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Forum Review

Thiamine-Dependent Processes and Treatment Strategies in Neurodegeneration

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ABSTRACT

Reductions in brain glucose metabolism and increased oxidative stress invariably occur in Alzheimer's disease (AD) and thiamine (vitamin B1) deficiency. Both conditions cause irreversible cognitive impairment; their behavioral consequences overlap but are not identical. Thiamine-dependent processes are critical in glucose metabolism, and recent studies implicate thiamine in oxidative stress, protein processing, peroxisomal function, and gene expression. The activities of thiamine-dependent enzymes are characteristically diminished in AD, and the reductions in autopsy AD brain correlate highly with the extent of dementia in the preagonal state. Abnormalities in thiamine-dependent processes can be plausibly linked to the pathology of AD. Seemingly paradoxical properties of thiamine-dependent processes may underlie their relation to the pathophysiology of AD: Reduction of thiamine-dependent processes increase oxidative stress. Thiamine can act as a free radical scavenger. Thiamine-dependent mitochondrial dehydrogenase complexes produce oxygen free radicals and are sensitive to oxidative stress. Genetic disorders of thiamine metabolism that lead to neurological disease can be treated with large doses of thiamine. Although thiamine itself has not shown dramatic benefits in AD patients, the available data is scanty. Adding thiamine or more absorbable forms of thiamine to tested treatments for the abnormality in glucose metabolism in AD may increase their efficacy. *Antioxid Redox Signal* 9, 1605–1619.

INTRODUCTION

Thiamine (vitamin B1) has been associated with neurodegenerative disease since the 1880s. Thiamine's role in neurological disease was used to isolate, purify, and identify thiamine in the 1930s, when the assay for its activity was the relief of spastic posturing in pigeons. Some of the first experiments on the biochemistry of neurological disease utilized thiamine deficiency as a model (121). Thiamine deficiency (TD) is a useful model of neurological diseases, since it leads to mild impairment of oxidative metabolism, to increased oxidative stress, and to selective loss of neurons in specific brain regions. These features also occur in a number of age-related neurodegenerative diseases, including Alzheimer's disease (AD). Reductions in the activities of thiamine-dependent enzymes characteristically occur in AD and in a number of other age-related neurodegenerative diseases, supporting the use of experimental TD as a model of important aspects of these diseases.

Thiamine-dependent enzymes catalyze important steps in intermediary metabolism, and intermediary metabolism in brain is characteristically diminished in AD and in other age-related neurodegenerative disorders that cause dementia. Recent studies demonstrate that thiamine-dependent enzymes are sensitive to oxidative stress and yet can also be the primary sources of H_2O_2 within the mitochondria. Thiamine itself can act as an antioxidant. Suggestions that thiamine may be involved in neu-

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rotransmission, transcription, and ion transport persist. TD also accompanies AIDS and can be induced by other treatments such as chemotherapy for cancer. Thus, investigating the role of thiamine in the nervous system function and dysfunction is still important 80 years after its purification and the elucidation of the role of its pyrophosphate derivative as a coenzyme.

THIAMINE AND THIAMINE ESTERS IN BRAIN AND THEIR RELATION TO NEURODEGENERATIVE DISEASE

Biology of thiamine

Thiamine (vitamin B1) is required in the diet; humans and other animals do not synthesize it. In tissues including the brain, thiamine is phosphorylated by two kinases (Fig. 1). They convert it to thiamine monophosphate and then to thiamine diphosphate (*i.e.*, thiamine pyrophosphate; TPP). A small portion of TPP may be converted to thiamine triphosphate. TPP is the cofactor for thiamine-dependent enzymes. Phosphatases acting on each phosphorylated thiamine derivative have also been identified.

Each thiamine derivative and processing enzyme may have unique functions. Recent discoveries have identified adenosine thiamine triphosphate (AThTP), or thiaminylated ATP. In *Escherichia coli*, AThTP accumulates specifically in response to carbon starvation, thereby acting as a signal rather than a cofactor. It also occurs in yeast, plant, and animal tissues (7).

Changes in the brain

Activities of the thiamine phosphate dephosphorylating enzymes thiamine diphosphatase (TDPase) and thiamine monophosphatase (TMPase) decline in AD brains. TDPase activities are significantly reduced in frontal cortex and temporal cortex of AD patients by 28% and 62%, respectively (52, 94). TMPase activities are reduced by 31% and 64% in frontal and temporal cortex, respectively (52). Reductions in enzyme activities are present both in affected areas of AD brain and also in relatively well-conserved tissue (52, 94). Concentrations of thiamine and thiamine esters are also altered in AD brains. Thiamine-diphosphate (TDP) levels are 18–30% less in multiple cortical brain areas in AD (52, 74); thiamine monophosphate (TMP) has been reported to be increased (52) or decreased by 28–30% (52). Decreased TDP concentrations and concomitantly increased TMP in autopsied brain tissue from AD patients and in CSF from AD support the suggestion that CNS thiamine phosphorylation-dephosphorylation mechanisms are disrupted in AD (52).

Changes in other tissues

Reductions in the concentrations of thiamine and its esters also occur in tissues other than brain in AD. The mean plasma levels of TPP (−40%) and of free and total thiamine (−25%) are significantly lower in AD patients (47, 48). Plasma TPP concentrations correlate with the Mini-Mental State Examination ($r = 0.41$, $p < 0.05$) in the AD patients group (79), that is, plasma thiamine continues to fall as the disease (dementia) gets worse. Serum and blood TMP are significantly reduced as well (47). A comparison of plasma and erythrocyte thiamine levels in a group of patients with Parkinson's disease to a group of patients with AD reveal that AD patients have significantly lower plasma thiamine levels than those with Parkinson's disease (49).

ROLES OF THIAMINE-DEPENDENT ENZYMES AND PROCESSES IN THE PENTOSE SHUNT, GLYCOLYSIS, AND TCA CYCLE, AND CHANGES IN AD

The major TPP-dependent enzymes are shown in Fig. 2. Transketoslase is thiamine dependent and is a primary enzyme in the pentose shunt. This metabolic pathway is critical for production of NADPH which is involved in free radical metabo-

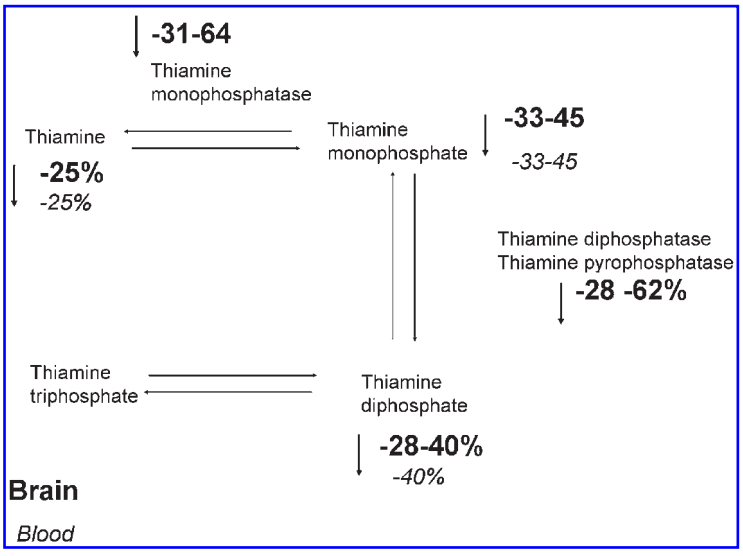

FIG. 1. Thiamine metabolism in brain and its changes in brain and plasma of patients with AD. The major pathways of thiamine metabolism in brain are shown. The *large, bold numbers* on top refer to changes in brain with AD. The *smaller numbers* refer to changes in blood with AD. The references for the reduction are in the text.

