mitochondrial oxidative phosphorylation [adapted from (Karp 2003)]. **b)** *Enolase reaction.* Step 9 in the glycolytic chain involves conversion of 2-phosphoglycerate (2-PGA) to phosphoenolpyruvate (PEP) by enolase [adapted from (Nelson & Cox 2009)].

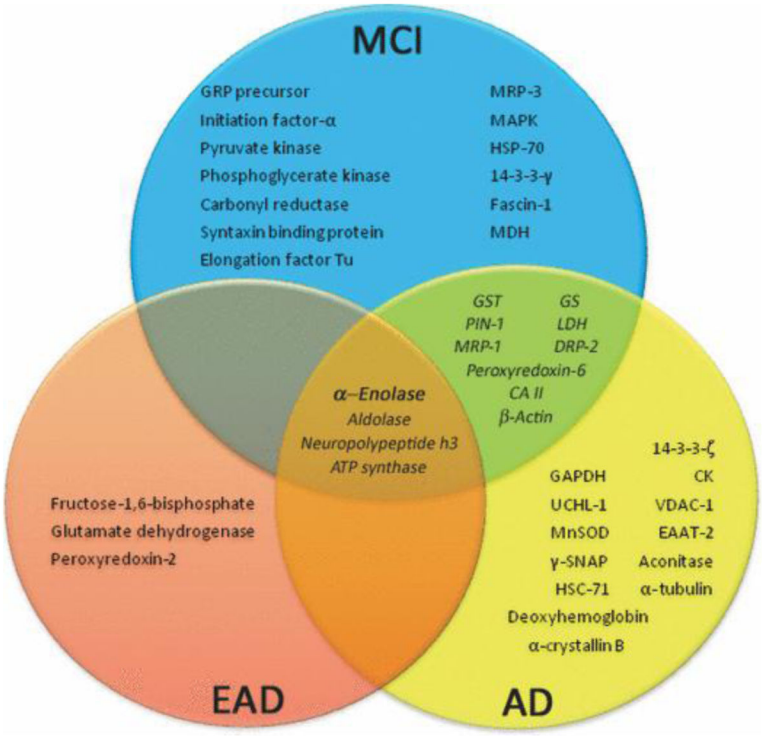

Figure 2.

Oxidatively modified and/or glutathionylated proteins in MCI, EOAD, and AD brain identified by redox proteomics studies from our laboratory (Perluigi et al. 2009, Butterfield et al. 2006a, 2006b, Sultana et al. 2006a, 2006b, Castegna et al. 2002, 2003, Reed et al. 2008a, 2008b, Newman et al. 2007). This diagram shows the interrelation of all proteins found to be oxidatively modified in MCI, EOAD, and AD brain from our laboratory. Abbreviations: GRP precursor, Glucose-regulated protein precursor; MRP-1, Multidrug-resistant protein; MAPK, Mitogen-associated protein kinase; HSP-70, Heat-shock protein-70; MDH, Malate dehydrogenase; GST, Glutathione S-transferase; GS, Glutamine synthetase; PIN-1, Peptidyl-prolyl cis/trans isomerase (PPIase) ; LDH, Lactate dehydrogenase; DRP-2, Dihydropyrimidinase-related protein-2; CAII, Carbonic anhydrase II; HSC-71, Heat-shock cognate-71; γ-SNAP, Soluble N-ethylmaleimide-sensitive factor attachment protein-γ ; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; UCHL-1, Ubiquitin carboxy-terminal hydrolase L-1; VDAC-1, Voltage dependent anion channel-1; CK, Creatine kinase; EAAT-2, Excitatory amino acid transporter-2; MnSOD, Manganese superoxide dismutase.

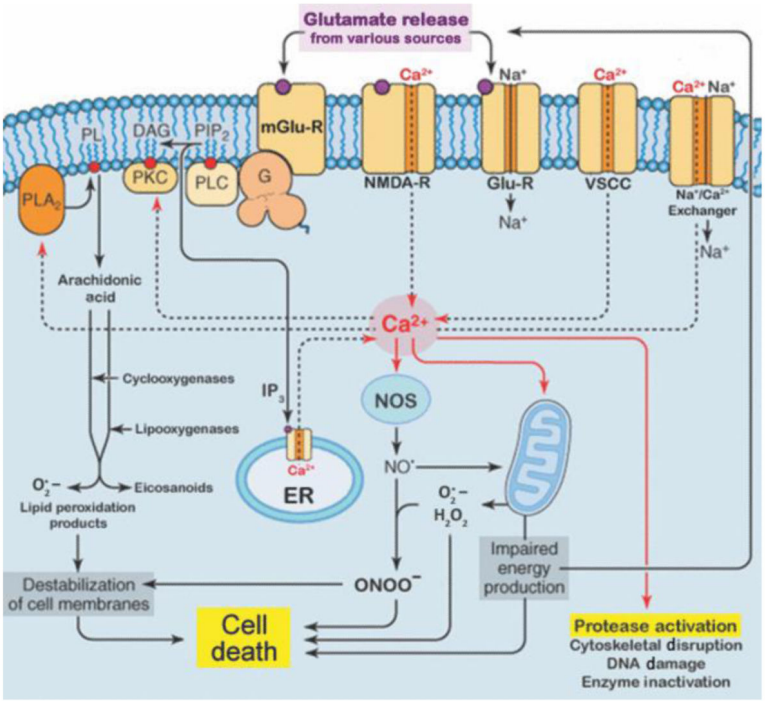

Figure 3.

