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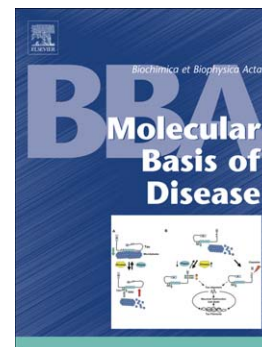
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**Cause and Consequence: Mitochondrial Dysfunction Initiates and Propagates
Neuronal Dysfunction, Neuronal Death and Behavioral Abnormalities
in Age Associated Neurodegenerative Diseases**

Gary E. Gibson^{a*}, Anatoly Starkov^b, John P. Blass^a, Rajiv R. Ratan^a, M. Flint Beal^b

^aDepartment of Neurology and Neuroscience, Weill Cornell Medical College of Cornell University at Burke Medical Research Institute, White Plains, NY 10605 USA

^bDepartment of Neurology and Neuroscience, Weill Cornell Medical College of Cornell University, New York, NY 10065 USA

*To whom correspondence should be addressed. Dr. Gary Gibson, Department of Neurology and Neuroscience, Weill Cornell Medical College of Cornell University at Burke Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605 USA
ggibson@med.cornell.edu

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Key Words.

Mitochondria, oxidative stress, transglutaminase, α -ketoglutarate dehydrogenase, calcium, reactive oxygen species

Abbreviations.

Alzheimer's Disease (AD)

amyloid precursor protein (APP)

amyloid β peptide (A β)

α -ketoglutarate dehydrogenase complex (KGDHC)

electron transport chain (ETC)

huntingtin (htt).

Huntington's Disease (HD)

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

3-nitropropionic acid (3NP)

Parkinson's disease (PD)

progressive supranuclear palsy (PSP),

reactive oxygen species (ROS)

transglutaminase (TGase)

tricarboxylic acid (TCA)

SUMMARY

Age-related neurodegenerative diseases are associated with mild impairment of oxidative metabolism and accumulation of abnormal proteins. Within the cell, the mitochondria appears to be a dominant site for initiation and propagation of disease processes. Shifts in metabolism in response to mild metabolic perturbations may decrease the threshold for irreversible injury in response to ordinarily sub lethal metabolic insults. Mild impairment of metabolism accrue from and lead to increased reactive oxygen species (ROS). Increased ROS change cell signaling via post transcriptional and transcriptional changes. The cause and consequences of mild impairment of mitochondrial metabolism is one focus of this review. Many experiments in tissues from humans support the notion that oxidative modification of the α -ketoglutarate dehydrogenase complex (KGDHC) compromises neuronal energy metabolism and enhance ROS production in Alzheimer's Disease (AD). These data suggest that cognitive decline in AD derives from the selective tricarboxylic acid (TCA) cycle abnormalities. By contrast in Huntington's Disease (HD), a movement disorder with cognitive features distinct form AD, complex II + III abnormalities may dominate. These distinct mitochondrial abnormalities culminate in oxidative stress, energy dysfunction, and aberrant homeostasis of cytosolic calcium. Cytosolic calcium, elevations even only transiently, leads to hyperactivity of a number of enzymes. One calcium activated enzyme with demonstrated pathophysiological import in HD and AD is transglutaminase (TGase). TGase is a cross linking enzymes that can modulate transcription, inactivate metabolic enzymes, and cause aggregation of critical proteins. Recent data indicate that TGase can silence expression of genes involved in compensating for metabolic stress. Altogether, our results suggest that increasing KGDHC via inhibition of TGase or via a host of other strategies to be described would be effective therapeutic approaches in age associated neurodegenerative diseases.

DIMINISHED METABOLISM, OXIDATIVE STRESS AND INCREASED TRANSGLUTAMINASE (TGASE) ARE COMMON TO NEURODEGENERATIVE DISEASES.

Huntington's disease (HD), Parkinson's disease (PD) and Alzheimer's disease (AD) are age-related diseases of diverse etiologies and pathologies, but abnormal mitochondrial function is common to all. All cases of Huntington Disease (HD) are caused by an expansion in the polyglutamine region of the protein huntingtin (htt). HD is one of several adult-onset neurodegenerative diseases that are caused by genes with expanded CAG triplet repeats within their coding regions and extended polyglutamine (Q) domains within the expressed proteins. How these mutations produce the clinical and pathological changes of the diseases is unknown. Mitochondrial abnormalities occur in autopsy brain samples [1] and animal models using the mitochondrial poisons malonate and 3-nitropropionic acid (3NP) mimic human HD [2]. In PD, identification of the rare specific gene mutations that cause PD has reinforced the relevance of oxidative stress and mitochondrial dysfunction in the disease. The proteins that are associated with familial PD—PTEN-induced putative kinase 1 (PINK1), DJ-1, α -synuclein, leucine-rich repeat kinase 2, and, possibly, parkin—are either mitochondrial proteins or are associated with mitochondria, and all interface with the pathways of oxidative stress and free radical damage [3-5]. Animal models using the mitochondrial poison 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPTP) mimic human PD [6]. In AD, the rare gene mutations in amyloid precursor protein (APP) or presenilin proteins lead to increased production of amyloid β peptide ($A\beta$), which depresses mitochondrial function, thereby altering calcium regulation, inducing oxidative stress and generally impairing metabolism. Pathological analysis and studies of peripheral tissues demonstrate these are common features of non-genetic forms of AD as well. The brains from patients with all age-related neurodegenerative diseases show extensive damage from oxidative stress in their brains at autopsy.

Reduced metabolism can be readily related to the clinical characteristics of neurodegenerative diseases. The relation of mitochondrial changes to clinical symptoms is particularly well documented in AD. Diminished glucose metabolism always accompanies AD and the reduction occurs at early stages of the disease, even pre-symptomatically. The decline in glucose metabolism with AD was shown in some of the earliest imaging studies of the human brain and has been recognized for many decades. The reduction in glucose metabolism is feasibly linked by robustly documented mechanisms to the decline in mental function, and diminished memory is particularly vulnerable. The sensitivity of memory to altered glucose metabolism may be because of the close link of metabolism and neurotransmitters that are known to be involved in memory, particularly acetylcholine [7]. In patients with APOE4 genotype that will be at greater risk to get AD later in their life, the decline precedes the clinical characteristics by decades [8]. Furthermore, mildly cognitively impaired patients that have a reduction in brain glucose metabolism all go on to develop AD [9-11]. Reductions in cerebral metabolism sufficient to impair cognition in normal individuals occur in AD. The degree of clinical disability in AD correlates well with the magnitude of the reduction in brain metabolism.

The question is no longer whether changes occur in mitochondria in neurodegenerative disease. Currently, the interesting questions are what is the cause and whether these mild changes in metabolism have any consequence on mitochondria or their ability to adapt to metabolic insults and if reversal of them can be beneficial to cells, animals or patients.

Damage from free radicals is common in brains from patients with age-related neurodegenerative disorders. Reactive Oxygen Species (ROS) are normally signaling molecules. For example, they regulate tricarboxylic acid (TCA) cycle enzymes such as KGDHC, and many transcription factors. However, if the level is too high they will damage multiple proteins. The sources of free radicals in neurons and other cells has been reviewed recently [12-13]. Changes in antioxidant enzymes or in key enzymes of glucose catabolism, especially those in the mitochondria, can lead to excess radical production. Evidence for free radical damage is extensive in brain. For example, immunostains for reactive aldehydes or peroxynitrite reveal that the damage caused by oxidative stress in AD brain is more extensive than are plaques and tangles (the defining feature of AD) [14]. Evidence of free radicals (i.e., isoprostanes) can even be observed in AD and PD urine, and these can distinguish AD and PD [15]. Increased levels of F2 isoprostane levels occur in plasma and urine of AD, but not PD patients [16]. Although the evidence for abnormal ROS in AD, PD and HD is overwhelming, the source of these ROS is not established.

In animal models of AD plaque formation, the changes in free radicals precede plaque formation. Levels of plasma, urinary and brain homogenate 8,12-iso-iPF(2 α)-VI isoprostanes are higher in Tg2576 than in control animals as early as 8 months of age. In contrast, a

surge of A β 1-40 and 1-42 levels as well as A β deposits in Tg2576 mouse brains does not occur until 12 months of age. This suggests that brain oxidative damage contributes to AD pathogenesis before A β accumulation in the AD brain [15].