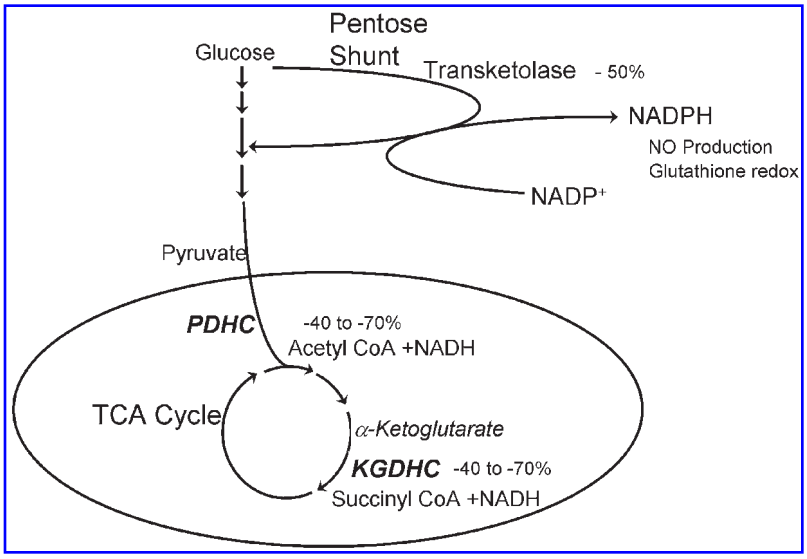

FIG. 2. The role of thiamine-dependent enzymes in brain and their changes in brain of patients with AD. Thiamine-dependent enzymes are key in the pentose shunt, in connecting glycolysis to the TCA cycle, and in the TCA cycle. Thus, reductions in thiamine-dependent enzymes are likely to have major effects on brain metabolism.

lism, both for $NO^{\cdot}$ synthesis and in the maintenance of the redox state of the free radical quencher glutathione. The other two major TPP-dependent enzymes in brain are large protein complexes.

The pyruvate dehydrogenase complex (PDHC) is composed of three protein subunits: E1p, E2p, and E3. There are many copies of each protein in the complex, and they are arranged in a specific manner (1). The activity is regulated in part by a kinase that inactivates the enzyme complex and a phosphatase that reactivates it. The PDHC reaction produces NADH and acetyl CoA and controls the entry of carbon into the TCA cycle.

The α-ketoglutarate dehydrogenase complex (KGDHC) is also composed of three proteins: E1k, E2k, and E3. The E3 enzyme is the identical protein in both the pyruvate and ketoglutarate complexes. There are many copies of each protein in the KGDHC complex, and they are also arranged in a specific array (1, 51). The KGDHC reaction produces NADH and succinyl CoA. The activity of KGDHC is among the lowest of the enzymes of the TCA cycle and it can be rate limiting, especially during oxidative stress (119). Detailed mechanisms for the reactions of these enzymes have been elucidated (51).

THIAMINE DEPENDENT ENZYMES AS A SOURCE OF REACTIVE OXYGEN SPECIES

Recent research indicates that PDHC and KGDHC can be significant sources of ROS in mitochondria. As noted above, each is a multienzyme complex. KGDHC is shown as an example in Fig. 3. The matrix soluble dihyrolipoyl containing dehydrogenases can generate superoxide radicals; production is stimulated by low NAD^+ availability or by high $NADH/NAD^+$ ratios. E3 is the likely source of the ROS. The amount of H_2O_2 generated by this enzyme is diminished in heterozygous E3 knockout mice (111). This is consistent with the idea that E3, a flavoprotein, is the source of the ROS from KGDHC. The absence of NAD^+ keeps lipoamide dehydro-

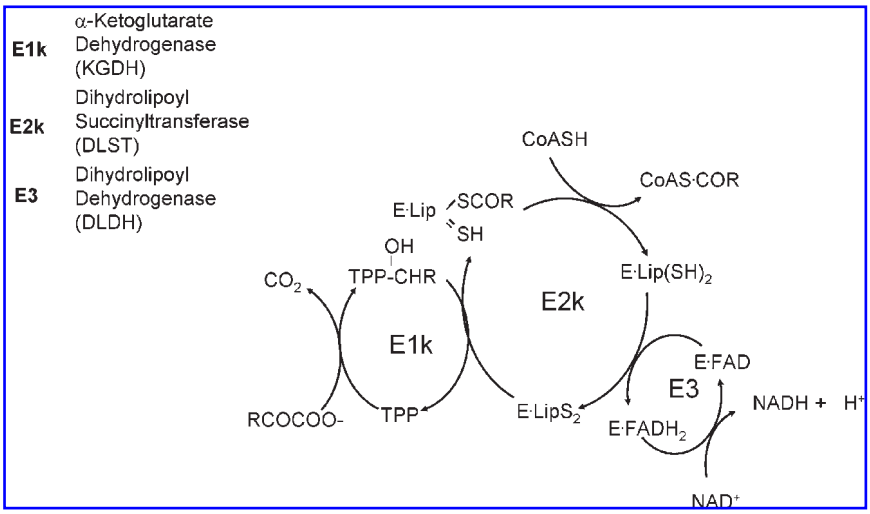

FIG. 3. The ketoglutarate dehydrogenase complex (KGDHC). KGDHC is composed of three subunits. Thiamine is a critical component of the reaction catalyzed by the E1k subunit, which may be the rate limiting step in the complex. The sulfhydryl groups of the E2 subunit can also act as antioxidants.

genase in the reduced state (*i.e.*, the intracellular environment of these enzymes is reducing). The reduced state increases the probability of lipoamide dehyrogenase reacting directly with oxygen to generate ROS. The production of ROS is physiologically important in regulation of the KGDHC enzyme in isolation, in mitochondria and synaptosomes, and in intact organisms.

Isolated enzyme

Isolated mitochondrial KGDHC and PDHC produce superoxide and H_2O_2. Isolated KGDHC incubated with coenzyme A (HS-CoA) and TPP starts to produce H_2O_2 after addition of α-ketoglutarate in the absence of NAD^+, a powerful inhibitor of KGDHC-mediated H_2O_2 formation. In contrast, the reduced form, NADH, stimulates H_2O_2 formation by KGDHC, and neither α-ketoglutarate nor HS-CoA is required. When all of the substrates and cofactors of the enzyme are present, the NADH/NAD^+ ratio determines the rate of H_2O_2 production. The higher the NADH/NAD^+ ratio, the higher the rate of H_2O_2 production (111, 118).

Lipoamide dehydrogenase, the E3 component of KGDHC and PDHC, catalyzes the transfer of reducing equivalents from the bound dihydrolipoate of the neighboring dihydrolipoamide acyltransferase subunit to NAD^+ (Fig. 3). This reversible reaction involves two reaction centers: a thiol pair, which accepts electrons from dihydrolipoate, and a noncovalently-bound FAD moiety, which transfers electrons to NAD^+. Studies of the complexes after converting the bound lipoate or FAD cofactors to nonfunctional derivatives indicate that the radicals are generated via FAD. In the presence of oxygen, the 2-oxo acid, CoA-dependent production of the superoxide anion radical is detectable. In the absence of oxygen, a protein-bound radical concluded to be the thiyl radical of the complex-bound dihydrolipoate is generated (17).

The production of ROS by PDHC and KGDHC may be important in a variety of pathological states. Zn-induced ROS production provides a clear example. Increased Zn has been implicated in many neurodegenerative conditions including stroke and AD. (29) Zn^{2+} accelerates the oxidase reaction described above up to fivefold, with an activation constant on the order of 90 n*M*. Activation is a consequence of Zn^{2+} binding to the reduced catalytic thiols, which prevents delocalization of the reducing equivalents between catalytic disulfide and FAD. The distinct effects of Zn^{2+} on different E3 activities represent a novel example of a reversible switch in enzyme specificity that is modulated by metal ion binding (38).

