

## Thiamin deficiency and brain disorders

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Thiamin plays a key role in the maintenance of brain function. Thiamin diphosphate is cofactor for several enzymes involved in glucose metabolism whereas thiamin triphosphate has distinct properties at the neuronal membrane. Thiamin metabolism in the brain is compartmented between neurons and neighbouring glial cells. Thiamin deficiency is commonly encountered in severe malnutrition associated with chronic alcoholism, HIV–AIDS and gastrointestinal disease where it frequently results in Wernicke's encephalopathy (the Wernicke–Korsakoff syndrome). Wernicke's encephalopathy is severely underdiagnosed according to clinical criteria in both alcoholic and HIV–AIDS patients. Magnetic resonance imaging reveals bilateral ventricular enlargement, mammillary body atrophy and cerebellar degeneration indicative of selective neuronal loss that is characteristic of Wernicke's encephalopathy. Several mechanisms have been proposed to explain this selective loss of neurons including a cerebral energy deficit resulting from reductions in activity of thiamin diphosphate-dependent enzymes, oxidative stress and N-methyl-D-aspartate receptor-mediated excitotoxicity. Both microglia and perivascular endothelial cells are sources of NO and oxidative stress in thiamin deficiency. Decreased activities of thiamin diphosphate-dependent enzymes (in particular  $\alpha$ -ketoglutarate dehydrogenase) have also been reported in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases independent of patient malnutrition. In these cases, decreased activities result from direct toxic actions of oxidative stress and  $\beta$ -amyloid produced as part of the neuronal cell death cascade in these disorders.

**Thiamin deficiency: Wernicke–Korsakoff syndrome: Alcoholism: AIDS: Neuronal cell death**

### Introduction

Thiamin (vitamin B<sub>1</sub>) is a water-soluble vitamin found in appreciable quantities in wholegrain cereals, yeast and some legumes. The thiamin content of these foods is sensitive to pH and to high temperatures. Thiamin deficiency remains an important healthcare issue in several world populations and the causes of thiamin deficiency in these populations include inadequate diets, the prolonged cooking of foods and the ingestion of certain foods containing significant quantities of thiaminases or anti-thiamin compounds. Populations at particularly high risk for the development of thiamin deficiency include pregnant and lactating women and their offspring. Currently, a minimum of 1 mg thiamin/d is recommended for adults with a further 0.4 mg/d during pregnancy to accommodate maternal and fetal growth as well as increased maternal energy intake.

Subsequently, in order to account for the thiamin secretion in milk and the increased energy consumption during lactation, a further increment of 0.5 mg/d is recommended during lactation.

Other populations at risk for the development of thiamin deficiency include chronic alcoholics and patients with HIV–AIDS (Butterworth *et al.* 1991), as well as patients with severe gastrointestinal disorders. Displaced individuals in refugee camps as well as victims of political trade embargos may manifest a high incidence of thiamin deficiency (Butterworth, 2001).

### Thiamin-deficiency disorders

The most common thiamin-deficiency disorders are beriberi and Wernicke's encephalopathy (WE; the Wernicke–Korsakoff syndrome). Beriberi occurs as one of two types.