Increased oxidative stress also occurs in PD [6], HD[17] and PSP [18]. The questions in these diseases are similar to those for AD. The evidence for oxidative damage is overwhelming but the source of the ROS and their pathological importance is unknown. Amongst the possibilities are mitochondria, NADPH-oxidase, iNOS xanthine oxidase, monoamine oxidase and the dehydrogenase complexes such as KGDHC. The strategies for understanding the therapeutic implications are similar in these diseases although the pathologies vary. Although the underlying basis of these changes in oxidation may be primary, determining what they are has proven elusive so the strategy has been to target downstream consequences.

REDUCTIONS IN KEY MITOCHONDRIAL ENZYMES MAY UNDERLIED THE REDUCTION IN GLUCOSE METABOLISM IN NEURODEGENERATIVE DISEASES

The simplest explanation for a reduction in metabolism is decline in the activity of metabolic enzymes. The reductions in the α -ketoglutarate dehydrogenase complex (KGDHC) activity in AD brain have been particularly well documented, and the reductions appear to be a common feature of neurodegeneration of various kinds. The initial studies [19] that showed this decrease in AD have been widely replicated [20-22], and there are no contravening reports. The severity of change and their correlation with the clinical dementia rating score vary with APOE4 genotype [23], but positive correlations occur whether or not patients carry any APOE4 allele. Reductions in KGDHC occur in PD [24], HD [2, 18] and progressive supranuclear palsy [18]. The reduction in KGDHC is not limited to the areas of pathology and neuron death. The precise chemical nature of the changes in KGDHC that lead to diminished activity in brains from AD patients are unknown. Results suggest that the decline is a post-translational modification. Although the activity is diminished in all forms of AD, the immunoreactivity is selectively altered in familial AD. In patients bearing the APP670/671 mutation the immunoreactivity of E1k and E2k are diminished whereas E3 is normal [25]. The reduced activity with normal subunit protein immunoreactivity is also found in PD [24] and progressive supranuclear palsy [18]. KGDHC activity was also reduced in putamen from patients with HD [2].

The reductions in KGDHC activities do not reflect general mitochondrial changes since some TCA cycle enzymes decline while others increase. Brains were from patients with autopsy-confirmed AD who had also had recent clinical dementia ratings (CDR) before death. Significant ($p < 0.01$) decreases occur in the activities of the enzymes in the first part of the TCA cycle that are combined dehydrogenases and decarboxylases: the pyruvate dehydrogenase complex (-41%), isocitrate dehydrogenase (-27%), and KGDHC (-57%). On the other hand, activities of the dehydrogenases in the second half of the TCA cycle increase: succinate dehydrogenase (complex II) (+44%) and malate dehydrogenase (+54%) ($p < 0.01$). Activities of the other four TCA cycle enzymes are unchanged. All of the changes in TCA cycle activities correlate with the clinical state ($p < 0.01$), suggesting a coordinated mitochondrial alteration. The highest correlation is with pyruvate dehydrogenase complex ($r = 0.77$) [26]. The results are consistent with a decline in these enzymes underlying the reduction in glucose metabolism, which is coupled to the clinical state of the patient. If we understood why the reductions occur, treatments might be devised to

reactivate them and hopefully thereby to restore brain metabolism. The relation of the changes in the TCA cycle enzymes to TGase and oxidative stress is discussed below.

A β is present in mitochondria and directly effects KGDHC and PDHC. Intracellular A β is present in mitochondria from brains of transgenic mice with targeted neuronal over-expression of mutant human APP and AD patients [27]. A β progressively accumulates in mitochondria and is associated with diminished enzymatic activity of respiratory chain complexes (III and IV) and a reduction in the rate of oxygen consumption. Mitochondria-associated A β was detected as early as 4 months, before extensive extracellular A β deposits [28]. Within the mitochondria A β will inhibit PDHC and KGDHC [29].

OXIDATIVE STRESS CAN LEAD TO THE AD-LIKE CHANGES IN THE TCA CYCLE.

The increased oxidative stress in brains from patients that died with neurodegenerative disease and the reduction in KGDHC are likely to be related. KGDHC is very sensitive to oxidants. Inhibition occurs in cells, mitochondria or isolated enzymes by H₂O₂, nitric oxide, peroxynitrite, chloroamine [30], hypochlorite [30], hydroxynonenal, hyperoxia, and acrolein [31]. Overproduction of monoamine oxidase produces increased ROS in-cells and inactivates KGDHC [32]. Similarly, if oxidants are overproduced by knocking out SOD, ROS increases and KGDHC is inactivated [33].

Peroxynitrite inhibits purified KGDHC in a dose- and time-dependent manner. Loss of enzymatic activity is associated with reduced immunoreactivity of E1k and E2k subunits, but not E3, and increased immunoreactivity of each component to a nitrotyrosine antibody, suggesting nitration of tyrosine residues. Nitrosylation was further confirmed by a combination of trypsin digestion and nano-LC-MS/MS analysis. Tyrosine residue 556 is nitrated in the E1k subunit, while no nitration is found in E2k and E3. Addition of GSH after peroxynitrite treatment of KGDHC completely restores the KGDHC activity and the immunoreactivity of E1k and E2k subunits. The immunoreactivity of nitrotyrosine is diminished to near control level upon addition of GSH. Such decreased immunoreactivity suggests that nitrotyrosine of KGDHC is further modified by possibly converting -NO₂ to -NH₂ in tyrosine residues to form aminotyrosine, or denitrating -NO₂ in tyrosine residues. Taken together, the data directly demonstrate the nitration of tyrosine residue of KGDHC. The findings indicate that the similar modifications of KGDHC may also occur in cultured cells, and regulation of KGDHC activity by these processes may be of significance in AD pathology as well as in normal regulation of KGDHC activity [34].

In addition, KGDHC can be inactivated by other means. KGDHC is very sensitive to other toxins such as cisplatin [35], MPTP [36], and 3-NP. In addition, KGDHC can be inactivated by transglutaminase [37].

TRANSGLUTAMINASE (TGase) IS ELEVATED IN MULTIPLE NEURODEGENERATIVE DISEASES.

TGase catalyzes a covalent bond between peptide-bound glutamine residues and either lysine-bound peptide residues or mono- or polyamines (Figure 1). Multiple lines of evidence suggest that TGase is involved in neurodegenerative diseases including frontotemporal dementia, AD, progressive supranuclear palsy (PSP), HD, and PD [38]. Transcription of TGase is increased by head trauma and stroke and glutamate toxicity [39] and by hypoxia (Wykoff). In age-related neurodegenerative diseases TGase enzyme activity is increased in selectively vulnerable brain regions, TGase proteins are associated with inclusion bodies characteristic of the diseases, and prominent proteins in the inclusion bodies are modified by TGase enzymes including tau, alpha-synuclein, and huntingtin protein. Evidence for N(epsilon)-(gamma-L-glutamyl)-L-lysine (GGEL) linkages is apparent in brain and CSF samples from AD and HD patients [40-42]. Four gamma-glutamylpoly amines are present in normal human CSF. All four gamma-glutamylamines are elevated in HD CSF. These findings support the suggestion that gamma-glutamylpolyamines are reflective of TGase activity in human brain, that polyamination is an important post-translational modification of brain proteins, and that TGase-catalyzed modification of proteins is increased in HD brain [42]. Thus, a wide range of studies suggest that activation and/or increased transcription of TGase could have wide ranging effects and serve as a therapeutic target.

INCREASED TGase CAN ALTER METABOLISM BY TRANSLATIONAL AND POST-TRANSLATIONAL CHANGES.

As described in the introduction, TGase is increased in multiple neurodegenerative diseases and in animal and chemical treatments that model these diseases. In contrast to its inactivating effect on TCA cycle enzymes, oxidative stress can upregulate TGase. For example, oxidative stress appears important in glutamate-evoked TGase upregulation in astrocyte cultures [43]. Glutamate causes a dose-dependent increase in ROS production. Pre-incubation with glutathione ethyl ester or cysteamine recovers oxidative status and reduces glutamate-increased tissue TGase. Thus, TGase up-regulation may be part of a biochemical response to oxidative stress [43]. An increase of TGase 2 expression may contribute to activation of microglia. After activation of BV-2 microglial cell lines by lipopolysaccharide, the expression of TGase 2 and iNOS increase in parallel. Furthermore, TGase inhibition reduces secretion of NO by up to 80%, in a dose-dependent manner. This observation suggests that TGase 2 controls iNOS transcription or iNOS message stability [44].

