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Role of Mitochondrial Dysfunction and Oxidative Stress in the Pathogenesis of Selective Neuronal Loss in Wernicke's Encephalopathy

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Abstract

Thiamine deficiency results in Wernicke's encephalopathy and is commonly encountered in chronic alcoholism, gastrointestinal diseases, and HIV AIDS. The earliest metabolic consequence of thiamine deficiency is a selective loss in activity of the thiamine diphosphate-dependent enzyme α-ketoglutarate dehydrogenase (α-KGDH), a rate-limiting tricarboxylic acid cycle enzyme. Thiamine deficiency is characterized neuropathologically by selective neuronal cell death in the thalamus, pons, and cerebellum. The cause of this region-selective neuronal loss is unknown, but mechanisms involving cellular energy failure, focal lactic acidosis, and NMDA receptor-mediated excitotoxicity have classically been implicated. More recently, evidence supports a role for oxidative stress. Evidence includes increased endothelial nitric oxide synthase, nitrotyrosine deposition, microglial activation, and lipid peroxidation. Reactive oxygen species production results in decreased expression of astrocytic glutamate transporters and decreased activities of α-KGDH, resulting in an amplification of cell death mechanisms in thiamine deficiency.

Index Entries: Thiamine deficiency; Wernicke–Korsakoff; oxidative stress; mitochondrial dysfunction; neuronal cell death; reactive oxygen species; nitric oxide synthase; thalamic lesions.

Introduction

Wernicke's encephalopathy or the Wernicke–Korsakoff syndrome (WKS) is a neuropsychiatric disorder characterized by ophthalmoplegia, gait ataxia, and memory loss. WKS is a common complication of chronic alcoholism and is also encountered in patients with human immunodeficiency virus–acquired immunodeficiency syndrome (HIV-AIDS) and other disorders associated with grossly impaired nutritional status. WKS results from thiamine deficiency. In chronic alcoholism, thiamine deficiency results

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from poor diet and impaired absorption of thiamine from the gastrointestinal tract. In addition, alcohol impairs the phosphorylation of thiamine both in peripheral tissues and in brain.

Neuropathologic evaluation of brain tissue from WKS patients reveals a pattern of selective symmetrical damage to mammillary bodies, thalamus, cerebellum, and pons *(1).* Cellular changes include neuronal loss, astrocytic proliferation, and microglial activation. The cause of this distinctive pattern of neuronal loss has not been fully elucidated. However, several theories involving cellular energy failure, focal lactic acidosis, blood–brain barrier breakdown, and NMDA-receptor-mediated excitotoxicity have been proposed. In experimental animals exposed to thiamine deficiency, both apoptotic and necrotic patterns of neuronal cell death have been observed.

In the 1930s, Peters and co-workers showed that thiamine deficiency in pigeons resulted in the accumulation of lactate in the brainstem of affected birds *(2).* Furthermore, they showed that the addition of small quantities of crystalline thiamine to the isolated brainstem tissue from thiamine-deficient birds in vitro resulted in normalization of lactate levels. These findings led to the formulation of the concept of "the biochemical lesion" in thiamine deficiency. Later studies showed that the enzyme defect responsible for the "biochemical lesion" was α-ketoglutarate dehydrogenase (α-KGDH) rather than pyruvate dehydrogenase (PDHC) (as had initially been suspected). α-KGDH and PDHC are major thiamine diphosphate (TDP)-dependent enzymes involved in brain glucose oxidation (Fig. 1).

In the brain, as in most mammalian cells, thiamine occurs predominantly (over 80%) in the form of TDP, the remainder being made up of thiamine monophosphate (10%) and thiamine triphosphate (5–10%), with only trace amounts of free thiamine. Thiamine is transported into the brain and phosphorylated by the action of thiamine pyrophosphokinase and inhibition of this enzyme by thiamine antagonists such as pyrithiamine results in decrease synthesis of TDP. Treatment of experimental animals with pyrithiamine results in a generalized reduction of TDP concentrations throughout the brain and an early selective loss in activity of α-KGDH in regions of the brain such as thalamus, which are destined to manifest selective neuronal cell loss *(3).* Decreased activities of α-KGDH following treatment with pyrithiamine are associated with decreased synthesis of glucose-derived excitatory and inhibitory amino acids, including glutamate, aspartate, and GABA, with a concomitant increase in lactate and alanine *(4)* consistent with decreased flux of carbon through the tricarboxylic acid cycle. Both the α-KGDH decreases and the changes in synthesis of amino acids are initially reversible following thiamine rehabilitation in pyrithiamine-treated animals *(4),* suggesting that these changes are an integral part of "the biochemical lesion" originally proposed by Peters.