Generation of radical species is accompanied by enzyme inactivation. The superoxide scavenger, superoxide dismutase, does not protect the enzyme; however, the thiyl radical scavenger, thioredoxin, which is also present in mammalian mitochondria, does prevent inactivation. Thus, the thiyl radical of the complex-bound dihydrolipoate induces the inactivation by $1e^-$ oxidation of the 2-oxo acid dehydrogenase catalytic intermediate. A product of this oxidation inactivates the first component of the complex. The inactivation prevents transformation of the 2-oxo acids in the absence of the terminal substrate, NAD^+ (17).

Mitochondria

In rat brain mitochondria, under conditions of maximum respiration, α-ketoglutarate supports a higher rate of H_2O_2 production than did other substrates. In the absence of ADP or in the presence of rotenone, H_2O_2 production rates correlate with the level of reduction of the NADPH/$NADP^+$ pair in mitochondria with a variety of substrates, but was independent of this ratio with α-ketoglutarate as substrate. In contrast, NAD^+ inhibited ROS production by permeabilized mitochondria utilizing a variety of substrates with the exception of α-ketoglutarate. Brain mitochondria from heterozygous knockout mice deficient in dihydrolipoyl dehydrogenase ($Dld^{+/-}$; $E3^{+/-}$) produced significantly less H_2O_2 than mitochondria isolated from their wild-type littermates. The data indicate that KGDHC can be a primary site of ROS production in normally functioning mitochondria (111).

Synaptosomes

In synaptosomes, the rate of H_2O_2 production increases by 2.5-fold with α-ketoglutarate as respiratory substrate, and aconitase activity decreases. The enzyme aconitase contains an iron–sulfur site that is particularly sensitive to free radicals such as H_2O_2. These observations therefore indicate that KGDHC can generate H_2O_2 *in situ* in mitochondria in amounts that can have significant biological effects (118).

Yeast

Recent studies in yeast indicate that production of ROS by thiamine-dependent processes may be physiologically important. Replicative life span in yeast is increased by calorie restriction and by augmented NAD^+. Strains that are defective in NAD^+ synthesis and salvage pathways exhibit decreased oxygen consumption and increased mitochondrial H_2O_2 release, reversed over time by calorie restriction. Measurements of total, oxidized, and reduced glutathione support the evidence that changes in release of H_2O_2 by mitochondria alter cellular redox state. The strains with null mutants for NAD^+ synthesis and salvage pathways also have decreased chronological longevity in a manner rescued by calorie restriction. Matrix-soluble dihydrolipoyl dehydrogenases are an important source of calorie restriction-preventable mitochondrial ROS. Indeed, deletion of the gene for dihydrolipoyl dehydrogenases prevents oxidative stress in strains with null mutants for NAD^+ synthesis and salvage pathways. Furthermore, pyruvate and alpha-ketoglutarate, substrates for dihydrolipoyl dehydrogenase-containing enzymes, promote pronounced reactive oxygen release in permeabilized wild-type mitochondria, while mitochondria in cells without dihydrolipoyl dehydrogenase do not produce H_2O_2. Together, these results substantiate the concept that mitochondrial ROS play an important role in yeast senescence, and that their production can be limited by caloric restriction. Furthermore, these findings support the suggestion that dihydrolipoyl dehydrogenase is an important and novel source of ROS that is involved in life span limitation. These results show that although the electron transport chain generates ROS, ROS generated by dihyrolipoyl dehydrogenase-containing enzymes

KGHC and PDHC are the main physiological source of these life-shortening chemical species, at least in yeast cells (114).

CHANGES IN THIAMINE-DEPENDENT ENZYMES IN AD

Changes in the brain

Thiamine-dependent enzymes are diminished in postmortem brains from patients with AD, and the decline is highly correlated to clinical dementia rating scores before the patient died. Early studies revealed AD-related decreases in transketolase activity [−50%; (42)], the activity of the pyruvate dehydrogenase complex [PDHC; −41% (109)] and that of the α-ketoglutarate dehydrogenase complex [KGDHC; −75% (42)] (see Fig. 2). Measurements of the activities of all of the enzymes of the TCA cycle in an independent replication study showed that the decline in the activities of PDHC and KGDHC is correlated highly to a clinical dementia rating score determined shortly before the patients slipped into their terminal stages ($r = 0.77$, $r = 0.52$, respectively) (16). The correlation is higher in $APOE4^+$ than $APOE4^-$ patients (40). In the absence of TPP in the assay mix, mean KGDHC activity is reduced to a slightly greater extent in all AD brain areas (75). The reduction in these enzymes is not a generalized loss in mitochondrial proteins. For example, the activities of two other enzymes of the TCA cycle, malate dehydrogenase (+44%) and succinate dehydrogenase (+54%), increase with AD (16). Other studies of other populations found even larger changes in PDHC (−70%), KGDHC (−70%), and similar decreases in TK (−52%) without changes in glutamate dehydrogenase, another mitochondrial enzyme important in brain metabolism. Importantly, these enzymes decline in all brain regions, not just in regions of damage visible by microscopy (19). These findings suggest a possible role for alterations of brain thiamine-dependent enzymes in the pathophysiology of Alzheimer's disease.

Changes in thiamine-dependent enzymes in the periphery

A generalized decrease of the physiological availability of thiamine would be expected to impair thiamine dependent processes in other organs as well as the brain. Low serum transketolase occurs more often in AD patients than in control subjects (96). Statistically significant reductions in transketolase but not PDHC and KGDHC occur in red blood cells and cultured fibroblasts from AD patients (42).

ALTERNATIVE FUNCTIONS OF THIAMINE THAT MAY BE CRITICAL IN NEURODEGENERATIVE DISEASES

These "traditional" roles of thiamine in metabolism that are described above are critical to brain function, and indirect evidence suggests that other important thiamine-dependent processes are also altered in AD. They include thiamine's antioxidant function (43). High concentrations of thiamine diphosphatase are normally present in the Golgi (18) and trans-Golgi network (54), which are critical sites of protein processing. Thiamine deficiency (TD) induces endoplasmic reticulum stress (122), a cellular response to unfolded proteins, including a programmed gene response. Recent studies indicate decreases in activities of thiamine-dependent enzymes including not only transketolase but also a newly discovered thiamine dependent enzyme (2-hydroxyphytanoyl-CoA lyase; 2-HPCL) that is critical to alpha-oxidation in peroxisomes (108).

THIAMINE AS AN ANTIOXIDANT

Although the antioxidant capacity of thiamine has not yet been fully characterized chemically, several indirect lines of evidence suggest that thiamine can act as an antioxidant. Thiamine inhibits lipid peroxidation in liver microsomes, reduces free radical oxidation of oleic acid *in vitro* (72), and elevates glutathione reductase activity following cardiac hypoperfusion (117). In mice, thiamine deficiency (TD) elevates many markers of oxidative stress. These markers are also elevated in brains from AD patients (23, 24), in which as noted above there is also a reduction in the activity of thiamine-dependent enzymes (42, 105). Homogenates of thalamus and cortex from thiamine-deficient rats produce 76% and 38% excess reactive oxygen species (ROS) compared to controls (68). In tissue culture, chronic TD elevates hydroxynonenal and increases DNA fragmentation, another sign of oxidative stress in cultured neurons, but not in microglia, endothelial cells, or astrocytes (89). The antioxidant vitamin E provides significant protection against death in TD neurons *in vitro* (87). Interestingly, thiamine has related actions in the heart; the elevation in lipid peroxidation, and reduction in glutathione reductase induced by cardiac hypertrophy are normalized by thiamine (117).