Results in cells and with isolated enzyme support a role for TGase in neurodegenerative diseases and as a possible cause of the decline in metabolism in neurodegenerative disorders. By its ability to cross link, TGase can inhibit enzymes such as glyceraldehyde-3-phosphate dehydrogenase, KGDHC [45] or aconitase [46], key enzymes of glycolysis and the TCA cycle. The cross links with aconitase have been observed in HD brains at autopsy [46], and most of the mitochondrial aconitase in HD caudate is present as high-Mr aggregates. Aconitase activity is markedly decreased in HD caudate (a region severely damaged by the disease) [47-48] and HD transgenic mice [49-50]. These findings suggest that an increase of TGase activity in HD caudate may contribute to mitochondrial dysfunction by incorporating aconitase into inactive polymers [46]. Although KGDHC is also a substrate for TGase [37], that linkage has not been shown in brains from patients that died with neurodegenerative diseases. TGase action on transcription may be

due to cross linking of transcription factors to diminish gene cascades that may normally enhance mitochondrial biogenesis or function [51]. For the inactivation of mitochondrial enzymes to occur under physiological or pathological conditions, TGase must be present in the mitochondria and appropriate substrates must be present. Studies reveal that TGase is present in mitochondria from both brain and liver [52]. However, the levels are very low.

Studies that diminish TGase activity *in vivo* support the suggestion that TGase is pathologically important. Studies in TGase knockout mice [53] as well with the TGase inhibitor cystamine [54-55] suggest TGase activation is a critical part of the HD disease process. Understanding the mechanism for that neuroprotection is important for designing better therapeutic approaches.

TGase is a multifunctional enzyme that may have beneficial effects. TGase is up-regulated in neurons exposed to oxygen and glucose deprivation and increased TGase expression protects neurons against oxygen and glucose deprivation induced cell death independent of its transamidating activity. TGase protects by interacting with HIF1 [56]. TGase attenuates the HIF1 hypoxic response pathway, and up-regulates the HIF-dependent proapoptotic gene Bnip3. These results indicate that TGase may play an important role in protecting against the delayed neuronal cell death in ischemia and stroke [56]

TGase can also affect mitochondrial function by acting at the level of transcription. Accumulating data suggests that the elevated TGase in neurodegenerative conditions may impair transcription and the ability to adapt. This implies that blocking TGase should promote transcription and be protective. Convincing evidence for this scenario has emerged in studies of HD. Transcriptional repression may be responsible for the inability of mutant huntingtin (mhtt) expressing cells to cope with the metabolic stress. Multiple groups have shown that mhtt is able to sequester needed transcription factors and other proteins necessary for transcription initiation. TGase, like mhtt, has been shown to alter specific transcription factors (e.g., Sp1) [57-58]. The transcription factor(s) found to be modified by TGase play a role in the expression of nuclear-encoded mitochondrial proteins such as cytochrome c and PGC-1 (PPAR γ -coactivator 1). Numerous metabolic proteins are repressed in HD models and HD patients [59-61]. These proteins (including cytochrome c (cyt c) and PGC-1 α) are under the control of nuclear transcription factors that contain glutamine (Q)-rich activation domains. Since the large number of Q residues in these activation domains provides a possible target for modification by TGase, experiments tested the ability of TGase to repress the transcription of metabolic genes (cyt c and PGC-1 α) that are down-regulated in HD models and patients. Transient transfection of a promoter/reporter of cyt c reveals a basal decrease in the cyt c promoter activity between htt and mhtt expressing cells exposed to serum starvation/restimulation. Co-transfection of TGase greatly reduces cyt c promoter activity. Pharmacological inhibition, as well as genetic deletion of TGase, induces cyt c promoter activity and increases mRNA levels of cyt c and PGC-1 α . Citrate synthase activity, the first step of the TCA cycle, also increases after TG inhibition [51]. Whether the action of TGase on transcription factors is a physiological regulatory mechanism that goes wrong if mutant huntingtin is present remains to be determined.

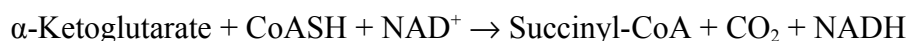
If the decreased ability of mutant huntingtin cells to respond to metabolic stress is due transcriptional repression and diminished mitochondrial activity, then increased expression of nuclear-encoded mitochondrial proteins (such as cytochrome c) should enhance mitochondrial

biogenesis and increase survival of mutant huntingtin (mhtt)-encoding neurons. If the inability of mhtt-expressing cells to respond to energetic stress is due the over-activity of the enzyme TGase that has been reported in HD patients and models alike, then inhibiting TGase should increase mitogenesis and be neuroprotective. Immortalized murine striatal neurons from mice with a huntingtin gene with a normal number of repeats as well as a separate strain with a pathological level of repeats were used to test whether TGase inhibitors can be neuroprotective. Inhibition of TGase increases the transcription of key mitochondrial proteins in cells expressing mutant huntingtin. Thus, over-active TGase within mhtt-expressing cells may silence the expression of nuclear-encoded mitochondrial proteins through the modification of transcription factors, and loss of this compensatory mechanism causes these cells to be more susceptible to the metabolic stress that is seen in HD [62]. The cells expressing mutant huntingtin are more sensitive to the mitochondrial toxin, 3-NP, which induces HD like changes in animal models. 3-NP acts by inhibiting complex II in the mitochondrial respiratory chain. To test whether TGase inhibition can prevent neuronal death due to mitochondrial dysfunction, the role of the TGase inhibitor ZDON and its inactive control in preventing death were tested. ZDON, but not its analog, enters the cell and inhibits TGase [51]. ZDON is neuroprotective, but the inactive analog is not in both striatal cell lines with mutant huntingtin and in myoblasts from HD patients. The neuroprotective efficacy of ZDON correlates with its ability to inhibit TGase.

The mechanism(s) by which TGase promotes harmful effects in HD remain(s) under study. TGase can act directly at gene promoters such as PGC-1 α (a coactivator) and cytochrome c to repress the ability of transcription factors to recruit the basal transcription factor machinery. TGase is bound to a complex of proteins that interact directly with the promoters of these target genes. Inhibition of TGase activity using pharmacological inhibitors or RNAi knockdown leads to increased promoter activity, mRNA levels and protein levels of the transcription factor PGC1 α and the protein cytochrome c. Finally, pharmacological inhibition of TGase activity leads to robust protection of HD cell lines as well as primary neurons and HD myoblasts against the mitochondrial toxicant, 3-NP. Together these studies suggest that transglutaminase inhibition overcomes mitochondrial dysfunction in HD by facilitating the expression of genes involved in mitochondrial biogenesis and function [63].

THE α -KETOGLUTARATE DEHYDROGENASE COMPLEX (KGDHC) IS A CALCIUM SENSITIVE CENTRAL ENZYME OF METABOLISM THAT PRODUCES AND IS CONTROLLED BY ROS

A compelling body of evidence indicates that KGDHC is one of the important targets of mitochondrial injury. An understanding of the multiple roles of KGDHC [64] is required to identify the upstream (i.e., causes) and downstream (i.e., consequences) effects which may provide additional therapeutic targets. KGDHC catalyses the reaction



The substrates and the products of this reaction are central to several key metabolic and signaling pathways (see Figure 2).

The α -ketoglutarate decarboxylase subunit (E1) catalyses the rate-limiting stage of the overall KGDHC reaction [65]. In brain mitochondria, the activity of KGDHC is the lowest of the TCA cycle enzymes and may be rate limiting for overall TCA cycle activity *in vivo* [66-67]. Consequently, the KGDHC reaction is essential for sustaining mitochondrial oxidative energy metabolism and neuronal viability.

Mammalian KGDHC is an integral mitochondrial enzyme tightly bound to the inner mitochondrial membrane on the matrix side [68]. It binds specifically to Complex I of the mitochondrial respiratory chain [69] and may form a part of the tricarboxylic acid cycle (TCA) enzyme super-complex [70]. Structurally, KGDHC is composed of multiple copies of three enzymes: α -ketoglutarate dehydrogenase (E1k subunit, EC 1.2.4.2), dihydrolipoamide succinyl-transferase (E2k subunit, EC 2.3.1.12), and lipoamide dehydrogenase (E3 subunit, EC 1.6.4.3). The E3 subunit of KGDHC is identical to that of pyruvate dehydrogenase; it is also known as dihydrolipoamide dehydrogenase (Dld). The other subunits are unique to KGDHC. All the subunits are encoded by nuclear genes. For enzymatic activity, E1k requires the cofactor thiamine pyrophosphate, which is tightly bound; E2k requires covalently bound dihydrolipoic acid; and E3 requires covalently bound flavin [71-73].