Glutamate excitotoxicity. This diagram depicts the many intracellular signaling events elicited by excess release and impaired uptake of glutamate, leading to neuronal death. Glu-R, AMPA/Kainate receptors; mGlu-R, metabotropic glutamate receptor; NMDA-R, *N*-methyl-D-aspartate receptor; VSCC, voltage-sensitive Ca^{2+} channel; PL, phospholipids; DAG, Diacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; G, G-protein; PLA_2, phospholipase A_2; PLC, phospholipase C; PKC, protein kinase C; ER, endoplasmic reticulum; H_2O_2, hydrogen peroxide; $NO^{\bullet}$, nitric oxide; $ONOO^-$, peroxynitrite; NOS, nitric oxide synthase; $O_2^{\bullet -}$, superoxide radical [adapted from (Siegel *et al.* 2006)].

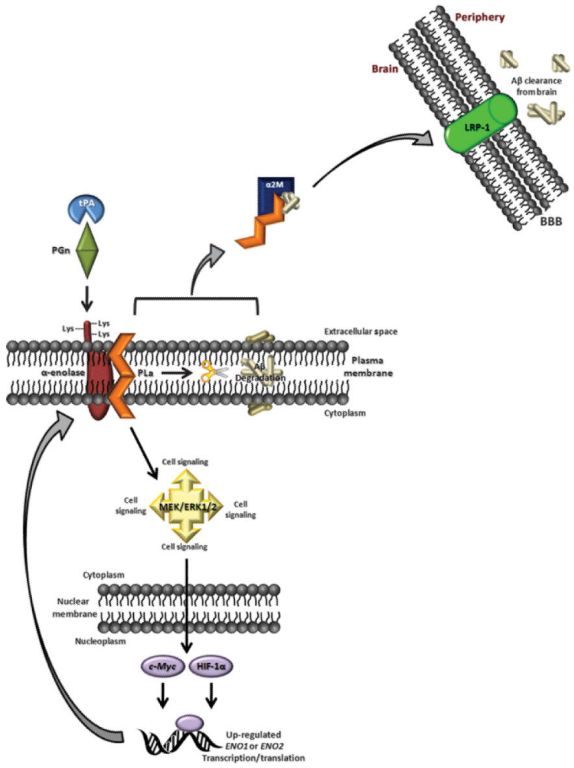

Figure 4.

Possible role of enolase in MCI, EOAD, and AD. This scheme illustrates an alternate role for enolase, in addition to glucose metabolism, in normal and/or MCI, EOAD, and AD brain. In this model, up-regulation and membrane integration of α-enolase, promotes surface-binding of the tPA/PGn complex, which produces the protease plasmin (PLa). Plasmin, in turn, can degrade Aβ peptides associated with the bilayer and activate the MAPK/MEK/ERK1/2 pathway, promoting up-regulation of ENO1 transcription, and therefore, production of α-enolase. In this way, up-regulation of enolase would catalytically amplify an internal signal for cell survival during AD progression. Moreover, by complexing with a2M, plasmin may also be involved with Aβ clearance from the brain via LRP-1 at the blood-brain barrier (BBB). Unfortunately, due to significant oxidative modification, it is hypothesized that enolase becomes unable to facilitate the initiation of these pathways, which would lead to the augmentation of neuronal death in brain of subjects with MCI, EOAD, and AD versus normal aged brain.

Table 1

Enolase functional diversity and/or involvement in disease:

Functional Diversity:

- Glycolytic/Gluconeogenesis enzyme
- Eye lens τ-Crystallin protein
- Plasminogen binding protein in:
  - ○ Various human diseases
  - ○Group A *Streptococci/Pneumococci* bacterial diseases
- *c-Myc* binding protein (MBP-1) and transcription factor in:
  - ○ Tumor formation
  - ○ Metastasis
  - ○ Tumor marker
- Heat-shock protein in yeast
- Hypoxic-stress protein
- Centrosome component in HeLa cells
- Toxin B in *Clostridium difficile* (?)
- Immunodominant antigen in:
  - ○ Invasive *Candidiasis* fungal disease
  - ○ *Candida albicans* and *Aspergillus* enolase-specific IgE responses
- Anti-centrosome antibody
- Anti-neutrophil cytoplasmic antibody (ANCA) in:
  - ○ Vasculitis
  - ○ Systematic lupus erythematosus
  - ○ Discoid lupus erythematosus
  - ○ Nephritis/Primary membranous nephropathy
  - ○ Inflammatory bowel diseases:
    - ■ Ulcerative colitis
    - ■ Crohn's disease
    - ■ Behçet's disease anti-endothelial antigen
  - ○ Liver diseases:
    - ■ Primary sclerosing cholangitis
    - ■ Primary biliary cirrhosis
    - ■ Autoimmune hepatitis
  - ○ Polyglandular candidal ectodermal dystrophy
  - ○ Cancer-associated retinopathy
  - ○ Endometriosis
  - ○ Acute rheumatic fever
  - ○ *Post-Streptococcal* neurological disorder/obsessive-compulsive disorder/Tourette's syndrome (?)

Other Roles/Disease Involvement:

- Site-specific organization of tubule/centrosome
- *Streptococcus intermedius/Streptococcus mutans*-mediated dental caries (?)
- *Plasmodium falciparum* Malaria

- Rheumatoid arthritis

(?), signifies unknown/debated enolase functions and/or disease involvement; [adapted from(Pancholi 2001)]