Thiamine treatment appears to be beneficial in a number of disorders in which free radicals are increased. One example is copper toxicity. Copper toxicity contributes to neuronal death in Wilson's disease and has been speculatively linked to the pathogenesis of Alzheimer's and prion diseases. Copper facilitates the formation of reactive oxygen species, and inhibits PDHC and KGDHC *in vitro* and in animal models of Wilson's disease *in vivo*. Thiamine therapy also extends life span from 6 months to greater than 16 months in rat models of Wilson's disease. Hydrogen peroxide, ethacrynic acid, or menadione, or the redox active metal (Cd^{2+}) kills neurons in culture and causes an early and marked reduction in both PDHC and KGDHC that precedes cortical neuronal death. The combination of thiamine and dihydrolipoic acid attenuates the reactive oxygen species-induced reductions in these enzyme activities, as well as subsequent neuronal death. Oral or i.p. thiamine administration either chronically or immediately before the insult reduces the size of middle cerebral artery occlusion-induced infarcts in mice (104). Thus, thiamine, or perhaps dihydrolipoic acid, or both together, may be potential therapeutic agents in the broad range of diseases mediated by free radical stress (102–104).

Thiamine's role as an antioxidant can also be demonstrated by testing neutrophil functions and mitogen-induced lymphocyte transformation in humans. Thiamine stimulates neutrophil motility *in vitro* and *in vivo* and increases lymphocyte transformation *in vivo*. Preincubation of neutrophils and lymphocytes with the combination of horseradish peroxidase/H_2O_2/halide inhibits migratory and proliferative responses. Inclusion of thiamine at concentrations which inhibit the peroxidase/H_2O_2/halide system protects the neutrophil migratory and lymphocyte proliferative responses from inactivation in this system. Thiamine may, therefore, increase neutrophil migration and lymphocyte transformation at least in part by protecting these cells from toxic oxidative products generated by the peroxidase/H_2O_2/halide combination (115). Thiamine protects against radiation damage—perhaps by acting as an antioxidant. When cultured lymphocytes are irradiated with X-rays, the DNA damage can be estimated as the frequency of micronuclei and apoptotic or necrotic morphological changes. A significant decrease in the fraction of apoptotic and necrotic cells occurs in lymphocytes irradiated in the presence of thiamine, compared to those irradiated in the absence of thiamine. The results indicate that thiamine can protect human cells from radiation-induced genetic changes (65).

Studies of thiamine deficiency (TD) provide indirect support for an interaction of nitric oxide or peroxynitrite with thiamine. In brain, TD induces the endothelial nitric oxide synthase isoform eNOS (23, 24, 66), increases lipid peroxidation (37), and increases tyrosine nitration in neurons within susceptible areas (23). Furthermore, genetic deletion of eNOS protects against TD (24). These results suggest (indirectly) that thiamine interacts with $NO^{\cdot}$ or its oxidation product N_2O_3. Those free radicals can modify proteins that regulate calcium homeostasis. This possibility was tested in fibroblasts. SIN-1 (3-morpholinosydnonimine) is frequently used as a model compound for a continuous release of $O^{\cdot}$, $NO^{\cdot}$, and/or NO. ROS induced by 3-morpholinosydnonimine (SIN-1) are reduced by low concentrations of thiamine (55).

O_2 reacts with $NO^{\cdot}$ to form $NO^{\cdot}{}_2$ (Reaction 1), which can react with $NO^{\cdot}$ to produce dinitrogen trioxide (N_2O_3) (Reaction 2, below) (60, 62, 63). N_2O_3 is known to be highly effective in nitrosating sulfhydryl groups (63). The nitosylated sulfhydryls can react with the thiol groups of proteins (RSH), of thiamine (TSH), and of glutathione (GSH) to form *S*-nitrosothiol (RSNO) (Reaction 3), TSNO (Reaction 4), or *S*-nitrosoglutathione (GSNO) (Reaction 5). Thus, thiamine may serve as an effective scavenger to neutralize nitrogen species produced by SIN-1 (see previous paragraph) by forming *S*-nitrosothiols.

1. $2\ NO^{\cdot} + O_2 \rightarrow 2\ NO^{\cdot}{}_2$
2. $NO^{\cdot} + NO^{\cdot}{}_2 \rightarrow N_2O_3$
3. $RSH + N_2O_3 \rightarrow RSNO + NO^{-}{}_2 + H^{+}$
4. $TSH + N_2O_3 \rightarrow TSNO + NO^{-}{}_2 + H^{+}$
5. $GSH + N_2O_3 \rightarrow GSNO + NO^{-}{}_2 + H^{+}$

Two different types of reaction sequence may explain the direct antioxidant effects of thiamine; they are shown in Fig. 4. The first involves opening of thiazole ring of the intermediate (Fig. 4, II) to form thiol thiamine (Fig. 4, III), which is oxidized to form TSNO (Fig. 4, IV), simultaneously reducing N_2O_3 to NO_2^{-}. The second pathway by which thiamine can act as an antioxidant may underlie its ability to quench t-butyl hydroproxide (t-BHP)-induced ROS. By transferring $2H^{+}$ from the NH_2 group of the pyrimidine ring of thiamine, an intermediate form of thiamine (Fig. 4, II) is transformed to tricyclic thiamine with a closed thiazole ring (Fig. 4, V) (112). Subsequently this tricyclic thiamine is oxidized to thiochrome (Fig. 4, VI) while reducing *t*-BHP (t-buOOH) to *t*-buOH (72, 77).

Evidence suggests that the interactions of thiamine with $NO^{\cdot}$, glutathione, thiol containing proteins, and oxidants may be important in cellular regulation of $NO^{\cdot}$ and may change the functions of the proteins involved (110). The reduced form of thiamine thiol (TSH) can be oxidized by the NO adduct of glutathione (GSNO) to form GSH and thiamine disulfide (TS-ST) with the release $NO^{\cdot}$ [Reaction 1]. GSH reacts with TS-ST through a thiol-disulfide exchange reaction to form thiol thiamine (TSH) and a combination of glutathione with thiamine disulfide (GS-ST) [Reaction 2].

1. $2\ TSH + GSNO\ (RSNO) \rightarrow TS\text{-}ST + GSH\ (RSH) + NO^{\cdot}$
2. $GSH + TS\text{-}ST \rightarrow TSH + GS\text{-}ST$

SUM OF THE TWO REACTIONS: $2TSH + GSNO + GSH \rightarrow TSH + GS\text{-}ST + NO^{\cdot}$

Thiamine disulfide, the mixed thiamine disulfide with glutathione, and nitric oxide are produced in the reaction. An exchange reaction between the thiamine thiol form and *S*-nitrosocysteine residues of proteins would permit nitric oxide to be released and mixed thiamine–protein disulfides to be formed. The mixed thiamine disulfides (including thiamine ester disulfides), as well as the thiamine disulfide form, are quite easily reduced by low molecular weight thiols to form the thiamine cyclic form with a closed thiazole ring. The results suggest a possible role of the thiamine thiol form in releasing deposited nitric oxide from low-molecular-weight *S*-nitrosothiols and protein *S*-nitrosothiols. Data suggest that this function may be important in regulation of blood flow (112).

PROTEIN PROCESSING AND THE ENDOPLASMIC RETICULUM (ER)

A common feature of many neurological diseases is altered processing of key proteins. Thus, AD and Huntingtons's (HD) and Parkinson's (PD) diseases are associated with the pathological accumulation of protein aggregates: β-amyloid in AD, huntingtin in HD, and α-synuclein in PD. The neuropatholgical hallmarks of AD are plaques and tangles, the former resulting from the aggregation of abnormally processed amyloid precursor protein (APP) and the latter from the aggregation of hyperphosphorylated tau proteins.

The localization of thiamine processing enzymes suggests they could be involved in protein processing, since critical components of protein processing occur in the endoplasmic reticulum (ER), trans-Golgi network, and Golgi apparatus where thiamine-processing enzymes also occur. Thiamine pyrophosphatase is a trans-Golgi marker enzyme (76, 80), although its function is not clear. Recent studies indicate that the TPP-dependent enzyme transketolase is localized around the endoplasmic reticulum (ER) (13).