The composition of KGDHC and its content exhibit tissue specificity. Neurons are particularly enriched in KGDHC compared to glia [74]. Immunogold electron microscopy with an antibody against the E1k component of the KGDHC demonstrates that KGDHC is heterogeneously distributed in mitochondria within individual astrocytes originating either from cerebellum or cerebral cortex [75]. Brain E1k appears to differ from heart. In the heart complex, only the known E1k is found. However, in brain the band corresponding to the brain E1k component includes a novel E1k protein. The functional competence of the novel brain isoenzyme and different regulation of E1k and E1kl by 2-oxoglutarate are apparent from kinetic studies [76]. Brain KGDHC also differs from the heart KGDHC in the ratio to the other components, lower apparent molecular mass and stability of isolated complex.

In addition to its traditional role to produce NADH in TCA cycle, KGDHC performs another uniquely important function by providing succinyl-CoA for heme synthesis which is initiated in mitochondria [77-78]. Heme is obligatory in the assembly of ETC and several antioxidant enzymes protecting the brain from oxidative stress.

Alternative functions are known for each of the KGDHC subunits. Conditions that destabilize the E3 homodimer enabled the mouse, pig, or human enzyme to function as a protease [79]. A truncated gene product (MIRTD) of E2k contributes to the biogenesis of the mitochondrial respiratory complexes [80]. E3 has diaphorase activity, being able to catalyze the oxidation of NADH to NAD by using different electron acceptors such as O_2 , labile ferric iron [81], nitric oxide [82], and ubiquinone [83-84]. In this capacity, E3 would primarily have a prooxidant role, achieved by reducing O_2 to a superoxide radical or ferric to ferrous iron. However, the ability to scavenge nitric oxide and to reduce ubiquinone to ubiquinol suggests that the diaphorase activity of E3 may also have an antioxidant role [82-84].

The regulation of KGDHC activity is rather complex and apparently makes it an integrating hub for cellular Ca^{2+} , metabolite, and ROS signaling systems. KGDHC is activated by low concentrations of Ca^{2+} and matrix ADP, and is inhibited by high NADH and its own product, succinyl-CoA [85-89]. In addition to this classical regulation, new data emerge that KGDHC activity is both controlled and controls the intracellular ROS levels. Several studies have shown

that KGDHC can produce free radicals at E2k and H_2O_2 at E3. These become physiologically significant when the $NAD^+/NADH$ ratio in the mitochondrial matrix is low (i.e., under reducing conditions) [90-91]. This production appears to be pathologically important since inhibitors of KGDHC can protect against *in vitro* associated with over-production of ROS, such as ischemia [92-93] and glutamate toxicity [94] while also blocking production of free radicals.

Excessive entry of calcium accompanies many cell injuries and mitochondria buffer rapid increases in calcium in neurons and most other cells. The role of calcium in activation of KGDHC has been reviewed reviewed in [95]. Mitochondria isolated from adult rat cerebral cortex and cerebellum generate extremely reactive hydroxyl (.OH) radicals, plus ascorbyl and other carbon-centered radicals when exposed to 2.5 μM Ca^{2+} , 14 mM Na^+ , plus elevated ADP under normoxic conditions, circumstances that prevail in the cytoplasm of neurons during excitotoxin-induced neurodegeneration [96]. In heart, the calcium that enters the mitochondria following stimulation activates both PDHC and KGDHC [85-86, 89]. KGDHC is more sensitive than PDHC to calcium [89].

The sensitivity of the KGDHC to oxidants may also be regulatory. For example, H_2O_2 is known to diminish NADH production by inactivating the KGDHC [32, 90], whereas its E2k component can sense the intramitochondrial redox state which affects the E2k dihydrolipoate/lipoate ratio [97]. It has also been shown that thyl radicals can inhibit E1k and that KGDHC and thyl radicals formed at E2k lipoate residues can interact with thioredoxin [98-99]. Although arguable in light of yet insufficient evidence, the ramification of this is that KGDHC may serve as a major regulatory element adjusting the metabolic output of TCA cycle according to the redox state of intramitochondrial thiols, pyridine nucleotides, and ROS production.

CONSEQUENCES OF MILD IMPAIRMENT OF KGDHC

Energy failure may be a late event following inhibition of KGDHC. α -Keto- β -methylvalerate (KMV), a structural analogue of α -ketoglutarate has reasonable specificity but low potency [66]. KMV inhibits acetylcholine synthesis before impairing energy metabolism as measured by ATP levels [66]. KMV promotes translocation of mitochondrial cytochrome c to the cytosol, and leads to activation of caspase-3 before the mitochondrial membrane potential or ROS production is altered. Thus, the results suggest a decrease in KGDHC activity induces gene cell death cascades before energetic failure. This suggests that treatments that affect these gene cascades may be effective in neurodegenerative diseases that are accompanied by mild impairment of oxidative metabolism.

Succinylphosphonate (SP) and its esters are potent and specific KGDHC inhibitors. SP and its phosphonoethyl (PESP) and carboxyethyl (CESP) esters at a concentration of 0.01 mM completely inhibit isolated KGDHC. In cells, 0.01 mM SP, PESP, or CESP inhibit KGDHC by 70%. The diethyl- and tri-ethyl esters were also inhibitory in the cell system, but only after preincubation, suggesting the release of their charged groups by cellular esterases. Thus, SP and its monoethyl esters target cellular KGDHC directly, while the di- and triethyl esters are activated in intact cells. When tested on other enzymes that bind α -ketoglutarate or related α -keto acids, SP has minimal effects and its two esters (CESP and TESP) are ineffective even at concentrations

one order of magnitude higher than those that inhibit cellular KGDHC activity. The high specificity in targeting KGDHC, penetration into cells, and minimal transformation by cellular enzymes indicates the usefulness of these compounds for studies of reduced KGDHC activity on neuronal and brain function [100].

Mild impairment of KGDHC by SP-esters alters the metabolic profile of intact cerebellar granule cells during incubation with [$1\text{-}^{13}\text{C}$]glucose and [$\text{U-}^{13}\text{C}$]glutamate. The results suggest increased α -ketoglutarate formation, increased transamination of α -ketoglutarate with valine, leucine, and GABA, and a new equilibrium position of the aspartate aminotransferase reaction. Overall, the findings also suggest that some carbon derived from alpha-ketoglutarate may bypass the block in the TCA cycle at KGDHC by means of the GABA shunt and/or conversion of valine to succinate. The results are consistent with the suggestion that impairing KGDHC increases the GABA shunt in order to maintain normal metabolism [101]. The shift in metabolism may make the cells less able to adapt to further insults.

Similar effects on metabolism were observed in cells by changing KGDHC activities by genetic manipulation of E2k. As described above, the KGDHC complex consists of three enzymes: E1k, E2k and E3. Any one of the three can be manipulated genetically to examine the consequences of a reduction in KGDHC activity. The results are complicated, but made more revealing, by the observation that manipulating each subunit does not necessarily have a parallel effect on the activity of KGDHC. Human embryonic kidney (HEK) cells were stably transfected with an E2k sense or antisense expression vector. Sense control (E2k-mRNA-100) was compared with two clones in which the mRNA was reduced to 67% of control (E2k-mRNA-67) or to 30% of control (E2k-mRNA-30). The levels of the E2k protein in clones paralleled the reduction in mRNA, and E3 proteins are not altered. Unexpectedly, the clone with the greatest reduction in E2k protein (E2k-mRNA-30) has a 40% increase in E1k protein. The activity of the complex was only 52% of normal in E2k-mRNA-67 clone, but was near normal (90%) in E2k-mRNA-30 clone [102].

Subsequent experiments tested whether a reduction in the KGDHC activity would alter cellular metabolism by comparing metabolism of [$\text{U-}^{13}\text{C}$]glucose in cell line (E2k100) to one in which the KGDHC activity was about 70% of control (E2k67). After incubation of the cells with [$\text{U-}^{13}\text{C}$]glucose, the E2k67 cells showed a greater increase in ^{13}C labeling of alanine and lactate compared to the E2k100 cells, which suggests an increase in glycolysis. Increased GABA shunt in the E2k67 cells was indicated by increased ^{13}C labeling of GABA compared to the control cells. GABA concentration as determined by HPLC also increased in the E2k67 cells compared to the control cells. The data are consistent with enhanced glycolysis and GABA shunt in response to a mild reduction in KGDHC [103]. The findings indicate that a mild change in KGDHC activity can lead to large changes in metabolism. The changes may allow maintenance of normal energy metabolism but still make the cells less able to adapt to perturbations such as occur with oxidants.