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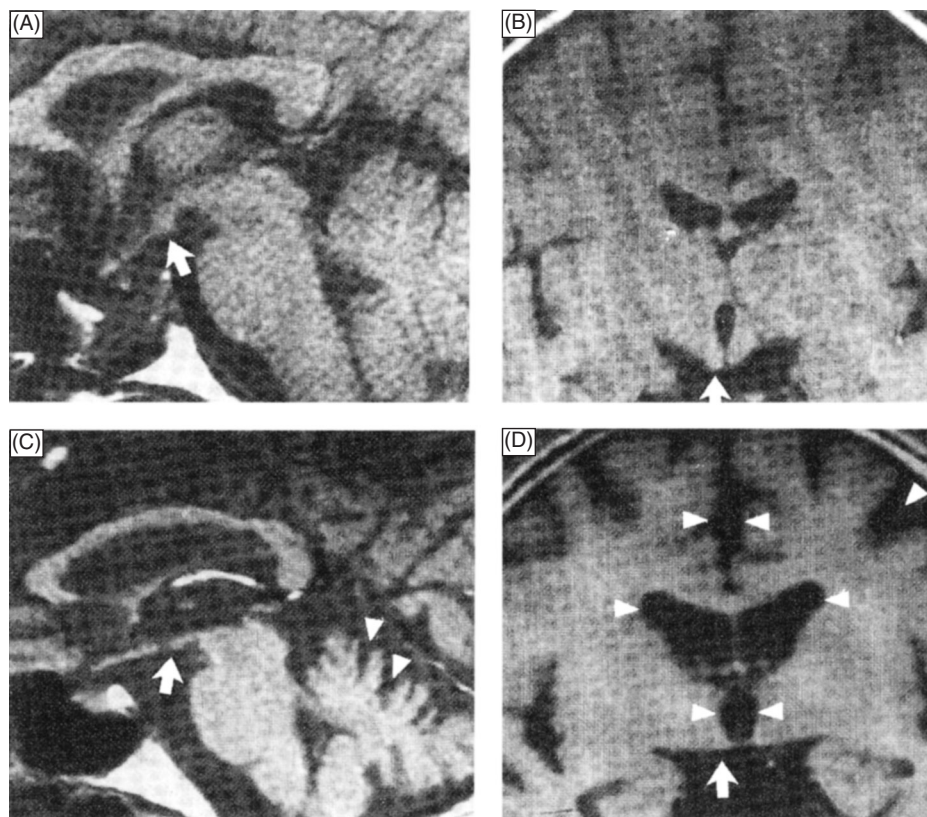
**Abbreviations:** AD, Alzheimer's disease;  $\alpha$ KGDH,  $\alpha$ -ketoglutarate dehydrogenase; TDP, thiamin diphosphate; TMP, thiamin monophosphate; TTP, thiamin triphosphate; WE, Wernicke's encephalopathy.

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Dry beriberi is characterized by peripheral neuropathy consisting of symmetrical impairment of sensory and motor nerve conduction velocities affecting the distal segments of the limbs more seriously than the proximal ones. Wet beriberi, on the other hand, is characterized by oedema, tachycardia, cardiomegaly and congestive heart failure in addition to peripheral neuropathy. Infantile beriberi occurs in the early months of life and, as in adults, may have both cardiac and neurological manifestations. WE occurs in association with chronic alcoholism and HIV–AIDS, as well as with severe gastrointestinal disease. It is occasionally encountered in patients with persistent vomiting. In the case of alcoholics, thiamin deficiency results from a combination of factors including an inadequate dietary intake of the vitamin due to poor nutrition, poor gastrointestinal absorption of thiamin as a consequence of gastric disease and a loss of liver thiamin stores associated with alcoholic liver disease. In addition, there is convincing evidence to demonstrate that ethanol *per se* directly inhibits the transport of thiamin in the gastrointestinal tract. Ethanol also inhibits the phosphorylation of thiamin to its active diphosphate ester, which is required for cellular energy metabolism and synthetic functions.

WE is seriously *underdiagnosed* both in alcoholic and non-alcoholic patients. It has been estimated that in alcoholic patients, the diagnosis of WE is missed in up to 80 % of cases (Harper, 1979). Similarly, a review of the literature describing WE in patients with HIV–AIDS revealed that 80 % of cases had again not been adequately diagnosed clinically during life (Butterworth *et al.* 1991). The principal reason for its consistent underdiagnosis results from the overuse of the classical textbook definition of WE, which requires that a triad of neuropsychiatric symptoms (ophthalmoplegia, ataxia, global confusional state) be present for diagnosis. In practice, it is rare that this triad of symptoms is present; rather, many patients diagnosed subsequently with WE present only with psychomotor slowing or apathy. It is important to suspect thiamin deficiency in all patients with grossly impaired nutritional status associated with chronic alcoholism, HIV–AIDS, gastrointestinal disease or persistent vomiting and to administer thiamin to these patients in a timely manner.

