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The α-Ketoglutarate–Dehydrogenase Complex

A Mediator Between Mitochondria and Oxidative Stress in Neurodegeneration

Gary E. Gibson,*[*,1,2] John P. Blass,[1,2] M. Flint Beal,[1] and Victoria Bunik[3]

[1]Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, NY; [2]Burke Medical Research Institute, White Plains, NY; and [3]A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

Abstract

Damage from oxidative stress and mitochondrial dysfunction occur together in many common neurodegenerative diseases. The enzymes that form the mitochondrial α-ketoglutarate–dehydrogenase complex (KGDHC), a key and arguably rate-limiting enzyme system of the tricarboxylic acid cycle, might mediate the interaction of these processes. KGDHC activity is reduced in numerous age-related neurodegenerative diseases and is diminished by oxidative stress. In Alzheimer's disease (AD), the reduction correlates highly to diminished mental performance. Thus, research has focused on the mechanisms by which select oxidants reduce KGDHC and the consequences of such a reduction. Diminished KGDHC in cells is associated with apoptosis without changes in the mitochondrial membrane potential. Studies of isolated mitochondria and of animal models suggest that a reduction in KGDHC can predispose to damage by other toxins that promote neurodegeneration. Diminished oxidative metabolism can be plausibly linked to pathological features of neurodegenerative diseases (e.g., reduced mental function, the plaques and tangles in AD). Thus, reductions in KGDHC might be central to the pathophysiology of these diseases. Studies of proteins, cells, animal models, and humans suggest that treatments to diminish, or bypass, the reduction in KGDHC might be beneficial in age-related neurodegenerative disorders.

Index Entries: Oxidative stress; Alzheimer's disease; α-ketoglutarate dehydrogenase; neurodegeneration.

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* Author to whom all correspondence and reprint requests should be addressed. E-mail: ggibson@med.cornell.edu

Introduction

Damage from oxidative stress and abnormal mitochondria are common features of many age-related neurodegenerative diseases. Data indicate that these are not inconsequential epiphenomena. In some cases, the abnormalities in oxidation and mitochondria could be primary, whereas in others, these could be essential parts of a cascade of events that cause the disease, including the pathology. The mitochondrial enzymes that form the α-ketoglutarate–dehydrogenase complex (KGDHC) provide a link between oxidative stress and abnormal mitochondria, because KGDHC is sensitive to oxidative stress and is a key and arguably rate-limiting system of the tricarboxylic acid (TCA) cycle. Although oxidative stress and abnormalities in mitochondria might be essential components of multiple diseases, the focus of this review is Alzheimer's disease (AD), because space limitations preclude discussing these mechanisms in all the diseases in which mitochondria and oxidative stress have been implicated.

Alzheimer's Disease

If changes in KGDHC, oxidative stress, and mitochondria are important in the pathophysiology of AD, they should be plausibly linked to the clinical and pathological symptoms. AD is characterized clinically by memory loss, a decline in cognitive abilities (thinking, understanding, and decision-making), and severe behavioral symptoms (agitation, aggression, depression, or wandering). Pathologically, brains from AD patients are characterized by plaques and tangles. The major protein in plaques is amyloid-β peptide (Aβ), a 40 to 42-amino-acid fragment that is derived from the amyloid precursor protein (APP). However, plaques contain many other proteins, including proteins that reflect exaggerated oxidative stress (e.g., heme oxygenase and ICAM-1) *(1).* Brains from AD patients also contain neurofibrillary tangles (NFTs), which are composed of abnormally hyperphosphorylated tau proteins. The tangles could also reflect oxidative stress *(2).* As described in detail in this article, KGDHC deficiency, oxidative stress, and mitochondria can be plausibly linked to the behavioral and pathological features of the disease.

Reduced Glucose Metabolism Occurs in AD and Could Contribute to Brain Dysfunction

Mitochondrial constituents play a key role in glucose utilization. Normal brain function depends on glucose metabolism. Although the brain represents only 2% of body mass, it consumes 20% of the glucose under physiological conditions. Glucose is nearly an exclusive substrate for brain oxidative metabolism. The oxidation of glucose by the pentose shunt and the TCA cycle includes three enzymes that require thiamine (i.e., vitamin B_1): transketolase, KGDHC, and the pyruvate–dehydrogenase complex (PDHC). Even 10 to 15% reductions in the availability of glucose or oxygen diminish brain function, including a decline in memory and judgment in humans *(3).* The occurrence of mental abnormalities at reduced concentrations of oxygen or glucose that do not affect ATP in animal models suggests that flux through the TCA cycle leads directly to altered function. Neurotransmitters that are linked to the the TCA cycle (e.g., acetylcholine, glutamate, and GABA) might mediate these interactions. In animals and humans, thiamine deficiency diminishes the activity of thiamine dependent enzymes in brain and causes severe memory deficits *(4).* Thus, decreased metabolism linked to reductions in the TCA cycle activity could lead to the clinical symptoms of AD.

The observation that diminished metabolism accompanies AD was first documented several decades ago. Some of the earliest studies on brain blood flow and metabolism in the 1940s found reductions in brains of patients with AD. Recent studies of brain metabolism in AD included longitudinal studies in individuals

that are at high risk to develop AD because of genetic variation. Previous studies demonstrated that cognitively normal, late-middle-aged carriers of the apolipoprotein E ε4 allele, a common susceptibility gene for late-onset Alzheimer's dementia, have abnormally low rates of glucose metabolism *(5,6).* Recent studies tested this possibility in younger patients. Clinical ratings, neuropsychological tests, magnetic resonance imaging, and glucose utilization studies were performed in subjects with the ε3/ε4 genotype and noncarriers of the ε4 allele. The young ε4 carriers have abnormally low rates of glucose metabolism. Thus, carriers of a common Alzheimer's susceptibility gene have functional brain abnormalities in young adulthood several decades before the onset of dementia *(7).* Patients with reduced metabolism and mild cognitive impairments often develop AD, and decreases in glucose metabolism help to predict cognitive decline in normal elderly subjects *(8–10).* The degree of impairment of cerebral oxidation of glucose correlates so closely with the degree of clinical impairment that it is used to follow the progression of the disease *(11).* Recent developments that allow plaques to be imaged in living patients show that brain regions with low metabolism tend to accumulate plaques in proportion to metabolic impairment *(12).* The observation that glucose administration might reverse some of the behavioral changes indicates that the deficits in metabolism are not just the result of neurodegeneration *(13).* Together, these results imply that abnormalities in glucose metabolism are pathophysiologically important, provide a measure that can be used to monitor the beneficial effects of treatments, and might offer an effective target for therapeutic approaches.