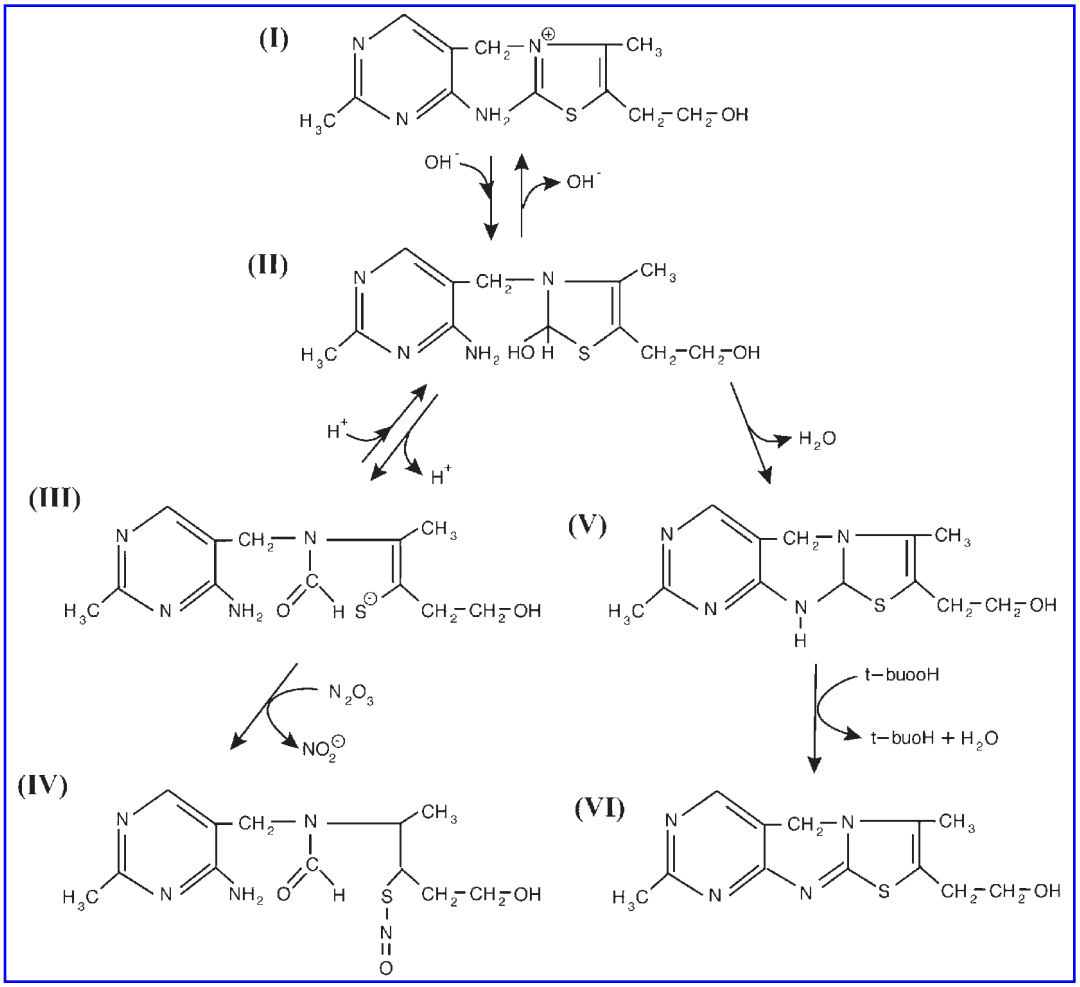

FIG. 4. Thiamine as an antioxidant. Two chemical pathways can account for the antioxidant effects of thiamine. The pathway on the *left* (I, II, III, IV) shows the interaction of $NO^{\cdot}$ and thiamine. The reactions on the *right* (II, V, VI) show the interaction with *t*-butyl hydroperoxide. The details are described in the text. The authors acknowledge the extensive help of Dr. Hsueh–Meei Huang in preparing this figure.

Even if thiamine-dependent enzymes are not involved directly in processing proteins, thiamine could be involved in processing through its effects on energy metabolism and oxidative stress. The ER is involved in post-translational protein processing and transport. The accumulation of unfolded proteins leads to ER stress. ER stress has several important effects: it diminishes gene translation, it enhances protein degradation, it increases levels of ER chaperones including glucose regulated protein (GRP) (78), it causes growth arrest and DNA damage-inducible protein (C/EBP-homologous protein), it leads to phosphorylation (reduced activity) of the translation initiation factor eIfalpha, and it causes cleavage of caspase-12. TD induces ER stress.in thalamus. The increases in GRP, GADD153, and phosphorylated eIF2alpha are maximal after seven days of TD, a time point which precedes neuronal death by 2 days (122). Electron microscopy also reveals differences in brain ER: enlarged lumens, deposition of vesicles, and an irregular packing pattern of rough ER (122).

Electron microscopic investigations on sciatic and plantar nerves of thiamine deficient rats show a pronounced distal axonal degeneration. The earliest alterations consist of an increase of mitochondria and a proliferation of vesicular elements of the ER. They are followed by loop formations of the axon membrane, clustering and disintegration of neurotubules and neurofilaments, axonal shrinkage, and finally myelin disruption. The distal accentuation of the early changes indicates a dying-back mechanism of axonal degeneration (92).

As noted above, plaques are one of the neuropathological hallmarks of AD. Plaques are formed primarily of amyloid β peptide ($A\beta_{1–40}$ and $A\beta_{1–42}$), a product of the processing of amyloid precursor protein (APP). Thiamine deficiency alters processing of amyloid precursor. Interruption of energy metabolism can increase the production of amyloidogenic fragments, so that reduction of the activities of thiamine-dependent enzymes of energy metabolism increase plaque formation (78). Changes in oxidative metabolism and oxidative stress *in vivo* induce AD-like pathology, and TD may be acting by a similar mechanism. Acute inhibition of energy metabolism with various pharmacological agents (insulin, 2-deoxyglucose, 3-nitropropionic acid, and kainic acid) in C57BL/6 wild-type and in

APP transgenic mice (Tg2576) elevated BACE1 levels and activity, and increased levels of Aβ (120). Inhibition of electron transport with azide increased formation of amyloidogenic fragments of APP (36). Oxidative stress can also promote abnormal processing of APP as shown by increase in plaques in Mn-SOD-deficient mice (70, 101) and a decrease in plaques in $NO^{\cdot}$-deficient mice (82). TD elevates nitric oxide synthase in macrophages/microglia in C57BL/6 mouse brain, and genetic knockout of eNOS protects animals against TD (20, 24). Crossing APP and PS1 double transgenic mice (Tg2596) with mice with genetically disrupted iNOS alleles reduces protein tyrosine nitration products, cerebral plaque formation, and Aβ levels (82). TD induced increases in iNOS and eNOS-derived $NO^{\cdot}$ may aggravate the Aβ pathology in Tg19959 mice.

Thiamine deficiency in normal wild-type mice promoted the formation of neuritic clusters that stained positively with several antibodies for amyloid precursor protein and amyloid precursor like proteins, but plaques were not formed (21, 22). Since murine APP does not aggregate, the results did not determine whether thiamine deficiency could lead to or promote plaque formation in humans. Recent studies report the effects of TD in mice overexpressing two familial AD mutations in human APP (KM670/671NL and V717F), namely the Tg19959 strain. TD significantly increases compact (Thioflavine-S or Congo red) and diffuse (4G8 or 6E10-immunoreactive) plaques. Quantification of plaques in cortex, hippocampus, olfactory bulb, cerebellum, thalamus, hypothalamus, and neostriatum reveals a marked acceleration of amyloid deposition in TD compared to saline-treated animals (61). These data demonstrate directly that TD accelerates plaque formation and alters APP processing.