In a study of thiamine-dependent enzymes in postmortem brain tissue from alcoholic patients, it was reported that decreased activities of PDHC, α-KGDH, and transketolase were confined to those cases in which the neuropathologic diagnosis of WKS had been made *(5).* Alcoholic patients dying in hepatic coma without WKS manifested brain TDP-dependent enzyme activities within normal limits. Furthermore, activities of a non-TDP-dependent enzyme glutamate dehydrogenase were unchanged in both WKS and non-WKS alcoholic brains. These findings provide evidence, for the first time, that reduction in thiamine-dependent enzymes are implicated in the pathogenesis of WKS in humans.

Mitochondrial Dysfunction, Lactic Acidosis, and Brain Energy Failure in Thiamine Deficiency

Oxidative decarboxylation of α-ketoglutarate (and pyruvate) are reportedly decreased in isolated mitochondria from the brains of pyrithiamine-treated rats *(6),* consistent with reductions in the activity of α-KGDH. Furthermore, normal rates of pyruvate decarboxyla-

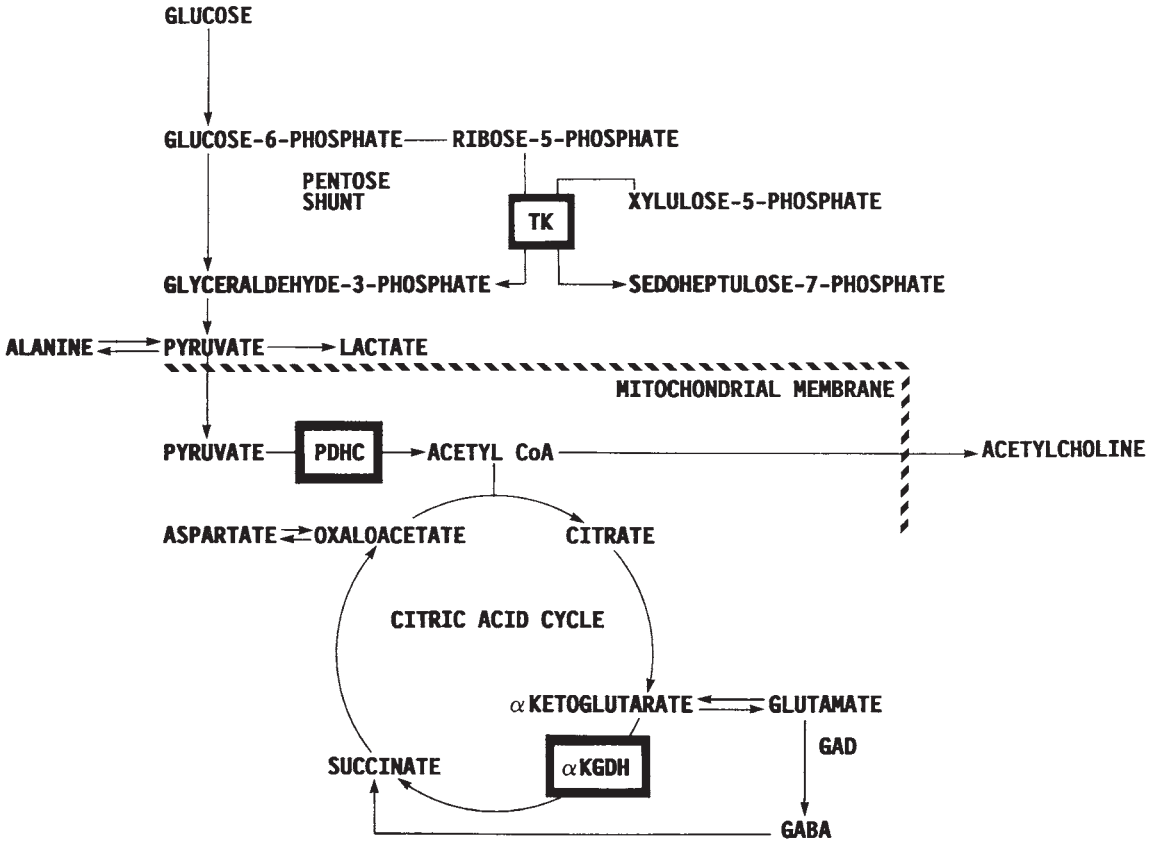

Fig. 1. TDP-dependent enzymes. TDP-dependent enzymes are implicated in brain glucose oxidation and pentose shunt pathway. Impaired activities of TDP-dependent enzymes result in decreased synthesis of glucose-derived neurotransmitters (acetylcholine, glutamate, GABA) and, ultimately, a cellular energy deficit and lactic acidosis. PDHC: pyruvate dehydrogenase complex; α-KGDH: α-ketoglutarate dehydrogenase; TK: transketolase.