Studies in transgenic mice support the hypothesis that cerebrovascular and metabolic abnormalities are early events in the pathogenesis of AD. Both cerebral blood flow and glucose utilization are diminished in transgenic mice overexpressing Swedish mutant amyloid precursor protein and APP-derived Aβ peptides. The reductions occur at an age that *precedes* plaque formation *(14).* Cerebral blood flow (CBF) is closely linked to the cerebral metabolic rate. The ability of the cerebral circulation to maintain flow in the face of changes in mean arterial pressure is impaired in transgenic mice that overexpress APP. The failure of autoregulation parallels impairment of the CBF response to endothelium-dependent vasodilators *(14).*

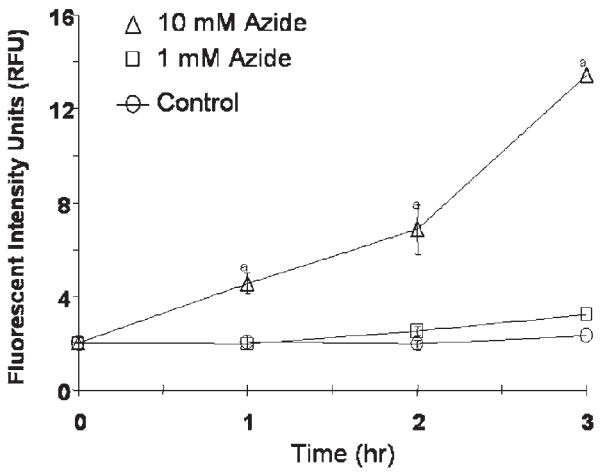

Fig. 1. Azide induces ROS production in microglia. (From ref. *15.*)

Oxidative Stress in AD

Oxidative stress (i.e., conditions in which production of free radicals or reactive oxygen species [ROS] exceeds the ability of the cell or brain to reduce them) accompanies impaired metabolism and neurodegenerative diseases. Disturbances in metabolism often increase free-radical production. For example, the blockade of respiration by azide increases production of ROS *(15).* The increases in ROS owing to blocking respiration can be large enough to inactivate enzymes that are sensitive to oxidative stress *(15)* (Fig. 1). Although the presence of ROS or free radicals is difficult to measure in the brain, markers of their damage to proteins, nucleic acids, carbohydrates, and

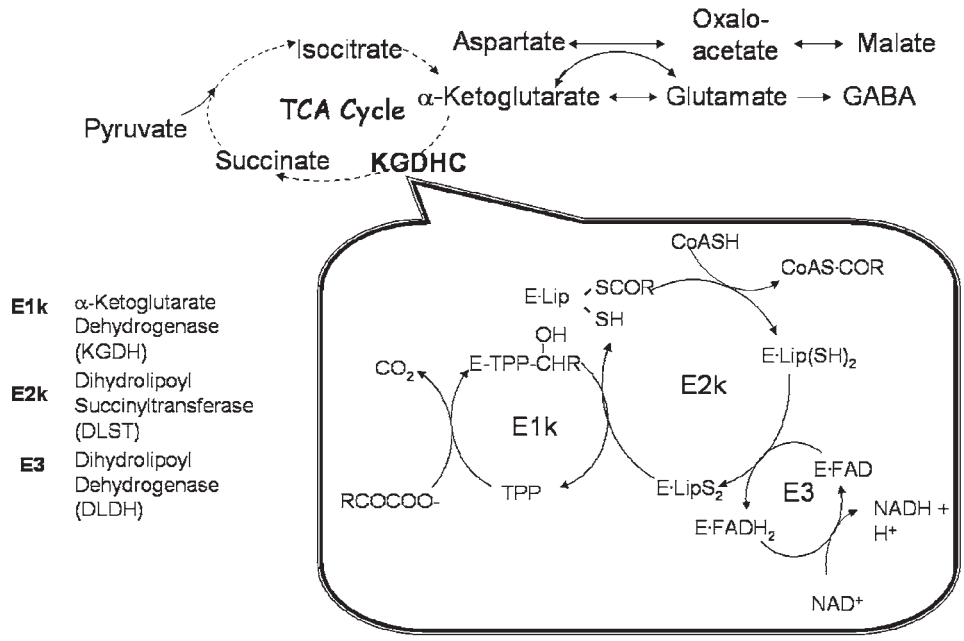

Fig. 2. Role of KGDHC in brain metabolism.

lipids are well documented to be elevated in AD. Evidence of oxidative damage in brain is more pervasive than plaques and tangles *(16).* Mitochondrial membranes contain significant amounts of arachidonic and linoleic acids, precursors of lipid peroxidation products, 4-hydroxynonenal (HNE), and 2-propen-1-al (acrolein), which are extremely reactive. Both alkenals are increased in AD brain. Acrolein damage colocalizes with tangles and also occurs in additional brain regions *(16).* Accumulating evidence indicates that oxidative stress occurs in early stages of the disease. In mice, isoprostanes accumulate in the urine of mice before plaques occur in the brain *(17).* Higher levels of isoprostanes occur in cerebrospinal fluid, plasma, and urine of patients with mild cognitive impairment than in controls *(18).* The evidence is overwhelming that the oxidative stress is an important component of AD.

Although evidence of oxidative stress occurs in multiple disorders, different diseases could have unique forms of oxidative stress. For example, elevated levels of isoprostanes differentiate AD from frontotemporal dementia *(19).* Furthemore, patients with Parkinson's disease have high levels of isofurans, but not F2-isoprostanes *(20).* Modification of a single step (e.g., KGDHC) by multiple oxidants could provide a cellular convergence point in different diseases.