TD can also lead to the formation of neurofibrillary tangles similar to those that occur in AD. Tangles occur in brains of severely thiamine-deficient patients with the Wernicke–Korsakoff syndrome, a severe form of thiamine deficiency seen in the United States, most often in chronic alcoholics (30-32). Neurofibrillary pathology is found in the nucleus basalis in these patients, but not any other brain region. Neurofibrillary tangles are not seen in age-matched controls and are infrequent in alcoholics without neuropathological signs of thiamine-deficiency. Neurofibrillary tangles are most numerous in those cases with cell loss in the nucleus basalis. These findings suggest that neurodegeneration of the nucleus basalis in chronic alcoholics proceeds through the formation of neurofibrillary tangles (31, 45).

The ER is altered in AD. Presenilins are localized to the ER, and one of the major enzymes involved in abnormal processing of amyloid precursor protein (BACE) is localized to Golgi. The ER is a highly oxidizing environment that is high in calcium. ER calcium is excessively high in cells from AD patients (45, 58) and in fibroblasts and neurons from transgenic mice bearing AD-causing mutations (69). Changes in the ER appear in brains from both sporadic and genetic forms of AD in humans. In sporadic AD, factors that cause oxidative stress prevent the clearance of Aβ from ER, leading to the accumulation of Aβ peptide. ROS stimulate Aβ formation by inhibiting the degradation of the enzyme BACE that releases it from APP [see reviews in Refs. 39 and 91]. Presenilins, which are important in many familial forms of AD, are localized to the ER, and presenilin mutations may lead directly to ER stress. Mutations in APP lead to formation of excess Aβ which can itself induce ER stress. These mechanisms provide means by which interactions with thiamine and thiamine deficiency could alter any and all of these processes.

THIAMINE AND GENE EXPRESSION

Thiamine may act directly at the gene level. Control of transcription is particularly well established in yeast. In yeast, three thiamine biosynthetic enzymes, the thiamine transporter, and an acid phosphatase (a thiamine pyrophosphokinase) have been shown to be transcriptionally regulated by thiamine (34, 84). In yeast, the gene encoding arginase is also suppressed by thiamine (84)

Activities of both transketolase and KGDHC decrease at the same rate and to proportionally roughly similar levels in response to thiamine deficiency in lymphocytes, when exogenous TPP is added to the activity assays. This observation implies roughly coordinate reduction of the transcription/translation of the genes encoding the apoenzymes forms of these two proteins. Differences between transketolase and alpha-KGDH become readily apparent when TPP is not added to the reaction mixture. Only 25% of the lost transketolase is present as apoenzyme, whereas 70% of the lost alpha-KGDH activity is present in the apo-enzyme form. For transketolase, the nonrecoverable activity is due mainly to a decrease in the synthesis rate of the protein during thiamine deficiency. These observations suggest that thiamine has a direct effect on the expression of the transketolase gene product (93).

Thiamine has also been implicated in post-transcriptional modification of RNA. Riboswitches are untranslated regions of messenger RNA, which adopt alternate structures depending on the binding of specific metabolites. Riboswitches specifically recognize TPP via conserved residues located within two highly distorted parallel “sensor” helices (116).

THIAMINE-DEPENDENT PROCESSES IN PEROXISOMES

Thiamine may alter lipid metabolism in peroxisomes. New studies indicate that thiamine is directly involved in alpha oxidation in peroxisomes (108). Peroxisomes are important in lipid metabolism, specifically of fatty acids in which beta-oxidation is blocked by a methyl or hydroxyl group in the beta position relative to the carboxyl moiety. 2-Hydroxyfatty acids, constituents of brain cerebrosides and sulfatides that are critical in myelination, are degraded by an alpha-oxidation system, generating fatty acids shortened by one carbon atom. The alpha-oxidation sequence of 3-methyl-branched fatty acids starts with an activation to the corresponding CoA-ester. Subsequently this acyl-CoA-ester undergoes a 2-hydroxylation by the peroxisomal phytanoyl-CoA hydroxylase. In a third step, the peroxisomal 2-hydroxyphytanoyl-CoA lyase (2-HPCL) disproportionates the carbon–carbon bond of the 2-hydroxy-intermediate into a 2-methyl(n-1)aldehyde and formyl-CoA, which is subsequently converted to formate and CO_2. Finally the aldehyde is dehydrogenated by an aldehyde dehydrogenase to the

corresponding acid, which, after its conversion to the acyl-CoA ester, can be a substrate for beta-oxidation. Experiments with cultured cells and animal models show that alpha-oxidation is controlled by the thiamine status of the cell/tissue/organism, and suggests that some pathological consequences of thiamine starvation could be related to impaired alpha-oxidation (108) These observations indicate that cleavage through the thiamine pyrophosphate-dependent 2-hydroxyphytanoyl-CoA lyase is the main pathway for the degradation of 2-hydroxyfatty acids. Long and very long chain 2-hydroxy fatty acids are constituents of brain cerebrosides and sulfatides, which mainly occur in myelin (25, 108).

Transketolase activity is also present in peroxisomes. The ultrastructural localization is similar to that of glucose-6-phosphate dehydrogenase activity, suggesting activity of the pentose phosphate pathway at these sites (13). Other TPP-dependent enzymes may also be present in peroxisomes.

Genetic lack of the α-hydroxylase leads to Refsum's disease, due to the accumulation of phytanic acid derived from the normal dietary constituents phytol and phytanic acid. This is a degenerative disease, albeit a very rare one. Neuropathy and ataxia are prominent abnormalities, as in TD.

OTHER FUNCTIONS OF THIAMINE THAT HAVE NOT BEEN DIRECTLY TESTED IN DISEASE

Thiamine in the form of TTP regulates large conductance chloride channels (6), serves as the phosphate donor to phosphorylate synaptic proteins (83), and modifies ion transport, especially sodium transport (4). Recent discoveries indicate the presence of an adenine nucleotide containing thiamine, namely, adenosine thiamine triphosphate (AThTP, 1), also named "thiaminylated ATP. In *Escherichia coli,* it accumulates specifically in response to carbon starvation, thereby acting as a signal rather than a cofactor. It also occurs in mammalian tissues including brain (7). Its functions in brain remain to be elucidated.

PLAUSIBLE LINKS OF THIAMINE-DEPENDENT PROCESSES TO THE PATHOPHYSIOLOGY OF AD

Plausible mechanisms link the decline in thiamine-dependent enzymes to particular aspects of AD. The relation to brain pathology is discussed above. Brain glucose metabolism is invariably diminished in symptomatic and even in presymptomatic AD (10, 12, 41). *In vivo* imaging studies of patients with genetic forms of AD indicate that the reduction in metabolism occurs decades before the patient expresses the disease clinically (95). The underlying basis for the decline is unknown, but one possibility is the decline in the activities of thiamine-dependent mitochondrial dehydrogenase enzyme complexes (See discussion above).

Reducing the activities of KGHC and PDHC diminishes oxidation by the brain. Impaired KGDHC function plays a key role in limiting the generation of NADH in the Krebs' cycle during the early phase of oxidative stress in synaptosomes (119). Impairing KGDHC also leads to release of cytochrome C and promotes caspase activation (56). A reduction in E3 decreases the activity of PDHC and KGDHC and increases oxidative stress in the brain (64). A reduction in E2k in cells diminishes their ability to reduce exogenously added oxidants and increases cell death in response to oxidants (106).

Thiamine-dependent processes are also documented linked to memory functions, as measured by neuropsychological testing in humans and behavioral testing in experimental animals. Indeed, thiamine deficiency in humans is classically related to severe memory deficits (5, 121). This may be due to the importance of oxygen and glucose metabolism in normal brain function (107) or through links to the cholinergic system which is altered by thiamine deficiency (5).

The role of KGDHC production of free radicals may exaggerate these changes. The diminished thiamine availability could impair KGDHC activity. This would impair brain function including memory, neurotransmitter synthesis, and release of apoptotic proteins including cytochrome c. The associated abnormalities in metabolism would promote free radical production by the electron transport chain. At later stages, this would lead to a reduced environment in which KHCHC would also produce H_2O_2 which would inactivate oxidant sensitive processes including KGDHC and aconitase.