tion and enzyme activities are promptly restored after the addition of TDP to the mitochondrial preparations. Subsequent studies in mitochondrial preparations from thiamine-deficient animals showed that state 3 respiration was significantly decreased using α-ketoglutartate as the substrate but was unchanged using succinate *(6)* (succinate is oxidized independently of TDP-dependent enzymes; Fig. 1).

Studies to elucidate the cellular metabolic consequences of thiamine deficiency in relation to mitochondrial function have been performed recently using cultured neuronal cell preparations. Cultured cerebellar granule cells exposed to the thiamine antagonist pyrithiamine manifest significant reductions in TDP, reduced activities of α-KGDH, increased lactate production indicative of decreased tricarboxylic acid cycle flux, and a significant lowering of cellular pH *(7)*. In an earlier study in which neuroblastoma cells were exposed to the thiamine transport inhibitor amprolium, decreases in activity of α-KGDH led to decreased oxygen consumption, uncoupling of mitochondria, and disorganization of cristae, all of which were restored following the addition of thiamine or succinate to the medium *(8)*. More prolonged exposure of either neuroblastoma cells or cerebellar granule cells to thiamine deficiency led to depolarization, lactate accumulation, decreased ATP production, and

cell necrosis *(7,8).* Pyrithiamine-treated rats likewise manifest disintegration of mitochondria and chromatin clumping in degenerating thalamic neurons *(9).* Decreases of ATP have also been described in the brainstem of pyrithiamine-treated animals *(10).* Significant acidosis has been described in vulnerable brain structures such as mammillary bodies and thalamus of pyrithiamine-treated rats *(11).*

Evidence for NMDA Receptor-Mediated Excitotoxicity in Thiamine Deficiency

It has been reported that the nature of the neuropathologic lesions observed in thiamine deficiency resembles that described in NMDA receptor-mediated excitotoxicity. Other evidence in favor of an excitotoxic mechanism of neuronal cell death includes the findings of increased concentrations of glutamate in brain extracellular (dialysate) fluid from pyrithiamine-treated rats *(12).* More recently, decreases in the expression of astrocytic glutamate transporters was reported in the brains of these animals *(13),* a phenomenon that could explain the increased extracellular brain glutamate concentrations. Also consistent with an NMDA receptor-mediated excitotoxic mechanism in the pathogenesis of the selective neuronal cell loss as a result of thiamine deficiency were the findings that administration of MK801, an NMDA receptor antagonist, resulted in the attenuation of necrotic damage in the thalamus of pyrithiamine-treated animals *(14).* However, a subsequent study attributed at least some of the neuroprotective effects of MK801 to its anticonvulsant and hypothermic properties *(15).*

Oxidative Stress and Selective Neuronal Cell Death in Thiamine Deficiency

There is increasing evidence to support a major role for oxidative stress in the pathophysiology of selective neuronal cell death resulting from thiamine deficiency. Increased production of reactive oxygen species has been reported in the brains of pyrithiamine-treated rats *(16)* and increased peroxidase activity indicative of oxidative stress is related to the neuropathology in human WKS *(17).* Other findings consistent with oxidative stress as a contributing factor to thiamine-deficiency-related neuropathology include reports of increase in heme oxygenase and ICAM-1 *(18)* as well as early induction of endothelial nitric oxide synthase (NOS) *(19)* and microglial activation *(20).*