Roles of KGDHC in Cell Function

The α-ketoglutarate–dehydrogenase complex is a key mitochondrial–enzyme complex. A branch-point metabolite α-ketoglutarate is generated in the TCA cycle during the oxidation of carbohydrates and fatty acids and by glutamate dehydrogenase during the oxidative deamination of glutamate (*see* Fig. 2). In addition, α-ketoglutarate is produced by transamination of glutamate as part of the malate–aspartate shuttle that transfers reducing equivalents from cytoplasm to mitochondria. Glutamate is an excitatory neurotransmitter that, in excess, produces neuropathology. Thus, the KGDHC-catalyzed reaction is significant for (1) energy

production, (2) neurotransmitter metabolism, and (3) metabolic interaction between mitochondria and cytoplasm. Disturbance of these functions might link KGDHC inactivation and neurodegeneration through (1) decreased energy production, (2) accumulation of glutamate, and (3) change in the normal flow of reducing equivalents and metabolites among cellular compartments. The significance of the oxidative decarboxylation of α-ketoglutarate by KGDHC is supported by whole-cell computational modeling. It shows that the α-ketoglutarate-involving reactions belong to the backbone of high-flux reactions, which is rather conserved in evolution *(21).* Metabolic adaptations of cells involve significant shifting of the fluxes through such reactions. This means that shifting the flux as a result of disturbed function of KGDHC would have an essential impact on the overall metabolism. The crucial role of KGDHC in the control of mitochondrial oxidative metabolism has been emphasized by the discovery of KGDHC dependence on Ca^{2+} *(22),* which occurs in response to Ca^{2+}-mobilizing hormones in a number of tissues, including the brain. The Ca^{2+} regulation of KGDHC underlies the activation of the TCA cycle, which comprises the metabolic response of mitochondria to Ca^{2+} signaling *(23,24).* This crucial position of KGDHC in metabolism and the association between the decreased KGDHC activity in the brain and neurodegeneration has stimulated extensive research *(25–28).*

The α-ketoglutarate–dehydrogenase complex is a complex including multiple copies of three proteins: E1k (α-ketoglutarate dehydrogenase), E2k (dihydrolipoyl succinyltransferase), and E3 (dihydrolipoamide dehydrogenase) (Fig. 2). E1k and E2k are specific to KGDHC, whereas E3 is common to all of the α-keto acid–dehydrogenase complexes; that is, the same enzyme is involved in oxidative decarboxylation of pyruvate and branched-chain α-keto acids as well. The consecutive action of the three catalytic components of KGDHC results in oxidative decarboxylation of 2-oxoglutarate, preserving the energy in the form of succinyl-CoA and NADH:

$$\alpha\text{-ketoglutarate} + NAD^+ + CoA \rightarrow \text{succinyl CoA} + CO_2 + NADH + H^+$$

The α-ketoglutarate–dehydrogenase complex is strongly regulated by energy-linked metabolites through a number of mechanisms. The E3-directed inhibition of KGDHC by an increase in the $NADH/NAD^+$ ratio has long been recognized as a mechanism of feedback control *(29).* However, NADH also acts as an allosteric inhibitor of E1k, with the inhibition relieved by micromolar Ca^{2+} and ADP. In the absence of NADH inhibition, ADP or Ca^{2+} activates E1k through allosteric binding *(30).* KGDHC function is also controlled by the ratio of complex-bound dihydrolipoate/lipoate, which allows KGDHC to sense the redox state of the surrounding medium *(31)* (*see* Fig. 3). After the concentrations of α-ketoglutarate, CoA and NAD(H) are transformed by the KGDHC catalytic action into the steady-state ratio of the complex-bound dihydrolipoate/lipoate, several regulatory mechanisms could be affected. An increase in the dihydrolipoate/lipoate ratio has three definable actions (*see* Fig. 3): (1) regulation of E1k by changing its cooperative properties *(32,33)* and irreversible inactivation *(34);* (2) production of ROS in the presence of oxygen *(35);* (3) reduction of disulfides *(36).* These reactions depend on catalytic competence of all of the KGDHC components and directly link KGDHC function to cellular redox state. In particular, thioredoxin, an ubiquitous thiol-disulfide oxidoreductase that participates in regulation of cellular redox status, efficiently protects KGDHC from the E1k inactivation when dihydrolipoate accumulates (Fig. 3, arrow 1). However, such alteration of the self-regulation of the complexes by thioredoxin is accompanied by stimulation of the ROS production (Fig. 3, arrow 2). A thioredoxin-dependent peroxidase, SP-22 interacts with the E3 component of the 2-oxo acid–dehydrogenase complexes *(37)* and is supposed to scavenge this increase in KGDHC-catalyzed ROS production *(31).*

The interaction of KGDHC with ROS-dependent pathways provides for novel function(s) of the complex. For instance, in some bacteria,

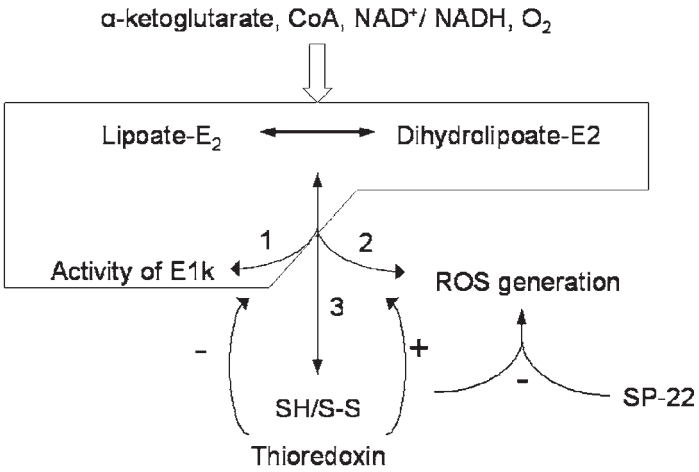

Fig. 3. Interaction of KGDHC to the cell redox state. (From ref. *31.*)

the KGDHC-bound dihydrolipoate intermediate, a thioredoxin-like protein, and a thioredoxin-dependent peroxidase comprise a specific antioxidant defense system *(38).* On the other hand, KGDHC can be an important source of cellular H_2O_2. This has been shown with isolated KGDHC *(32,35)* and further supported in the experiments on mitochondria *(39).* Whereas the succinate-driven ROS production at Complex I is completely abolished by the uncoupler-induced mitochondrial membrane depolarization, a significant fraction (30%) of H_2O_2 formation in the presence of NADH-linked substrates persists under maximal uncoupling and maximal NAD(P)H oxidation. This suggests ROS production independent of Complex I by specific dehydrogenases, including KGDHC *(32).*

The E3 component of KGDHC is a multifunctional enzyme that might be involved in drug metabolism and ROS production owing to a number of its catalytic activities. Apart from the native oxidoreductase reaction between (dihydro)lipoamide and NAD(H), E3 catalyzes nicotinamide transhydrogenase, inorganic electron transferase, quinone diaphorase, and nitroreductase reactions (*40* and references therein). The role of the metal ions on partial E3-catalyzed reactions including ROS generation was studied with the isolated E3 component. Zn^{2+} inhibits E3-catalyzed NADH-dependent lipoamide reduction and stimulates up to fivefold NADH-dependent oxygen reduction yielding ROS (superoxide anion radical and hydrogen peroxide) *(41).* The differential effects of metal ions on different E3 activities were also observed with Hg^{2+} and Cu^{2+} *(42).* Both ions nearly completely inhibited lipoamide reduction, concomitantly stimulating NADH oxidase activity of E3, although to a different extent (about 20-fold stimulation by Hg^{2+} and 2-fold by Cu^{2+}). The data point to a reversible switch in enzyme catalytic activities that is modulated by metal ion binding. Competition between different metal ions for the E3-binding site might provide a way to regulate the ROS-generating activity of E3.