The response to TD is exaggerated by aging (35). The magnitude of this effect is modified by the genetic background of the mice. Although KGDHC activities in brain are not altered by aging or genetic background (*i.e.*, mouse strain), the response of KGDHC to TD is exaggerated by aging. In contrast, transketolase is altered by the genetic background but not by aging (35).

WHY ARE THIAMINE-DEPENDENT ENZYMES AND PROCESSES DIMINISHED WITH AD?

As described above, convincing data suggests that multiple thiamine-dependent processes are diminished in AD and other neurodegenerative diseases. Furthermore, TD mimics important aspects of the changes in AD. Two pressing questions are: (a) Why are these thiamin-dependent processes diminished? and (b) Is there a feasible way to either reverse or bypass these deficits? There are at least four plausible explanations for the reduction in thiamine dependent processes: (a) AD patients may be thiamine deficient; (b) thiamine-dependent enzymes are sensitive to oxidants and may have been inactivated; (c) thiamine-dependent enzymes may be vulnerable to proteases; (d) thiamine transport may be inhibited. Evidence suggests each of these mechansims can occur, but their relative contributions are unknown. The cause of the deficits will obviously alter the clinical approach to trying to ameliorate the deficits.

TD in AD as shown by TPP activation

The reduction in so many thiamine-dependent processes stimulates the question of whether AD patients are actually TD. A

surprisingly large number of studies suggest that either AD patients are thiamine deficient or that thiamine metabolism is altered. As indicated previously, a large number of thiamine esters are diminished in brains and blood from AD patients. A more standard way to assess thiamine deficiency is to measure the response of thiamine-dependent enzymes (especially transketolase activity) to the addition of TPP. With normal thiamine in the diet, the binding of TPP to transketolase is so tight that no activation occurs. However, in TD animals or individuals, TPP activates red blood cell transketolase. As discussed below, TPP activates transketolase from red blood cells of AD patients significantly more than for controls, implying some sort of functional thiamin deficiency in AD (31). TPP also activates thiamine-dependent enzymes from brain. TPP produces a greater stimulatory effect on KGDHC activity from AD brains (23–280% mean stimulation) as compared with the controls (−4% to +50%), suggesting some of the diminished activity may be related to reduced endogenous TPP levels in AD brain (74, 75). In the absence of TPP in the assay mix, mean KGDHC activity is reduced to a slightly greater extent in all AD brain areas (74, 75). Thus, a significant proportion of patients with AD may also be TD, which may have an impact on the pathophysiology, including cognition. Unfortunately, currently used assays may not be adequate to assess thiamine status (48). Although no studies contradict the idea of a functional thiamine deficiency in AD, such negative findings may not have come to publication.

ALTERNATIVE EXPLANATIONS

Modification of thiamine-dependent proteins

An alternative explanation for the changes in response to thiamine in AD could be a modification of the thiamine-dependent proteins. A precedent exists for such a possibility. A subgroup of alcoholics develop Wernicke–Korsakoff syndrome and are thiamine deficient. Studies with fibroblasts suggest that the subgroup of patients may have a Km mutation in transketolase which leads to their developing symptoms of TD with degrees of thiamine deficiency that are clinically silent in normal individuals (9). Although the precise alteration in transketolase is controversial, the existence of a modification this thiamine-dependent enzyme in Wernicke–Korsakoff patients has proven reproducible. Whether the change is genetic or reflects post-translational effects however remains unclear.

Several different lines of evidence suggest TK is modified in AD. A study of transketolases (TK) in red blood cells from 21 patients with AD and 24 age-matched controls revealed that TK from AD erythrocytes had nearly double the activity coefficient and more than doubling of the Km for TPP, compared to the controls. These findings suggest structural abnormalities of TK rather than vitamin B1 deficiency in AD (33). Other data suggest abnormalities in proteinase actions on this enzyme. Cultured fibroblasts from patients affected by AD exhibit peculiar alterations of TK. Abnormalities consisted of enzyme forms having unusually high pI. These have been proposed to be markers of the disease in living patients. There is evidence for an underlying imbalance of transketolase proteolysis in AD fibroblasts due to a relative increase/derangement of the cysteine proteinases "cathepsins" (88).

No mutations have been identified so far in the gene for transketolase that would explain the altered transketolase proteins or transketolase enzyme activities found in neurodegenerative diseases, diabetes, or cancer. Recent studies suggest a second transketolase enzyme (TKTL1) in humans. During the evolution of the vertebrate genome, mutations in this transketolase gene (TKTL1) have led to tissue-specific transcripts of different sizes, which encode an enzymatically active transketolase protein as well as different smaller protein isoforms. The mutations within the TKTL1 gene caused a mutant transketolase enzyme with an altered substrate specificity and reaction mechanism, which did not however correlate with any disease state (28). Thus, protein modification could explain reported changes in TK I. It seems unlikely, however, that a similar modification could be occurring in all of these thiamine-dependent enzymes encoded on different genes.

No changes in protein structure of KGDHC subunits from AD patients have been reported. Western blots either reveal no change in protein content or, in APP mutant forms of AD, a reduction in their amount. However, no changes in migration on gels have been reported. In some populations (Ashkenazi Jewish males), there is a strong association between abnormal single nucleotide polymorphism in E3 and AD (14, 15), but not E2k (15). Perhaps, TD-induced changes in ER folding or processing could give rise to more general changes.

Thiamine-dependent enzymes are very sensitive to oxidants. KGDHC has been studied most extensively. KGDHC is inactivated by a variety of oxidants including peroxynitrite, NO (90), hydroxynonenal (57), H_2O_2 (in m*M* concentrations), chloroamine (μM concentrations), and sodium hypochlorite (in n*M* concentrations). H_2O_2 diminishes KGDHC activity in synaptosomes (26), fibroblasts (43, 46), and N2a cells. PDHC and KGDHC in intact mitochondria are inactivated by hydroxynonenal (HNE) (57), a marker of oxidative stress that is elevated in brains of both AD patients and thiamine-deficient animals. Treatment of rat heart mitochondria with HNE selectively inhibits KGDHC and PDHC, while other NADH-linked dehydrogenases and electron chain complexes are unaffected. Although all forms of AD have reduced brain KGDHC activities, protein levels as determined by immunoreactivity decline in some forms of AD but not others, as noted above. Even related oxidants can produce differing and relatively specific changes, similar to those which occur in AD brain. Peroxynitrite and $NO^{\cdot}$ inactivate KGDHC activities in cells and in isolation (59, 89). However, peroxynitrite, but not $NO^{\cdot}$, diminishes immunoreactivity of E1k and E2k. The first pattern is similar to that observed in AD patients bearing the APP670/671(44) mutation. The second pattern is similar to that seen in AD patients with no known genetic basis (75).

KDGHC activity is reduced in animal and cell models when oxidative stress increases. Transgenic superoxide dismutase (53) knockout mice have reduced KGDHC activities in their brains (53). KGDHC is diminished in cells that overexpress monoamine oxidase (MAO). Increased substrate (*i.e.*, more ROS) exaggerates the reduction in KGDHC (67). Exposure of CHO cells to hyperoxia inactivates KGDHC to a greater extent than numerous other enzymes including succinate dehydrogenase, NADH dehydrogenase, and α-glycerol phosphate dehydrogenase (99, 100).

Discoveries related to other diseases suggest that deficiencies of thiamine transport could lead to the changes in AD. Thiamine is accumulated into cells through the activity of plasma

membrane thiamine transporters (*e.g.*, hTHTR1). Genetic evidence links mutations in hTHTR1 with the manifestation of thiamine responsive megaloblastic anemia (TRMA), a condition also associated with diabetes mellitus, sensorineural deafness, and retinal disorders. Results reveal a spectrum of mutant phenotypes, underscoring that TRMA can result from decreased thiamine transport activity underpinned by changes in either hTHTR1 expression level, cellular targeting, and/or protein transport activity. The disorder is helped by administration of excess thiamine (86, 113). Additional transport deficiencies are associated with neurological disease. SLC25A19 mutations cause Amish lethal microcephaly (MCPHA), which markedly retards brain development and leads to alpha-ketoglutaric aciduria. The mutation alters the thiamine pyrophosphate (TPP) transport into the mitochondria. The mitochondria of Slc25a19(−/−) and MCPHA cells have undetectable and markedly reduced ThPP content, respectively (71).