The levels of E2k protein modify the response to oxidative stress. Subsequent experiments tested whether the response to an oxidant challenge more closely paralleled the E2k protein levels or KGDHC activity. Growth rates, increased DCF-detectable reactive oxygen species, and cell death in response to added H_2O_2 are proportional to E2k proteins, but not to complex activity. These results were not predicted because subunits unique to KGDHC have never been manipulated in mammalian cells. These results suggest that in addition to its essential role in

metabolism, the E2k component of KGDHC may have other novel roles [102]. For example, the results are consistent with the suggestion that a truncated gene product (MIRTD) of E2k contributes to the biogenesis of the mitochondrial respiratory complexes [80].

Alterations in E2k protein levels alter gene expression. Although KGDHC activities are diminished in AD brains, the activities of MDH are increased. To test the possibility that this is secondary to the decline in KGDHC, MDH activity and message levels were determined in cells with varying E2k protein levels. Activity of MDH is increased with KGDHC deficiency but only in the E2k23 lines. Of the three lines tested, these have the greatest decrease in E2k but nearly normal KGDHC activities. In contrast to the increase in MDH activities, the message levels of both cytosolic and mitochondrial MDH decline in the E2k23 lines. H_2O_2 reduces KGDHC activities in cells, but increases MDH activity and the message levels for both the mitochondrial and cytosolic MDH — but only in the lines most deficient in E2k. This raises the possibility of a coordinate regulation of gene expression of the TCA cycle [104]. The interaction of KGDHC with α -ketoglutarate oxidases such as the HIF prolyl hydroxylases that have the same substrate (ketoglutarate) and same product (succinate) is not clear. Thus, an increase in α -ketoglutarate due to impaired KGDHC may increase HIF prolyl hydroxylases.

Treatment of purified KGDHC with oxidized glutathione (GSSG) leads to glutathionylation of all three KGDHC subunits [105]. H_2O_2 converts reduced glutathione (GSH) to oxidized glutathione (GSSG), which reacts with protein thiols [104]. Cellular glutathione level has been manipulated in HEK cells by two means to determine whether the level of E2k alters the effects of glutathione on KGDHC. Both buthionine sulfoximine (BSO), which inhibits glutathione synthesis without altering redox state, and H_2O_2 diminished glutathione to a similar level after 24 h. However, H_2O_2 , but not BSO, reduced KGDHC activities, and the reduction was greater in the E2k-23 line with the lowest E2k protein. These findings suggest that H_2O_2 moved glutathione to the protein. These findings suggest that the E2k may mediate diverse responses of KGDHC to oxidants [104]. When cardiac mitochondria are challenged with H_2O_2 , NADH production and oxidative phosphorylation decline. Upon consumption of H_2O_2 , mitochondrial function is restored. These alterations are due, in large part, to reversible glutathionylation and inhibition of KGDHC [106]. In addition, the differential response of KGDHC activities to BSO and H_2O_2 together with the *in vitro* interaction of KGDHC with GSSG suggests that glutathionylation is one possible mechanism underlying oxidative stress-induced inhibition of the TCA cycle [104].

The ability of KGDHC to produce H_2O_2 suggests that inhibiting KGDHC could be neuroprotective, while the diminished production of NADH would suggest that impairing KGDHC is damaging. As discussed above, inhibiting KGDHC under normal conditions appears damaging. However, if the cell is experiencing life threatening conditions, inhibition of KGDHC appears to protect. The first study demonstrated protection by impairing KGDHC in microglia. The KGDHC inhibitor KMV (see above) dose-dependently reduced ROS production and LDH release from a hypoxic microglial cell line [92]. KMV also reduced ROS production and enhanced the cell viability following H_2O_2 but failed to reduce the SIN-1 and sodium nitroprusside (SNP) toxicity. KMV also reduced caspase-3 and -9 activation under stress. These results suggest that KMV protects BV-2 cells from stress and acts by reducing ROS production through inhibition of ROS production by KGDHC.

Inhibition of KGDHC can also be protective in neurons. KGDHC inhibitors were added to glutamate poisoned neurons. Their subsequent inhibition of KGDHC decreases the mitochondrial potential [107]. The production of O_2^- was detected by reaction with hydroethidine. The distribution of the resulting fluorescence of mt-DNA-ethidium coincides with that of the mitochondrial marker Mitotracker, pointing to the mitochondrial site of the hydroethidine-detected ROS in response to glutamate. The SP inhibitors described above inhibited glutamate-induced ROS production by about 20-44%. The decreases in neuronal ROS by specific inhibitors of KGDHC suggest that KGDHC is a source of ROS in cultured neurons responding to glutamate. However, at higher concentration the KGDHC inhibitor increased ROS production compared with glutamate alone, presumably due to secondary effects arising upon the strong KGDHC inhibition. This suggests that different mechanisms are invoked at different concentrations. They support the suggestion that inhibition of KGDHC ROS production can protect against *in vitro* excitotoxicity [107].

IMPAIRMENT OF KGDHC *IN VIVO* INDUCES OXIDATIVE STRESS AND COMPROMISES THE ABILITY OF THE BRAIN TO RESPOND TO OTHER INSULTS.

Three different models test mechanistic interactions of KGDHC in neurodegeneration. They are presented from the most complex to the one that only affects one enzyme. (1) thiamine deficiency (TD; i.e., vitamin B1 deficiency) which primarily affects KGDHC and transketolase (a critical enzyme of the pentose shunt), although thiamine is also a cofactor for PDHC; (2) E3^{+/-} mice which have reduced activities of KGDHC and PDHC and (3) E2k^{+/-} mice, which have a primary deficit in only KGDHC. A comparison of the three models helps distinguish critical variables that are necessary to cause particular deficits.

Thiamine deficiency. Thiamine deficiency (TD) is a model of neurodegeneration induced by mild impairment of oxidative metabolism. Thiamine is required for PDHC, KGDHC and transketolase. KGDHC is particularly sensitive to TD. Further, KGDHC is more sensitive to TD in aged mice than young mice [108]. TD produces a time-dependent, selective neuronal death in specific thalamic brain regions. Loss of neurons and elevation in markers of neurodegeneration are preceded or accompanied by markers of oxidative stress that mimic the changes in brains from AD brain including: (1) changes in microglia including increased redox active iron, (2) induction of nitric oxide synthase and heme oxygenase-1, a marker of oxidative stress, and (3) altered processing of amyloid precursor protein that exacerbates plaque formation in an AD-like manner. Endothelial cells also show changes in early stages of TD including induction of intracellular adhesion molecule-1 and of endothelial NOS. Thus, the metabolic deficiency including the decline in KGDHC is sufficient to induce oxidative stress and an inflammatory response [109]. Interestingly, TGase message levels are dramatically increased (as much as 11 fold) in areas of brain damage in TD [110]. Whether the induction of TGase is secondary to the oxidative stress induced by TD has not been tested. Nor has it been tested whether impairing TGase would be protective. Mild impairment of oxidative metabolism can induce a variety of physiological changes: oxidative stress, increases in TGase message, an inflammatory response, altered APP processing and neuronal death [109].

E3 deficiency. The E3 component of KGDHC is a critical subunit common to KGDHC and PDHC (as well as to the branched chain dehydrogenase complex, which however has minimal enzymic activity compared to the other two complexes). E3 is responsible for ROS produc-

tion by both complexes. The E3^{+/-} mice do not show any generalized neuron loss. They have increased oxidative stress as measured with malondialdehyde. However, mice that are deficient in E3 are more vulnerable to brain damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), malonate and 3-nitropropionic acid (3-NP), toxins that have been proposed as models of PD and HD, respectively. Administration of MPTP causes a greater depletion of tyrosine hydroxylase-positive neurons in the substantia nigra of E3^{+/-} mice than in wild-type littermate controls. Striatal lesion volumes produced by malonate and 3-NP are increased in E3^{+/-} mice [2]. Isolated brain mitochondria treated with 3-NP show that both succinate-supported respiration and membrane potential are suppressed to a greater extent in E3^{+/-} mice. These findings provide further evidence that mitochondrial defects may contribute to the pathogenesis of neurodegenerative diseases [2].