Not only E3 but also the E2k component of KGDHC can have functions outside the complex. For example, a truncated product of the DLST gene coding for E2k is involved in biogenesis of a protein of the respiratory chain *(43).* A polypeptide derived from E2k has been found in the plasma membrane *(42).* The existence of yet unknown functions of E2k might be related to its high abundance in synaptic membranes *(44).*

Thus, KGDHC has a crucial role in the maintenance of the oxidative state of the brain. The complex as a whole and its individual components possess diverse functions and a number of oxidant-sensitive sites, including the catalytically essential sulfhydryl groups. These factors make KGDHC both an essential and particularly vulnerable site in metabolism, supporting the suggestion that it might serve as a convergence point in neurodegenerative processes.

Activities of Mitochondrial Enzymes in Autopsy Brain From Patients With Neurodegenerative Diseases

Alzheimer's Disease

Changes in KGDHC structure and function might be critical to the metabolic changes in AD. Measurements of KGDHC activities in autopsy brains suggest that reductions in KGDHC might underlie the diminished metabolism that occurs in genetic *(45)* and sporadic *(46)* cases of AD. Four independent groups report that KGDHC is diminished in brains from patients with AD, and no contravening reports exist (*see* ref. *26*). Diminished activities occur in brain regions with severe pathology, as well as in areas that show minimal pathology. This might indicate that the diminished KGDHC activity precedes the development of pathology. The immunoreactivities of the three components are selectively altered in various brain regions in sporadic forms of AD *(47).* Changes in the immunoreactivity of the three KGDHC components do not correspond to the reduction in activity *(47).* This suggests that posttranslational modifications that decrease KGDHC activities might have occurred. Brains from patients bearing the APP 670/671 mutation have reduced protein levels of E1k and E2k, but not E3, as determined by immunoreactivity *(45).* Posttranslational modifications that affect immunoreactivity or changes in gene expression could account for the differences. These findings suggest that the reductions in KGDHC activity in AD can occur because of multiple mechanisms. The relation of the decline in KGDHC to the pathophysiology of AD varies with apolipoprotein genotype. In patients with one apolipoprotein E4 allele, the correlation of KGDHC activity to the clinical dementia rating is very high (r = 0.7) (Fig. 4). In the same subpopulation, the correlation with plaques (r = 0.11) and tangles is very low (r = 0.32) *(46).* Thus, KGDHC activity declines with the severity of AD in brain and the changes appear to be pathophysiologically important.

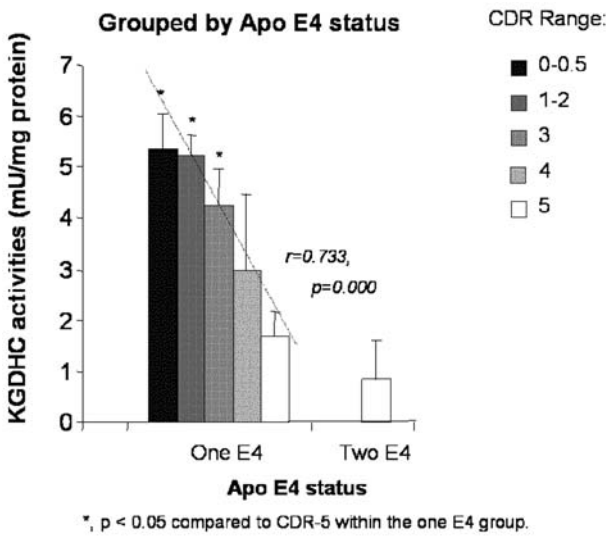

Fig. 4. KGDHC activities, apolipoprotein E4 alleles, and clinical dementia rating (CDR). (From ref. *47.*)

In some cases of AD, the decline in KGDHC activity could be related to genetic abnormalities in E1k, E2k, or E3. This possibility has received considerable attention, but a clear conclusion remains elusive. Association of the candidate gene for E2k with late-onset AD (LOAD) risk has been suggested on the basis of case-control studies. Positive reports have correlated different E2k alleles with LOAD, whereas other groups have failed to find any

significant association *(48–50).* Analysis of complex genotypes or haplotypes based on five single-nucleotide polymorphism (SNP) loci failed to identify a LOAD risk allele, suggesting that further studies of E2k in relation to AD are not warranted *(51,52).* Recent reports suggest that a polymorphism in E3 might be associated with AD. Genotyping studies of E3 in predominantly Ashkenazi Jewish Caucasians revealed association between AD a complex diplotype composed of four SNPs, which is independent of ApoE status. The association with the E3 genotypes was restricted to the male population in both a Caucasian series ($p = 0.0009$, $n = 83$) and an Ashkenazi Jewish subseries ($p = 0.017$, $n = 49$) *(53).* Replication was tested in a total of 255 cases and 198 controls. Evidence of a significant association was found for ApoE ($p < 0.001$) and E3 ($p = 0.022$) *(54).* Further analysis in more populations is required before a claim of a new AD causing gene mutation can be made.