CLINICAL TRIALS

Whether thiamine-dependent processes can be used as a therapeutic target is unclear. Thiamine has been shown to be protective against copper toxicity and middle cerebral artery occlusion in rats by quenching free radical formation (104) and thiamine derivatives have been reported to improve peripheral neuropathy in diabetes (50).

Examples of thiamine treatments of patients that have diseases other than AD

Thiamine treatment is very effective for some patients with PDHC deficiency. Several mutations of the pyruvate dehydrogenase (E1) alpha subunit have been reported. The DNA sequence of some patients reveal X-linked E1alpha subunit point mutation (F205L and L216F) within the TPP-binding region in exon 7. These patients display very low PDHC activity in the presence of a low (1×10^{-4} m*M*) TPP concentration, but their PDHC activity significantly increases at a high (0.4 m*M*) TPP concentration. Therefore, the PDHC deficiency in these two patients is due to a decreased affinity of PDHC for TPP. Treatment of these patients with thiamine results in a reduction in the serum lactate concentration and clinical improvement, suggesting that these patients have a thiamine-responsive PDHC deficiency (81).

Thiamine-responsive megaloblastic anemia syndrome is an autosomal recessive disorder disease that is caused by mutations in the SLC19A2 gene encoding a high-affinity thiamine transporter. Active thiamine uptake into cells is disturbed. Clinical features include many large immature and dysfunctional red blood cells (megaloblasts), mild reduction in platelet count, and decreased white blood cells, sensorineural deafness, and diabetes mellitus. Treatment with pharmacological doses of thiamine ameliorates the megaloblastic anemia and diabetes mellitus (86).

Wernicke–Korsakoff syndrome

Wernicke–Korsakoff syndrome is caused by thiamine deficiency. Wernicke encephalopathy is the first acute phase and Korsakoff psychosis is the long-lasting, chronic stage. The most common cause is alcoholism, but the syndrome can also be associated with excessive vomiting, AIDS, cancers that have spread through the body, very high levels of thyroid hormone, chemotherapy, and certain other conditions. The Wernicke syndrome is characterized by confusion, unsteady gate, and paralysis of eye muscles. The most striking abnormality in the Korsakoff psychosis is difficulty in learning new information. Individuals tend to "confabulate" (*i.e.*, make up information they cannot remember). Prompt treatment of patients in the early stage with intravenously injected thiamine typically protects against the development of the memory difficulties of the Korsakoff psychosis.

Alzheimer's disease

The suggestions of altered thiamine metabolism in AD have encouraged some limited clinical trials. Like most other therapies of AD such as antioxidants, the therapeutic effects have been no more than minimal. Although treatment with large doses of thiamine has not been beneficial, the data are not totally negative. Global cognitive rating by the Mini-Mental State Examination was higher during 3 months with 3 g/day of oral thiamine hydrochloride than with niacinamide placebo. Behavioral ratings, however, did not differ significantly, nor did clinical state when it was judged subjectively (11). A 12-month trial did not reveal differences (85). Higher dosages (3–8 gm/day) may have had a mild beneficial effect in AD patients. Fursultiamine (TTFD), a derivative of thiamine, at an oral dose of 100 mg/day had a mild beneficial effect in AD patients in a 12-week open trial. Only mildly impaired subjects showed cognitive improvement (78). The review for the Cochrane Database Syst Rev review concluded that the data are inadequate to allow a conclusion about the efficacy or lack of efficacy of thiamine in the treatment of AD (97, 98).

STRATEGIES TO TREAT NEURODEGENERATIVE DISEASES IN WHICH REDUCTION IN THIAMINE-DEPENDENT PROCESSES OCCURS

The lack of effectiveness of thiamine in treating AD, even though reasonable evidence suggests that thiamine-dependent processes are diminished, suggests that alternative treatment strategies may be required. High dose vitamins are used to treat many inheritable diseases. They tend to be effective when a mutation increases the Km for the coenzyme derived from the vitamin. A thorough review of the literature suggests that the proportion in a disease gene that is responsive to high concentration of a vitamin or substrate may be one-third or greater (2). With AD, oxidation may alter many proteins, thereby decreasing their affinity for their substrates or coenzymes. However, treating with a single cofactor may not be adequate. For example, the thiamine-dependent complexes also use NAD, FAD, and dihydrolipoic acid as cofactors and have a magnesium requirement. In some patients with mutations in pyruvate decarboxylase, the use of thiamine plus lipoic acid is more effective than thiamine alone (73). Thus, a cocktail of cofactors or their precursors may be necessary. Testing cocktails and their components in clinical trials may be logistically difficult and very expensive. Tissue culture models, including cells derived from

the affected patients and from matched healthy controls, may be an efficient way to decide which therapeutic components and which combinations should go on to formal clinical trials (27).

In addition to cofactors or their precursors, inclusion of metabolites to bypass partial metabolic blockade may be useful way to try to correct metabolic insufficiencies. In transgenic mouse models of Huntington disease, activation of the thiamine-dependent enzyme PDHC is protective (3). In confused patients with possible Wernicke–Korsakoff syndrome (*i.e.*, symptomatic thiamine deficiency), intravenous administration of thiamine is routinely followed by intravenous injection of glucose (121). In thiamine-deficient Wernicke patients, driving glucose metabolism by injecting glucose without prior injection of thiamine can increase damage. However, even in WK, if the symptoms have gone on too long, thiamine will not alleviate memory loss, presumably because of permanent brain damage. Both lessons likely apply to the treatment of AD. Thus, the thiamine clinical trials have not used other supplements. One recent study showed benefit to AD patients with a cocktail of glucose, malate, and an antioxidant (8). Perhaps addition of thiamine or other cofactors might increase the efficacy of that preparation.

In summary, a large body of data indicates that thiamine-dependent processes are altered in a number of neurodegenerative diseases, including AD. Although the cause of the reduction is not clear, thiamine-dependent processes may provide attractive clinical targets. A combination of activating them by adding cofactors (or other activators) as well as methods to bypass the biochemical lesion(s) appears tenable.

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ABBREVIATIONS

AThTP, adenosine thiamine triphosphate; AD, Alzheimer's disease; MCPHA, Amish lethal microcephaly, $A\beta_{1–40}$ and $A\beta_{1–42}$, amyloid β peptide; APP, amyloid precursor protein; HS-CoA, coenzyme A; Dld, dihydrolipoyl dehydrogenase, ER, endoplasmic reticulum; TTFD, fursultiamine; GRP, glucose regulated protein, GSH, glutathione, HD, Huntingtons's disease; HNE, hydroxynonenal; s-HPCL, 2-hydroxyphytanoyl-CoA lyase; KGDHC, α-ketoglutarate dehydrogenase complex; MAO, monoamine oxidase; SIN-1, 3-morpholinosydnonimine; PD, Parkinson's disease; ROS, reactive oxygen species; RSNO, *S*-nitrosothiol; GSNO, *S*-nitrosoglutathione; RSNO, *S*-nitrosothiol; *t*-BHP, *t*-butyl hydroperoxide; TD, thiamine deficiency; TDPase, thiamine diphosphatase; TDP or TPP, thiamine diphosphate; TS-ST, thiamine disulfide; TMPase, thiamine monophosphatase; TMP, thiamine monophosphate; TRMA, thiamine responsive megaloblastic anemia; TSH, thiamine thiol; TK, transketolases; TKTL1, transketolase enzyme.

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