Microglial Activation in Thiamine Deficiency

Neuropathological studies in animals with experimental thiamine deficiency consistently show early damage to glial cells rather than neurons *(21–23).* Morphological changes include swelling of glial soma and vacuolation of astroglial end feet. Studies in human WKS patients likewise show changes in astroglia together with microglial proliferation, which is apparent even in regions of the brain showing little if any neuronal cell death *(24).* A systematic study of astroglial integrity in thiamine deficiency using cell-specific markers (ED-1 for microglial activation; glial fibrillary acidic protein for astrocytic proliferation) revealed early increases of microglial activation in brains of pyrithiamine-treated animals *(20).* Increased ED-1 immunolabeling was confined to vulnerable brain structures such as the thalamus, inferior colliculus, and vestibular nuclei and was found to precede neuronal cell loss, breakdown of the blood–brain barrier, and reactive astrocytosis in the brains of these animals (Fig. 2). These findings suggest that metabolically compromised neurons (as a result of thiamine deficiency) could initiate, by cell-to-cell signaling pathways, microglial activation as an initial cellular event. Similar proposals were previously made to explain selective neuronal cell death in experimental cerebral ischemia *(25).*

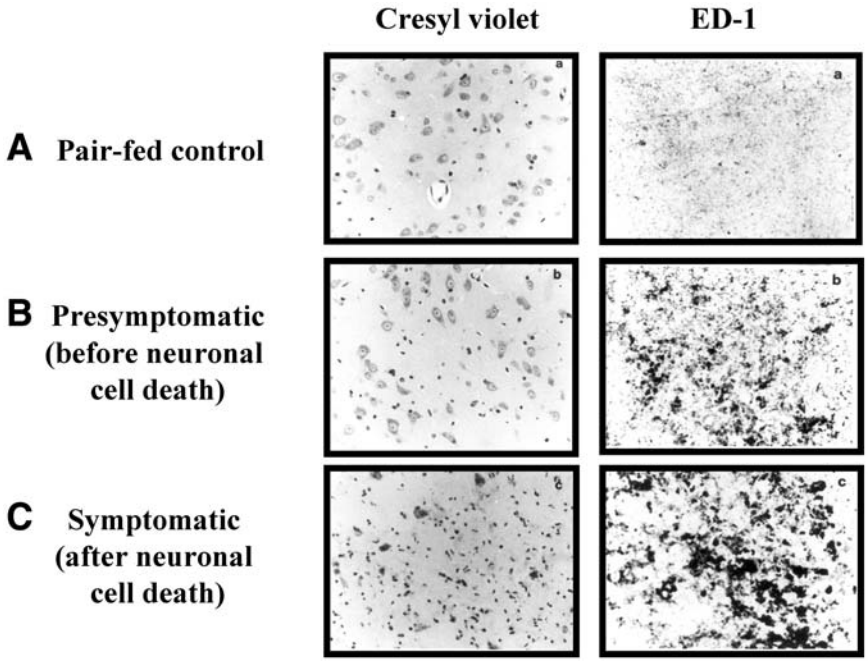

Fig. 2. Microglial activation (ED-1 immunolabeling of microglia) as a function of the appearance of neuronal cell death in medial thalamus of rats at presymptomatic (before neuronal cell death, **panel B**) and symptomatic (after neuronal cell death, **panel C**) stages of thiamine deficiency compared to the pair-fed control **(panel A)**. Note the significant ED-1 immunostaining of microglia prior to the onset of neuronal cell death.

eNOS-Derived NO in the Thiamine-Deficient Brain

A recent study showed significant increases of expression of the endothelial isoform of nitric oxide synthase (eNOS) in the brains of rats treated with pyrithiamine *(19).* Increased eNOS expression was apparent prior to the onset of neurological symptoms and was restricted to vulnerable medial thalamus and inferior colliculus (Fig. 3A,B); eNOS expression in the spared cerebral cortex was unaltered by pyrithiamine treatment. In contrast to the early increase in the expression of eNOS, the expression of inducible (iNOS) and neuronal (nNOS) isoforms were not significantly altered at this stage of thiamine deficiency (Fig. 3C). These findings add to a growing body of evidence to suggest that NO production by vascular endothelial cells is an early cellular event in thiamine deficiency *(26,27).* It has also been demonstrated that targeted disruption of the eNOS (but not the iNOS or nNOS) gene results in reduced extent of neuropathological damage in thalamus of pyrithiamine-treated animals *(27).*

The suggestion that eNOS-derived NO is implicated in neuronal cell death mechanisms in thiamine deficiency contrasts with current views in cerebral ischemia in which increased eNOS-derived NO is thought to play a neuroprotective role by virtue of its vasodilatory potential *(28).* Studies in cultured cell preparations indicate that eNOS-derived NO could limit apoptotic cell death *(29).* Apoptosis has been described in brain under thiamine-deficient conditions *(30);* however, apoptosis was limited to medial thalamus in these studies. The extent to which eNOS-derived NO impacts either positively or negatively on neuronal cell death in thiamine deficiency awaits future studies. Increased production of NO via eNOS