Brain KGDHC Activity in Other Age-Related Neurodegenerative Diseases

The decline in KGHC activity in the brains of patients with different neurodegenerative diseases suggests that this is a mechanism that these neurodegenerative diseases have in common. Reduced activity has been found in brains from patients with Parkinson's disease *(55),* Huntington's disease *(56),* Wernicke–Korskoff disease *(4),* and progressive supranuclear palsy *(57,58).* All of these measures were made in a blinded fashion, and the differences cannot be accounted for by postmortem intervals, acidosis, or other known variables, except the diseases. Oxidative stress has been implicated in all of these diseases. In most of these diseases, protein levels of KGDHC as determined by immunoreactivity do not change, which suggests that posttranslational modification of the protein underlies the loss in activity. Remarkably, KGDHC activity is not diminished in brains of patients that died with schizophrenia, a dementing disorder without neurodegeneration *(59).*

Age-Related Changes in KGDHC in Other Organs

The degree of inactivation of respiration with cardiac reperfusion increases with age and is paralleled by modification of KGDHC by HNE. E2k shows a high HNE-induced modification with age in the heart *(60,61).*

Inactivation of KGDHC by Oxidative Species or Toxins May Link the Changes in Oxidative Stress, Depressed Metabolism, and Neurodegeneration

Inactivation of KGDHC by Oxidative Species

Considerable evidence suggests that KGDHC can be inactivated by oxidative stress in isolation, in cells, and in animals. KGDHC is sensitive to a variety of oxidants including peroxynitrite *(15),* NO *(15),* hydroxynonenal *(61),* H_2O_2 (in millimolar concentrations) *(62),* chloroamine (in micromolar concentrations) *(62),* and sodium hypochlorite (in nanomolar concentrations) *(62).* H_2O_2 diminishes KGDHC activity in synaptosomes *(63),* fibroblasts *(64),* N2a cells *(62),* and isolated mitochondria. The addition of H_2O_2 to mitochondria reversibly inactivates KGDHC through glutathionylation, followed by further irreversible damage to the protein *(65).* The addition of *t*-butyl hydroperoxide to mitochondria also inactivates KGDHC *(66).* Treatment of yeast cells with H_2O_2 causes oxidative damage to KGDHC, as measured by an increase in carbonyl groups *(67).* KGDHC is among the most sensitive proteins to the addition of hydrogen peroxide or menadione in aerobically respiring yeast or bacteria *(67).* The addition of H_2O_2 outside of cells reduces KGDHC activity in proportion to the increase in ROS, as measured with a common fluorescent probe *(64)* (Fig. 5). Catecholamines enhance E3 inactivation by the copper Fenton system [Cu(II)/H_2O_2], whereas E3 counteracts the prooxidant effect of catecholamines by scavenging

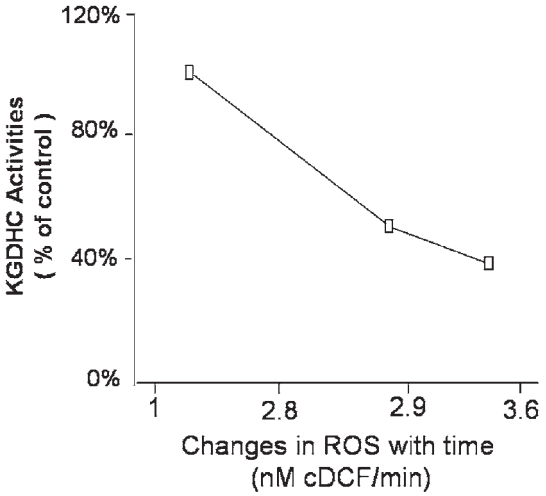

Fig. 5. Relation of KGDHC activities to H_2O_2-induced ROS formation. (From ref. *61*.)

Oxidant	Type of AD	E1	E2	E3
NO	APP 670/671	↓	↓	↔
Peroxynitrite	Sporadic AD	↔	↔	↔

Fig. 6. Changes in protein levels of E1k, E2k, and E3 with oxidant challenge *(15)* resemble those in brains from genetic *(45)* and nongenetic *(47)* forms of AD.

hydroxyl radicals *(68).* Related oxidants could produce different changes in immunoreactivity in E1k, E2k, and E3. Both KGDHC activity and immunoreactivies of E1k and E2k decline following treatment with NO *(15)* or in brains from patients bearing the APP67O/671 mutation *(45).* In contrast, KGDHC activity declines without changes in immunoreactivities of E1k, E2k, or E3 following treatment with peroxynitrite *(15)* or in brains of patients with sporadic AD *(47)* (Fig. 6). Both KGDHC and PDHC are sensitive to acrolein and HNE. Acrolein modifies the complex-bound lipoic acid, which decreases production of NADH by the complexes *(69).* HNE-mediated reductions in KGDHC are responsible for the reduction in respiration in the heart in reperfusion injury *(60,61).* Because KGDHC can be inactivated by multiple mechanisms, different inactivation processes could occur in various forms of AD, in different diseases, or between brain regions.

The α-ketoglutarate–dehydrogenase complex is also sensitive to ROS that are generated within the cells or within the brains of intact mice. This was first shown in Chinese hamster ovary cells. The cells were made hyperoxic and the enzymes that were inactivated were examined. KGDHC was the most sensitive *(71,72).* Similarly, the addition of azide, an inhibitor of respiration, to microglia increases ROS production and this leads to inactivation of KGDHC *(15)* (Fig. 1). Transfection of cells with monoamine oxidase increases ROS production and inactivates KGDHC. The inactivation is exaggerated if additional substrate is provided to increase ROS further *(73).* Genetic knockout of superoxide dismutase 2 (SOD2) in mice increases ROS production. A proteomic screen revealed that one of the most sensitive enzymes to the SOD2 knockout was the E1k of KGDHC *(74).* Thus, the sensitivity of KGDHC to oxidative stress provides a link between oxidative stress and brain dysfunction.

The α-ketoglutarate–dehydrogenase complex is more sensitive to oxidative stress than are commonly used measures of oxidation. As described earlier (Fig. 5), the addition of H_2O_2 to cells increases ROS, as detected by a common fluorescence probe and impairs KGDHC. If the antioxidant Trolox is added, the fluorescent-detectable ROS decline, but inhibition of KGDHC is not reversed. In fact, activity declines even more. Thus, KGDHC continues to be affected by ROS even after a decrease in the ROS concentration measured by common probes *(75).* To test the relative sensitivity of KGDHC to markers of oxidative stress in human brains, direct comparisons were made in brains from patients who died with progressive supranuclear palsy. In the cortex, malondialdehyde increases and KGDHC declines. KGDHC also declines in the cerebellum. However, malondialdehyde is not increased. Other measures of oxidative stress, including protein nitration and carbonyl formation, are also unchanged. This

suggests that reduced KGDHC might be a better indicator of the primary ROS action than are commonly used measures of oxidative stress in human brain *(57,58).*