Since E3 can be a source of free radicals in KGDHC and PDHC, a reduction in E3 may be expected to decrease ROS. Indeed, ROS mitochondria isolated from these mice produce less ROS. However, in living mice a reduction in the E3 component of KGDHC elevates free radical production as shown by an increase in malondialdehyde in their brains [2]. Further, the production of these ROS is exaggerated by toxins that are used as models of PD and HD. These data provide direct evidence that imbalance ROS metabolism occurs in animal models with only mild reductions in glucose metabolism. Thus, both TD and E3 deficiency enhance the production of oxidative stress. Further, the production of these ROS is exaggerated by toxins that are used as models of PD and HD. Thus, under the metabolic conditions that have been studied *in vivo*, reductions in PDHC and KGDHC can initiate oxidative stress.

Reduction of E2k in mice. To determine the consequences of diminished brain KGDHC activity, the *dlst* gene encoding the E2k subunit of KGDHC was disrupted. This protein is unique to KGDHC. E2k^{+/-} heterozygous mice had lower message and protein levels for E2k, leading to reduced brain KGDHC activity by about 50%. Behavioral assessment of adult DLST^{+/-} mice revealed abnormalities in a discriminative fear-conditioning paradigm and in high-speed rotorod performance. Spatial memory in a water maze task was unaltered. Neurons cultured from DLST^{+/-} mice have increased resting calcium levels. Furthermore, oxidative stress as assessed by malondialdehyde is increased in DLST^{+/-} mice. Thus, a deficiency in KGDHC is by itself sufficient to induce oxidative stress in the brain [111].

MILD IMPAIRMENT OF METABOLISM PROMOTES PATHOLOGIES RELATED TO AGE-RELATED NEURODEGENERATIVE DISEASES.

Abnormal mitochondrial function alters APP processing. Altered processing of amyloid precursor protein (APP) is prominently associated with changes in AD, but it is likely altered in other neurodegenerative diseases as well. Increased APP expression and intracellular accumulation of its toxic fragments including the carboxy terminal fragment of APP have been associated with acute neuronal death. APP C-terminal and β -amyloid domains occur in rat substantia nigra pars reticulata neurons targeted for delayed degeneration following a neurotoxic striatal lesion [112]. These findings suggest that intranuclear APP C-terminal fragments (CTF) play a role in genomic events contributing to delayed neuron degeneration [112]. These CTF also appear to be involved in neuron death following TD. During TD, CTF of APP appears in the nuclei of neurons that will die 5-6 days before neuron death [113]. Thus, very mild interruptions of metabolism and of KGDHC can lead to altered APP processing leading to accumulation of CTF of APP in the nuclei of neurons that will die.

Mild impairment of energy metabolism can also lead to the formation of Alzheimer-like plaques. In Tg19959 transgenic mice over expressing a double mutant form of APP, TD exacerbates amyloid plaque pathology, and enlarges the area occupied by plaques by 50% in cortex and by 200% in both hippocampus and thalamus. TD increases A β (1-42) levels by about three fold, beta-CTF (C99) levels by 33% and β -secretase (BACE1) protein levels by 43%. Thus, TD induces plaque formation throughout the brain even though neuron loss is restricted to a small region [114].

Alterations of metabolic/oxidative activity modify APP processing in a variety of different models. Genetic ablation of inducible nitric oxide synthase protects against plaque formation in mice [115]. Insulin, 2-deoxyglucose, 3-nitropropionic acid, and kainic acid induce acute energy inhibition, and simultaneously induce the BACE enzyme which cleaves APP [116]. Cerebral A β 1-40 levels in plaque bearing mice increase to approximately 200% of control at seven days after treatment, demonstrating that energy inhibition can be amyloidogenic [116]. Energy deprivation induces phosphorylation of the translation initiation factor eIF2 α to eIF2 α -P, which increases the translation of BACE1 [117]. These results strongly suggest that eIF2 α phosphorylation increases BACE1 levels and causes A β overproduction, which could be an early, initiating molecular mechanism in sporadic AD [117]. Thus, these studies demonstrate that impairing energy metabolism can promote plaque formation.

Abnormal mitochondrial function can also lead to tangle formation. A reduction in mitochondrial function can also lead to tangle formation. Tangles are hyperphosphorylated tau proteins, and a hallmark of typical pathology in AD brains. They also occur in brains from patients with Wernicke Korsakoff syndrome (i.e., human thiamine deficiency)[118]. Insulin deficiency and diabetes causes a mild hyperphosphorylation of tau at early stages, and at later stages massive hyperphosphorylation of tau occurs. However, neither mild nor massive tau phosphorylation induced tau aggregation in mice or rats, i.e., true tangles [119]. Rotenone, an inhibitor of electron transport, causes loss of neurons in the substantia nigra and in the striatum. Spherical deposits of alpha-synuclein, which accumulates in Parkinson disease, are observed in a few cells, but cells with abnormal cytoplasmic accumulations of tau immunoreactivity are significantly more numerous in the striatum after rotenone [120] or annonacin, a natural complex I inhibitor [121], treatment. Many tau+ cell bodies also stain positively for thioflavin S (which stains Alzheimer plaques and tangles), for nitrotyrosine and for ubiquitin [122]. The data suggest that chronic respiratory chain dysfunction might trigger a form of neurodegeneration in which accumulation of hyperphosphorylated tau protein predominates over deposits of α -synuclein [120]. SOD2 deficiency which increases ROS also increases tau phosphorylation [123].

Abnormal energy metabolism and oxidative stress also alter cellular calcium. Glucose metabolism and cellular calcium are closely linked. Mitochondrial calcium uptake is diminished in mitochondria from AD fibroblasts[124]. The changes in calcium regulation in mitochondria from E2k deficient mice are consistent with altered mitochondrial buffering. Endoplasmic reticulum (ER) calcium is a major calcium store within the cell, ER calcium is increased in cells bearing AD causing presenilin-1 mutations [125]. ER calcium is also increased in non-genetic forms of AD. ER calcium is also sensitive to oxidants. A screen of a variety of times and dosages of revealed that specific oxidants can alter calcium in an AD-like manner. These oxidant-induced changes can be blocked by specific antioxidants. The antioxidants that blocked the effects on ER calcium also ameliorated the deficits in ER calcium in cells from AD patients [126].

KGDHC changes with aging. The most important factor for developing age-related neurodegenerative diseases is aging. One possibility for a role of KGDHC in age related neurodegeneration is that a factor with aging making the brain vulnerable with age. In mice, the baseline activity of brain KGDHC does not change with age, but with increased age it does become more sensitive to other metabolic insults such as thiamine deficiency [108]. KGDHC activity also diminishes with age in heart, and the number of hydroxynoneal (HNE) adducts is increased [127]. Hearts from old rats exhibit significantly higher HNE-modified mitochondrial proteins when compared with those from young rats. The protein most markedly modified by HNE with age is the E2k component of KGDHC [127]. Replicative life span in *Saccharomyces cerevisiae* is increased by glucose limitation and by augmented NAD⁺. The increased life span due to glucose limitation is related to diminished mitochondrial ROS production. The data indicate that E3 is an important source of glucose-mediated preventable mitochondrial ROS production. The results substantiate the concept that E3 is an important and novel source of ROS limiting life span [128].

Neurogenesis is impaired by reductions in KGDHC. Although the precise role of neurogenesis in the adult brain is unknown, one interpretation is that it permits the brain to respond to insults. Thus, reduction in neurogenesis would make the brain less able to respond [129]. Mitochondrial dysfunction can influence the number of neural progenitor cells in the hippocampus of adult mice [111]. Neurogenesis is impaired in three models of impaired TCA cycle enzymes including E2k^{+/-}, E3^{+/-} [111] and thiamine deficiency [129]. Adult brain neurogenesis was assessed using immunohistochemistry for the immature neuron markers, doublecortin (Dcx) and polysialic acid-neural cell adhesion molecule, as well as a marker for proliferation, proliferating cell nuclear antigen (PCNA). Both E3 and E2k-deficient mice showed reduced Dcx-positive neuroblasts in the subgranular zone (SGZ) of the hippocampal dentate gyrus compared with wild-type mice. PCNA staining reveals decreased proliferation in the SGZ of E2k-deficient mice. An analogous pattern of change was observed in thiamine-deficient mice [129].

Mitochondrial dynamics.