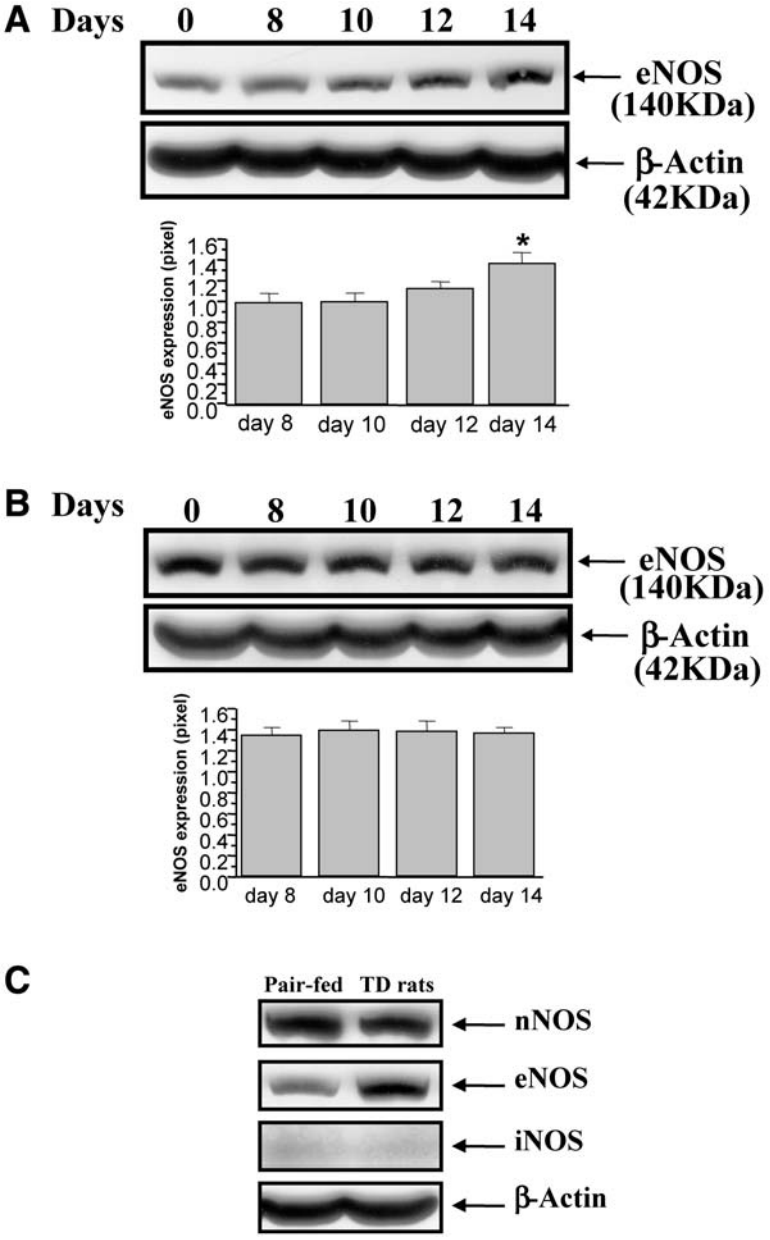

Fig. 3. Selective induction of eNOS expression in the medial thalamus of thiamine-deficient (TD) rats. **(A)** Increased eNOS starts as early as d 12 in the medial thalamus of TD rats (*$p < 0.05$ compared to d 8 by analysis of variance). **(B)** No significant changes of eNOS expression in the cerebral cortex of TD rats at any time-points. **(C)** Increased eNOS expression but no induction of either nNOS or iNOS in medial thalamus following 12 d of thiamine deficiency.

induction offers one plausible explanation for observations of early increases in nitrotyrosine immunolabelling of thalamic neurons in pyrithiamine-treated animals *(27)*.