Inactivation of KGDHC by Other Pathological Toxins

The α-ketoglutarate–dehydrogenase complex is sensitive to a variety of compounds thought to be important in neurodegenerative diseases. For example, compounds that are used in models of Parkinson's disease or might cause Parkinson's disease (MPP^+ and isoquinoline derivatives) inhibit KGDHC *(76,77).* 5-*S*-Cysteinyldopamine (5-S-CyS-DA) increases in Parkinson brains. Its oxidation product inhibits KGDHC *(68,78).* 3-Nitroproprionic acid (3-NP), a compound used to model Huntington's disease, also inhibits KGDHC *(56).* Oxidation of 5-hydroxytryptamine by superoxide, nitric oxide, or peroxynitrite forms tryptamine-4,5-dione. When incubated with intact rat brain mitochondria, this compound strongly inhibits KGDHC *(79).* KGDHC also seems particularly sensitive to environmental toxicants that work through β-lyase reactions. For example, several halogenated cysteine S-conjugates [e.g., *S*-(1,1,2,2-tetrafluoroethy)-L-cysteine] inactivate KGDHC *(80).* KGDHC can also be inactivated by transglutaminase *(81),* which is elevated in brains from patients with Huntington's disease and AD.

Consequences of Diminished KGDHC

Animal Models of Diminished KGDHC

A decline in KGDHC appears to affect neuron survival and to make neurons more sensitive to other insults, including aging. Thiamine deficiency (TD) and a genetic reduction in E3 (Dld +/− mice) have been used to test the effects of diminished KGDHC on brain function. TD produces neuronal death in select brain regions (i.e., selective neuronal vulnerability). Although TD kills neurons, it does not kill endothelial cells, astrocytes, or microglia. Indeed, it seems to activate these other cell types. The mechanisms for the TD-induced neuronal death are not well understood, but disturbances in the coenzyme and regulatory *(82,83)* functions of neuronal thiamine phosphates might well result in oxidative stress. TD diminishes the activity of KGDHC more than the activities of other thiamine-dependent enzymes. Many of the same markers of oxidative stress that have been observed in AD brain accompany neuronal death in TD *(84).* At later stages of TD, abnormalities in APP processing also occur. The selective neuronal loss in TD is not related to a specific regional loss of KGDHC protein. Hence, the loss of KGDHC activity resulting from the lack of the thiamine diphosphate coenzyme might lead to oxidative stress and to neuronal death both directly and by predisposing to other insults *(85).*

Recent studies tested the effects of heterozygote knockouts of E3 on the response to toxins that induce neurodegeneration *(56).* The activity of KGDHC is diminished by about one-half in the brain and liver of these mice. The mice do not show any changes in phenotype. However, they have increased vulnerability to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), malonate, and 3-NP, which have been proposed for use in models of Parkinson's disease and Huntington's disease. MPTP produces a greater depletion of tyrosine hydroxylase-positive neurons in the substantia nigra of Dld +/− mice than that seen in wild-type littermate controls. Striatal lesion volumes produced by malonate and 3-NP are larger in Dld +/− mice (Fig. 7). In addition, the 3-NP-injected mice have a reduction in KGDHC, although 3-NP is regarded as an inhibitor of succinic dehydrogenase. Thus, these in vivo studies suggest that a reduction in KGDHC predisposes to damage from other challenges, reducing the ability of nerve cells to adapt to stress *(56,85).*

Cell Models of Diminished KGDHC

Diminishing KGDHC activity through thiamine deficiency or by direct inhibition of

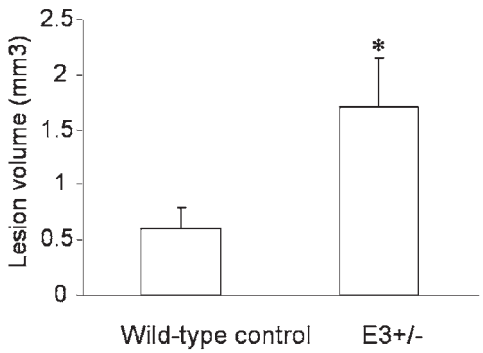

Fig. 7. Lesion volume in controls and E3 +/– mice following malonate. (From ref. *56*.)

KGDHC by chemical inhibitors has been used to test the role of KGDHC in cell models. The responses of multiple cell types to TD were studied in culture. Just as in the in vivo experiments, in vitro studies of neurons show that TD induces a marked reduction in KGDHC activity, increases oxidative stress (i.e., hydroxynonenal accumulation), and promotes apoptotic cell death *(86)*. However, the same degree of TD did not affect microglia, astrocytes, or endothelial cells. Thus, neurons appear to be particularly vulnerable to reductions in KGDHC.

The mechanisms by which diminished KGDHC predisposes to damage has been examined in two cell lines by inhibiting KGDHC with α-keto-β-methyl-*n*-valeric acid (KMV). This inhibitor is relatively specific, but it must be used at high concentrations *(87)*. In PC12 cells, inhibition of KGDHC activity by KMV as measured *in situ* does not alter mitochondrial membrane potential, but is associated with the release of cytochrome-*c* from mitochondria into the cytosol, reduction in basal cytosolic $[Ca^{2+}]_i$, and diminishing endoplasmic reticulum calcium stores *(88)*. The increased release of cytochrome-*c* associated with diminished KGDHC activity would be expected to activate other pathways, including cell death cascades. This possibility was tested in N2a cells. Concentrations of KMV that do not alter the mitochondrial membrane potential promote translocation of mitochondrial cytochrome-*c* to the cytosol, activate caspase-3, and increase extracellular lactate dehydrogenase (a measure of cell death). Inhibition of the mitochondrial permeability transition pore by cyclosporin A partially blocks the KMV-induced release of cytochrome-*c* and lactate dehydrogenase *(89)*. Thus, impairment of KGDHC could be associated with partial opening of the permeability transition pore prior to the loss of mitochondrial membrane potentials. This alters calcium dynamics, promotes release of cytochrome-*c* and activates caspase-3.

Mitochondrial and Synaptosomal Models of Diminished KGDHC

Studies of the enzymes controlling the availability of NADH for the respiratory chain under H_2O_2-induced oxidative stress in intact isolated nerve terminals determined that although the most vulnerable enzyme is aconitase, it is the inhibition of KGDHC that limits the amount of NADH available for the respiratory chain *(90)*. Thus, under these conditions, KGDHC appears to be rate limiting in the TCA cycle.