Extensive evidence implicates mitochondrial dynamics in neurodegenerative diseases. Whether these changes lead or follow the changes in mitochondrial function described in this review is unknown. These dynamic processes include transport of mitochondria down axons and dendrites as well as mitochondrial fission and fusion. Axonal transport is energy dependent and the movement of mitochondria can be selectively impaired by glutamate, zinc, rotenone or uncouplers [130]. Thus, any interruption of energy metabolism (i.e., a loss of KGDHC activity) may diminish transport. The resulting diminished mitochondrial function in the nerve ending would likely diminish synaptic function. Similarly, a balance of fusion and fission is necessary for normal mitochondrial and neuronal function. Changes in fission/fusion alter mitochondrial function and vice versa. Disruption of the mitochondrial fission and fusion equilibrium lead to neurodegeneration [131]. Genetic mutations in fusion proteins cause neuropathy. When genetic defects lead to lack of fusion, the enzymes encoded by the mitochondrial (mt)DNA are most affected, whereas the expression of nuclear DNA-encoded enzymes increase, apparently to compensate for the mitochondrial dysfunction. The results also show that functional mitochondria due to loss of fusion are lost in the neuronal terminals more than in the cell body [131-132]. Dominant optic atrophy is caused by mutations in the OPA1 gene, which encodes a dynamin-re-

lated GTPase required for mitochondrial fusion [133]. In *Drosophila*, increased mitochondrial fission occurs in PD models, such as mutations in PINK1, and proteins that are mutant in genetic forms of PD promote fission, and their inhibition leads to excessive fusion [134]. In mammalian cells, PINK1 mutants or knockdown promotes excessive fission [135-137]. Parkin and PINK2 function in a pathway in which defective mitochondria are targeted for autophagy [138]. Mutant huntingtin alters mitochondrial morphology and dynamics. Both rotenone and β -amyloid initiate mitochondrial fission. Mitochondrial fission is an early event in ischemic stroke and diabetic neuropathies [139]. Recent studies show that APP, through A β production, causes an imbalance of mitochondrial fission/fusion that results in mitochondrial fragmentation and abnormal distribution, which contributes to mitochondrial and neuronal dysfunction [140]. In AD brains, mitochondria are distributed away from axons in pyramidal neurons, and this is replicated in cultured neurons using oligomeric A β [141-142]. As described in other sections of this review, mild impairment of oxidative metabolism can lead to overproduction of A β . Thus, mild impairment of oxidative metabolism can lead to increased A β which can imbalance fusion/fission. Thus, precedent exists that suggests alteration in fusion/fission could lead to alterations in mitochondrial proteins and that alterations in energy metabolism can cause changes in fusion/fission. Together they would form a cascade of events to promote neurodegeneration

THERAPEUTIC APPROACHES.

The goal of these studies is to develop new therapeutic strategies. The data reviewed above suggest several innovative approaches.

1. **Bypass the metabolic block.** Since the degree of cognitive deterioration in AD correlates to the reduction in cerebral metabolic rate, this is a reasonable therapeutic target. An attempt to treat this abnormality in AD patients is based on feeding the patients glucose and malate to bypass the diminished activities of PDHC and KGDHC. In addition, the antioxidant resveratrol is included. Encouraging results of a preliminary double-blind clinical trial do not allow confident conclusions but do support the need for more intensive examination of this approach to AD treatment [143].

2. **Reactivate KGDHC.** KGDHC is diminished in brains of patients that died with neurodegenerative diseases. The lack of change in protein levels suggest that this is related to a post-translational modification. Thus, reversal of the modification should be beneficial. Glutathionylation of KGDHC due to H₂O₂ reverses when H₂O₂ is diminished [106]. Nitration of isolated KGDHC can also be reversed by glutathione [105]. The inactivation of KGDHC in cells by 4-hydroxy-2-nonenal can be protected against by DL-lipoamide, DL-lipoic acid, reduced glutathione and cysteine [144]. Prolonged treatment with trolox (a vitamin E like compound) increases KGDHC by three fold [126]. *In vivo* treatment with particular lipoic acid derivatives can increase heart KGDHC two to three fold [145]. Although reversing the inactivation of one enzyme may seem like a too focused approach, one should remember that even relatively small increases in the activity of enzymes involved in a number of inborn errors of metabolism have been associated with clinically significant benefits. Furthermore, it is likely that compounds that reactivate KGDHC may also reactivate other processes that have been inactivated by similar mechanisms. For example, the same conditions that inactivate KGDHC alter endoplasmic reticulum calcium in a manner that is reminiscent of AD [126].

3. Inhibit TGase activity. Numerous papers try to block the actions of TGase in order to treat multiple disorders including skin and gastrointestinal disorders. The inhibitors of TGase are generally directed to blocking the cross linking. The inhibitors would act to block the crosslinking of enzymes such KGDHC and aconitase, and also to alleviate the inhibition of transcription. The compound used the most with the CNS is cystamine. Treatment in R6/2 transgenic HD mice, using the transglutaminase inhibitor cystamine, significantly extends survival, improves body weight and motor performance, and delays the neuropathological sequelae [54-55]. Free N[σ]-(γ -L-glutamyl)-L-lysine (GGEL), a specific biochemical marker of TGase activity, is markedly elevated in the neocortex and caudate nucleus in HD patients. TGase activity and GGEL levels are significantly altered in HD mice. Both TGase and GGEL immunoreactivities colocalized to huntingtin aggregates. Cystamine treatment normalized transglutaminase and GGEL levels in R6/2 mice. These findings are consistent with the hypothesis that transglutaminase activity may play a role in the pathogenesis of HD, and they identify cystamine as a potential therapeutic strategy for treating HD patients [54-55]. However, the specificity of cystamine is questionable, so the studies need to be replicated with more specific TGase inhibitors that enter the brain.

Rather than being a direct inhibitor of TGase in brain, cystamine may provide an alternative substrate for TGase. Cystamine [β -mercaptoethanolamine (MEA) disulfide] is reduced within cells to MEA which is largely responsible for the in vivo effects of its disulfide precursor. The amine group of MEA acts as a TGase substrate resulting in the formation of N[β]-(γ -L-glutamyl)-MEA bonds. The formation of such bonds would compete with the generation of other TG-catalyzed reactions that may contribute to neurodegeneration such as polyamination, protein cross-linking, deamination and the covalent attachment of ceramide to proteins. Structure-function studies also indicated that the mercapto group of MEA significantly influences the substrate behavior of this compound [146]. Whether the more specific inhibitor of TGase, ZDON, that overcomes transcriptional inhibition associated with mutant huntingtin can be beneficial in mice or humans is unknown [51]

4. Prevent upregulation of TGase. The ability of glutathione to prevent glutamate-evoked TGase upregulation was tested in astrocyte cultures. A 24 h exposure to glutamate caused a dose-dependent depletion of glutathione intracellular content and increased the ROS production. Preincubation with glutathione ethyl ester or cysteamine recovered oxidative status and was effective in significantly reducing glutamate-increased TGase. These data suggest that TGase upregulation may be part of a biochemical response to oxidative stress induced by a prolonged exposure of astrocyte cultures to glutamate [43]. Thus, the available data suggest that both the KGDHC deficit and the induction of transglutaminase increase in neurodegenerative diseases can be “treated” with glutathione. One way to increase glutathione is by administration of tri-terpenoids, or other compounds, which activate Nrf2/ARE pathway which activates the enzymes involved in glutathione synthesis [147]

5. Act on redox sensitive transcription factors. Chronic exposure to ROS in neurodegenerative diseases likely activates cascades of genes. Even if initially protective, prolonged activation may be damaging. Thus, therapeutic approaches based on modulation of these gene cascades may lead to effective therapies. Genes involved in several pathways including antioxidant defense, detoxification, inflammation, etc. are induced in response to oxidative stress in neurode-

generation. However, genes that are associated with energy metabolism, which is necessary for normal brain function, are mostly down-regulated. Redox sensitive transcription factors such as activator protein-1 (AP-1), nuclear factor κ B (NF- κ B), specificity protein-1 and hypoxia-inducible factor (HIF) are important in redox-dependent gene regulation. PPAR γ co-activator (PGC-1 α) is a co-activator of several transcription factors and is a potent stimulator of mitochondrial biogenesis and respiration. Down-regulated expression of PGC-1 α has been implicated in HD and in several HD animal models [59-61]. Its role in regulation of ROS metabolism makes it a potential candidate player between ROS, mitochondria and neurodegenerative diseases[148]. Potential therapeutic strategies based on the updated understandings of redox state-dependent gene regulation may be productive [149].