Conclusion

In summary, thiamine deficiency leads to selective neuronal cell death by multiple mech-

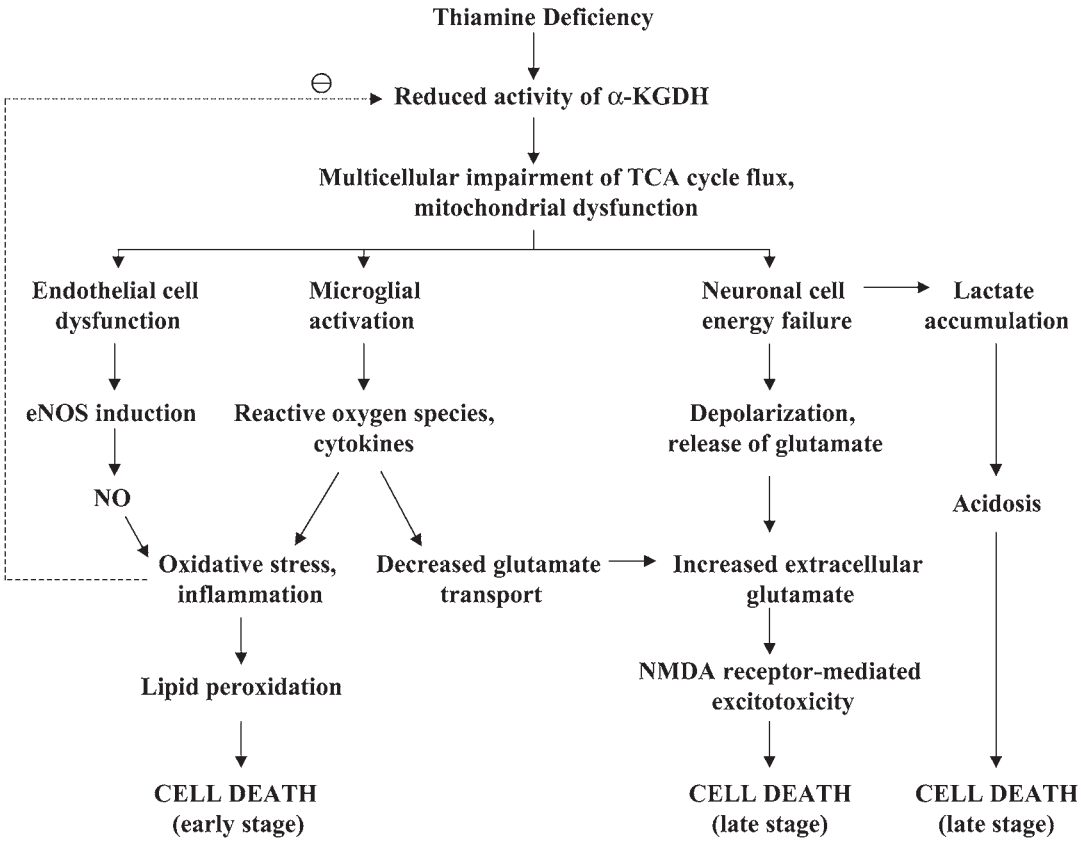

Fig. 4. Schematic representation of potential consequences of impaired α-KGDH and mitochondrial dysfunction in thiamine deficiency.

anisms as shown schematically in Fig. 4. The enzyme most sensitive to thiamine deficiency, α-KGDH, is reduced in activity at early stages, resulting in mitochondrial impairment and reduced tricarboxylic acid cycle flux in endothelial cells, neurons, and microglia. Initially, this metabolic insult results in endothelial cell dysfunction and induction of eNOS, which is accompanied by microglial activation and production of reactive oxygen species and cytokines. Together, these changes lead to oxidative stress and inflammation, lipid peroxidation, and cell death. At late stages of thiamine deficiency, impaired neuronal glucose (pyruvate) oxidation results in impending cellular energy failure, depolarization, glutamate release, and lactate accumulation. Increased extracellular glutamate at late stages leads to hyperstimulation of NMDA receptors and excitotoxic cell death in the thalamus. Increased glutamate in the extracellular space of the brain in thiamine deficiency results not only from depolarization release from synaptic terminals but also from malfunction of astrocytic glutamate transporters owing to the action of reactive oxygen species. Reactive oxygen species are also known to adversely affect α-KGDH activities, leading to a vicious cycle of metabolic impairment and increased oxidative-stress-related brain damage. Other mechanisms involved in cell death in thiamine deficiency include focal lactic acidosis. Apoptotic cell death in thiamine deficiency is limited to vulnerable thalamic structures. The precise cause of the regional selectivity of neuronal cell death as a result of thiamine deficiency remains

poorly understood but could relate to regional cerebral glucose or energy requirements, to regional differences in thiamine turnover rates, or to regional antioxidant capacities. Further studies are required in order to address these issues.

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