The effects of diminished KGDHC activity on mitochondrial function has been tested in isolated mitochondria from mice in which KGDHC activity is reduced by about one-half through genetic manipulation of the E3 component as well as by testing inhibitors of KGDHC. Mitochondria isolated from mice deficient in E3 activity are more sensitive to other metabolic insults. Brain mitochondria isolated from mice that are treated with 3-NP show that both succinate-supported respiration and membrane potential are suppressed to a greater extent in the E3-deficient mice. In vivo treatment with 3-NP induced more pronounced inhibition of SDH activity in brain mitochondria isolated from E3-deficient mice than controls. In vivo treatment with 3-NP also severely inhibits KGDHC activity *(56)*. Studies with the KGDHC inhibitor KMV suggest that inhibition of KGDHC in mitochondria might contribute to cell death by induction of the mitochondrial permeability transition (MPT). Decreasing KGDHC

activity in liver mitochondria by KMV facilitates MPT induction in response to calcium. Mechanistic studies suggest that this effect is independent of resting state 4 mitochondrial membrane potential, Ca^{2+} transport, and overall oxygen consumption. In isolated forebrain (nonsynaptosomal) mitochondria, KMV exerts essentially coincident inhibitory effects on KGDHC, $\Delta\Psi$, Ca^{2+} transport, and Ca^{2+} retention. Studies with ruthenium red and cyclosporin A implicate both Ca^{2+} cycling and the MPT in these responses. These data suggest that impaired KGDHC activity might contribute to neurodegeneration by facilitating mitochondrial recruitment into cell death cascades *(91).*

Experimental assessment of the in vivo consequences of a reduction in KGDHC is complicated. Reductions in mitochondrial respiration during cardiac reperfusion increase with age and depend on inactivation of KGDHC paralleled by modification of protein with HNE *(60,61).* Others fail to detect the inactivation of the rat heart KGDHC with age, although the increase in HNE adduct to the protein is still apparent *(70).* The apparent contradiction could reflect different conditions of the KGDHC activity test. More than 50% of the complex-bound lipoate can be removed or modified without significant influence on the overall activity assayed in the presence of saturating substrate concentrations *(92–94).* However, cells with the decreased lipoate content of the complexes are at physiological disadvantage (e.g., show decreased growth rates) *(93,94).* Thus, the influence of a partial modification of KGDHC (e.g., with HNE) on the complex activity can be detected in one system (e.g., upon measuring mitochondrial respiration as in *[70]*), but is not obvious in another (e.g., when KGDHC is assayed at substrate saturation conditions in extracts). The opposite relation of KGDHC activity and related physiological functions has been observed *(73).* A 40% decline in KGDHC occurs before any effect on mitochondrial energy metabolism appears in PC12 cell lines. However, the spare KGDHC threshold is nearly abolished in cells with elevated monoamine oxidase B levels, which increases H_2O_2 production *(73).*

Relation of KGDHC, Mitochondria, and Oxidative Stress to Pathology Associated With AD

Plaques

Amyloid-β peptide interacts with metabolism including KGDHC. The genetic forms of AD that most clearly implicate Aβ in the pathophysiology of AD are those that contain a mutation in APP that leads directly to increased formation of Aβ. However, even in these genetic forms of AD, metabolism and oxidative stress are likely to be involved in the neurodegenerative process. These patients have diminished KGDHC in their brains *(45).* Investigations by several groups suggest that Aβ induces ROS *(95–99)* and increases H_2O_2 in cells *(98–101).* Antioxidants protect against Aβ-induced cell death *(95–103).* Nanomolar concentrations of Aβ impair a general measure of cellular dehydrogenase activity *(104).* Aβ reduces the activities of KGDHC, pyruvate dehydrogenase, and complex IV of the electron transport chain *(105,106).* Recent studies indicate that alcohol dehydrogenase is a direct molecular link from Aβ to mitochondrial toxicity *(107).* Thus, even in forms of AD where the primary deficit is in APP processing, changes occur in oxidative metabolism and KGDHC, which appear to be important components of the disease.

Interruption of metabolism can lead to plaque formation. Patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes have Alzheimer-type pathology *(108).* A sustained high concentration of cytosolic calcium that can be caused by metabolic disturbance induces the production of intraneuronal $A\beta_{42}$ *(109).* Thiamine-deficient mice and rat brains show abnormal processing of APP, as demonstrated by immunostaining of neuritic processes *(110).* Oxidative stress (i.e,

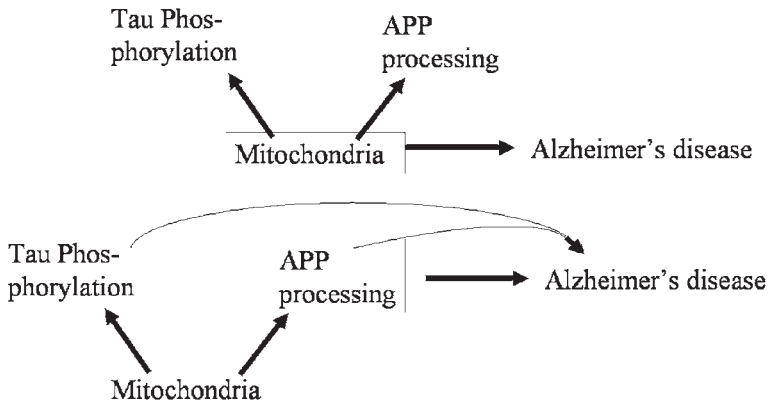

Fig. 8. In some cases, abnormal mitochondria could lead to the known pathologies. In other cases, mitochondria could change in response to other abnormalities but still be central.

treatment with H_2O_2) increases Aβ in the mammalian lens *(111)* and in SH-SY5Y cells *(112,113).* Blockade of complex IV of the electron transport chain with azide increases production of amyloidogenic fragments of APP *(114,115).* Mercury, which poisons metabolism and induces oxidative stress, increases Aβ production in SY5Y cells *(113).* Thus, in vivo and in vitro studies demonstrate that metabolic perturbations and oxidative stress increase Aβ production and alter APP processing in the direction of plaque formation. These data provide experimental support for the hypothesis that metabolic dysfunction can promote Aβ accumulation.