Therapies normally associated with antioxidant or energy metabolism may work by more general gene mechanisms. For example, results show a protective effect of two mitochondrial antioxidant/nutrients, R-03B12013lipoic acid and acetyl-L-carnitine in a chronic rotenone-induced cellular model of PD. When combined, lipoic acid and acetylcarnitine work at 100 to 1000 fold lower concentrations than they did individually. The combined pretreatment increases mitochondrial biogenesis and decreases production of ROS reactive oxygen species through the up-regulation of the peroxisome proliferator-activated receptor- γ (PGC-1 α) — a possible underlying mechanism [150].

Experiments with HIF1-1 provide a specific example of this approach. The transcription factor hypoxia-inducible factor-1 (HIF1-1) mediates the activation of a large cassette of genes involved in adaptation to hypoxia in surviving neurons after stroke. Pharmacological or molecular approaches that inhibit HIF prolyl 4 hydroxylases lead to profound sparing of brain tissue and enhanced recovery of function. The strategy appears to involve HIF-dependent and HIF-independent pathways and more than 70 genes and proteins activated transcriptionally and post-transcriptionally [151]. One of the genes activated by HIF is TGase [152], and TGase regulates HIF [56].

CONCLUSION

A surprisingly large number of features of age-related neurodegenerative diseases can be accounted for by changes in KGDHC, TGase, oxidative stress and calcium (Figure 4). Therapeutic approaches that ameliorate these deficits are likely to be therapeutically useful.

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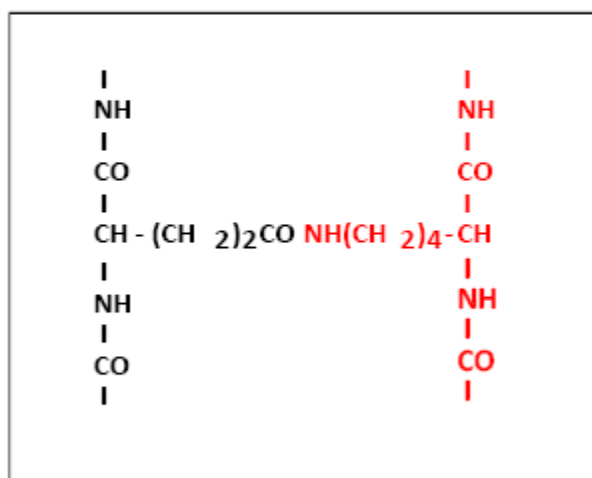
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Figure 1. Transglutaminase (TGase) is elevated in Alzheimer's Disease and expanded CAG-disorders



N ϵ -(γ -glutamyl)lysine cross link

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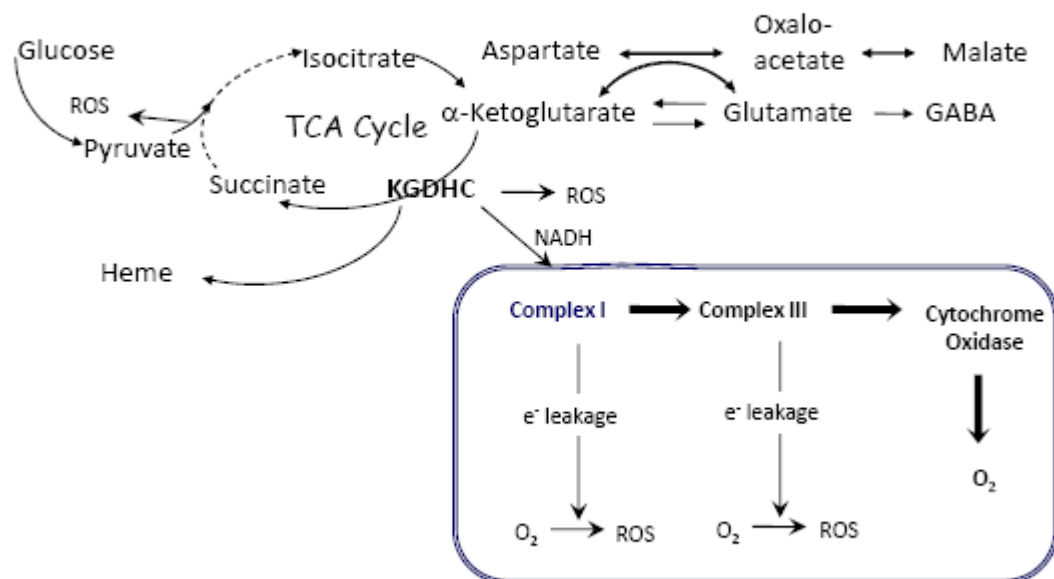
Figure 2. Role of KGDHC in Brain Metabolism

Figure 3. Subunit composition of KGDHC. KGDHC produces oxidants or NADH depending on redox state. Low NAD⁺ promotes ROS formation

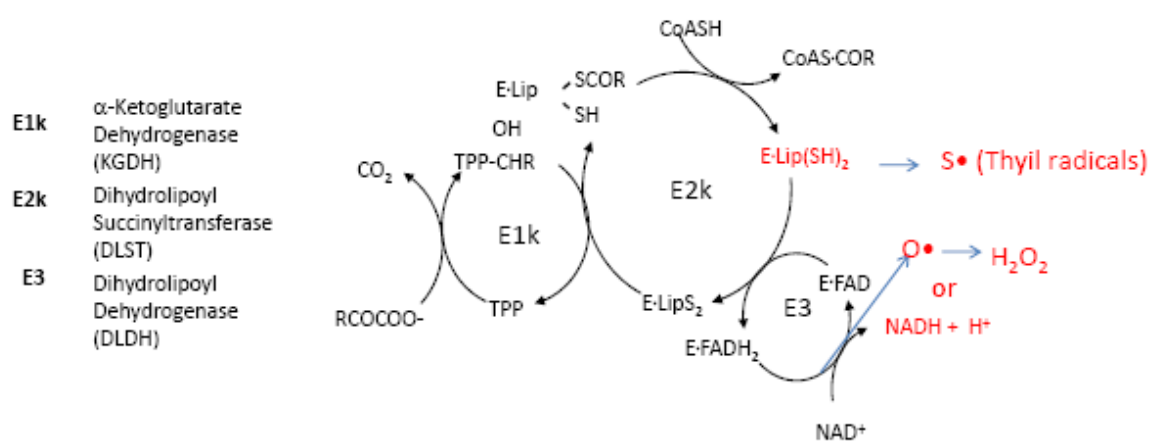
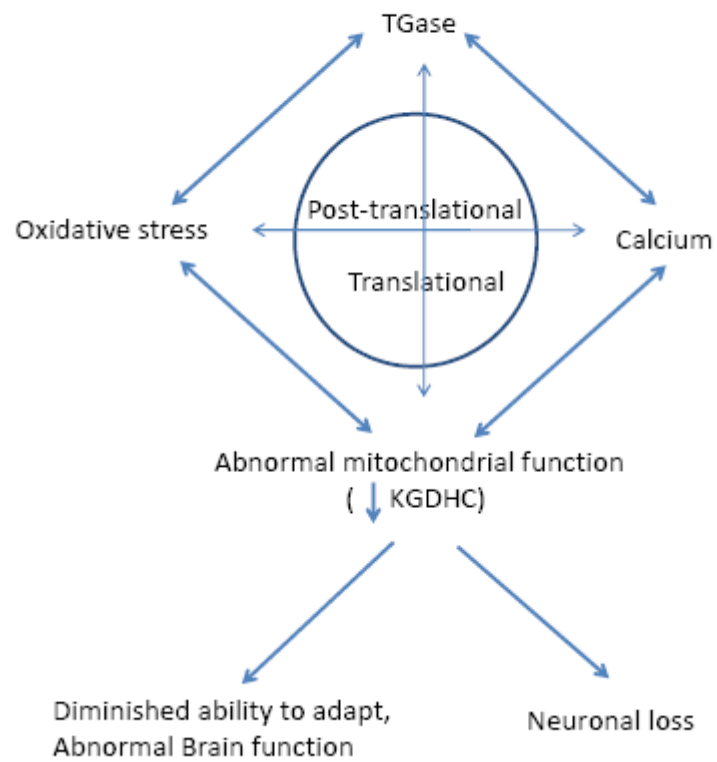


Figure 4. Interaction of TGase, oxidative stress, calcium lead to abnormal mitochondrial function and diminished ability to adapt.



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