Tangles

Tangle formation can also be secondary to impairment of metabolism and oxidative stress. Wernicke–Korsakoff patients, who have reduced activities of thiamine-dependent enzymes including KGDHC, have tangles in their brains *(116).* Metabolic insults increase immunoreactivity of antigens that react with tangle antibodies in cultures of neurons *(117)* and fibroblasts *(118).* Glucose deprivation elevates ROS *(119)* and increases tau phosphorylation *(119).* Mercury, which alters metabolism and induces oxidative stress, also increases tau phosphorylation in SY5Y cells *(113).* Induction of in vivo alterations of glucose metabolism in mice by starvation or intraperitoneal injections of either insulin or deoxyglucose profoundly alter glucose metabolism. These treatments cause tau hyperphosphorylation with patterns resembling those in early AD brains *(120).* Together, the results indicate that oxidative stress and metabolic abnormalities can promote tau phosphorylation. Tangles might also lead to altered glucose metabolism. Patients with frontotemporal dementia, which is characterized by tangles, also have diminished glucose metabolism *(121).*

Thus, published data support the suggestion that altered oxidative metabolism could contribute to AD and to the formation of plaques and tangles, the characteristic lesions of AD. As shown in Fig. 8, the disease could result directly from the mitochondrial deficit or it could follow the formation of plaques and tangles.

Methods for Protecting or Reactivating KGDHC Might Be Beneficial

Understanding the role of KGDHC in neurodegeration could help to lead to new therapeutic strategies. Diminishing the oxidative

stress related to TD protects neurons. For example, knockouts of eNOS or ICAM-1 diminish oxidative stress and reduce neuronal death in thiamine-deficient mice *(122,123).* Lipoic acid, an essential cofactor for KGDHC, exerts antioxidant activity through a number of mechanisms involving its thiol–disulfide redox couple or metal-binding properties. Lipoic acid supplements to aged rats decrease the levels of lipid peroxidation and oxidized glutathione and increase the levels of reduced glutathione and the activities of mitochondrial enzymes such as KGDHC *(124).* Lipoic acid was also shown to attenuate the age-related loss in glutathione synthesis through the activation of Nrf2 transcription factor, leading to increased levels of glutathione-synthesizing enzymes *(125).* Acute administration of lipoic acid, an essential cofactor for KGDHC, increases cerebral metabolism in damaged areas of a Parkinson's disease model, suggesting reversal of the KGDHC deficit *(126).* Thus, lipoic acid supplementation enhances the activities of mitochondrial enzymes and antioxidant status and thereby protects mitochondria from aging. Treatment of a small number AD patients in an open trial with lipoic acid led to a stabilization of cognitive functions, demonstrated by mini-mental state examination, AD Assessment Scale (ADAS) and its subscale *(127).*

Trolox is similar to vitamin E, but it is water soluble. Treatment of cells with Trolox can dramatically increase KGDHC in cultured cells *(64)* (Fig. 9). Antioxidants that act on KGDHC might be effective in treating KGDHC-related neurodegenerative diseases. Nigral cell death in Parkinson's disease is associated with decreased glutathione levels, impaired complex I activity, and diminished KGDHC.

The effects of HNE on highly purified KGDHC were investigated. HNE decreases KGDHC activity when it is added to isolated protein or to cells. KGDHC is protected from HNE-induced inactivation by lipoamide, lipoic acid, reduced glutathione, and cysteine *(128).* Inactive KGDHC from H_2O_2-treated mitochondria was reactivated with dithiothreitol or

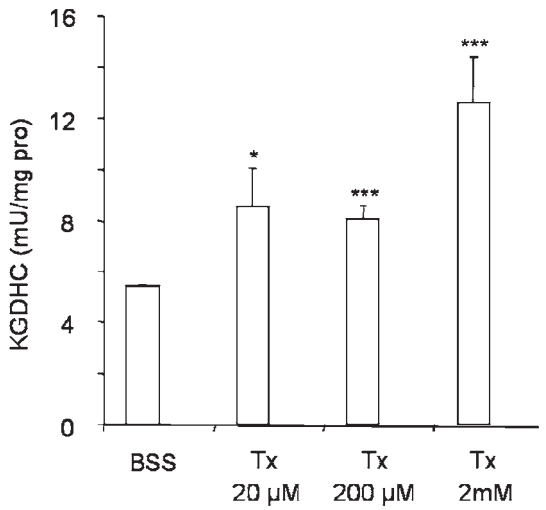

Fig. 9. Five-day Trolox treatment and KGDHC activities. (From ref. *64.*)

glutaredoxin, implicating modulation of KGDHC activity through enzymatic glutathionylation and deglutathionylation *(65).*

Activation of pathways to stimulate metabolism might also be an effective strategy. Activation of pyruvate dehydrogenase by dichloroacetate enables mouse models of Huntington's disease to live longer *(129).* Thiamine deficiency or excess causes major changes in mitochondrial thiamine diphosphate, the cofactor form of the vitamin used by the keto acid–dehydrogenase complexes. Excessive thiamine increases thiamine diphosphate and KGDHC in the liver *(130),* but this has not yet been tested in the brain. Treatment of AD patients with thiamine or thiamine derivatives, which has been reported to have some beneficial effects, could also reverse the loss in KGDHC and neurodegeneration *(131,132).* On the other hand, bypassing the KGDHC-catalyzed step to overcome the mitochondrial deficit could also be beneficial. The result of a trial in AD patients demonstrates that this approach has clinical relevance. A formulation that contains glucose, a Krebs cycle intermediate, and an antioxidant shows positive responses in both open trials and in a prospective double-blind placebo-controlled trial *(133).*

Although data are clearly preliminary, this finding reveals a positive trend, demonstrates the feasibility of addressing energy imbalance by diet, and supports our proposition that impairment of KGDHC leads to neurodegeneration.

Conclusion

Alterations in oxidative metabolism and in KGDHC appear to be important components of age-related neurodegenerative diseases. In AD, which the review emphasizes, evidence suggests that although in some cases the mitochondrial abnormality and KGDHC deficit might be secondary to the formation of plaques and tangles, the KGDHC reduction might lead to neuronal dysfunction. The reverse might also occur (i.e., the KGDHC deficit might precede the development of pathology). The reviewed data suggest that KGDHC is a valuable target of therapeutic intervention whether its inactivation is primary or secondary. Even if the reduction in KGDHC results from other primary events, the KGDHC inactivation would strongly aggravate the consequences of other changes. Thus, impairing oxidative metabolism might lead to induction of AD pathology and to the symptoms. Studies in mice and preliminary studies in humans suggest that the oxidative deficits, including the alterations in KGDHC, might be valuable therapeutic targets.

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