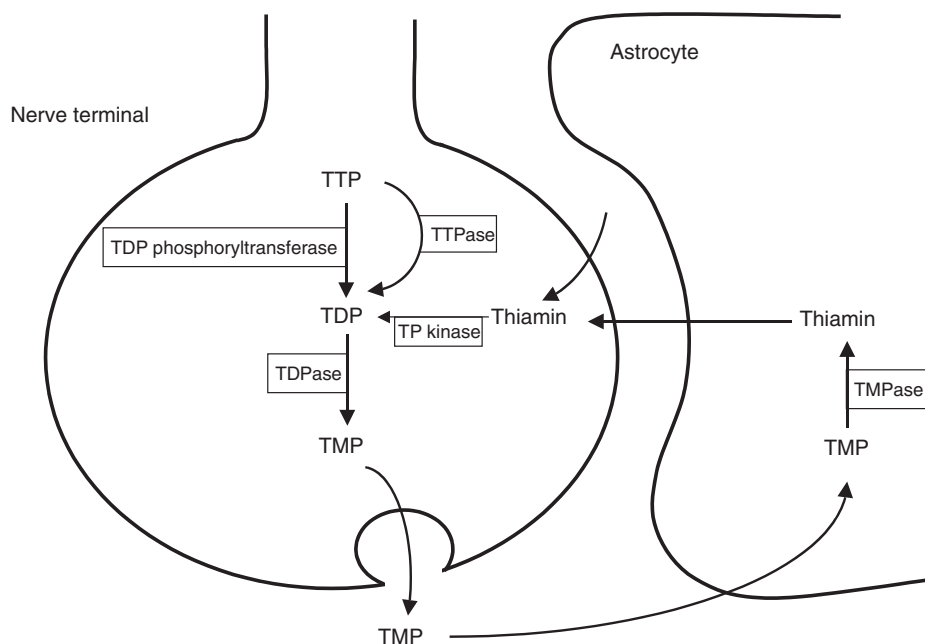
In the meantime, a definitive diagnosis of WE can nowadays be accurately made using magnetic resonance imaging (Charness & DeLaPaz, 1987) as shown in Fig. 1.



**Fig. 1.** Magnetic resonance imaging in a control subject (A and B) compared with a patient with chronic Wernicke's encephalopathy (WE) (C and D). T<sub>1</sub>-weighted images of sagittal (A and C) and coronal (B and D) sections at the level of the mammillary bodies are shown. The WE patient shows shrinkage of the mammillary bodies which is particularly apparent in the coronal section (←). Enlargement of the third and lateral ventricles is also apparent in the WE patient (△) confirming loss of periventricular tissue. Also apparent in the WE patient is cerebellar degeneration consisting of widening of the sulci, in the sagittal section (△). (Modified from Charness & DeLaPaz, 1987.)

### Thiamin and brain cell function

Thiamin taken up into the brain is phosphorylated to thiamin diphosphate (TDP) by the enzyme thiamin pyrophosphokinase. TDP is an essential cofactor for enzymes involved in brain glucose metabolism such as transketolase, pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ KGDH). TDP is then further phosphorylated to thiamin triphosphate (TTP) or is dephosphorylated to thiamin monophosphate (TMP). It is becoming clear that thiamin phosphorylation–dephosphorylation is a compartmented process in the brain. Evidence for this concept is derived from studies of the cellular localization of the thiamin phosphorylating and dephosphorylating enzymes as well as the phosphate esters themselves. Thiamin phosphate esters are significantly more concentrated in neurons compared with other brain cells (Laforenza *et al.* 1988). Moreover, TDPase activities are 20-fold higher in neurons whereas TMPase is expressed primarily by glial cells. In nerve terminals, TTP is rapidly synthesized from TDP by the action of TDP phosphoryltransferase but the TTP ester does not accumulate to high concentrations; rather it is rapidly hydrolysed to TDP by the action of TTPase, an enzyme that is also enriched in nerve terminals. Nerve stimulation results in the release of thiamin, which is mainly in the form of TMP (Cooper & Pincus, 1979). Taken together, these findings suggest that trafficking of thiamin, and TMP occurs in the brain as shown in a simplified schematic manner in Fig. 2.



**Fig. 2.** Proposed trafficking of thiamin and its monophosphate ester (TMP) between nerve terminals and astrocytes in the brain. Thiamin diphosphate (TDP) is an essential cofactor for transketolase, pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase, enzymes involved in brain glucose and energy metabolism. Thiamin triphosphate (TTP), on the other hand, has direct actions on neuronal membranes, particularly at the site of the chloride channel and the acetylcholine receptor. Two neuronal pools of TDP have been proposed, namely a rapid turnover pool associated with TMP and TTP synthesis and a slow turnover pool associated with cellular energy metabolism. TP kinase, thiamin pyrophosphokinase.

Support for this concept is derived from studies in neurological disorders such as Alzheimer's disease (AD), which is associated with significant loss of neurons and a concomitant decrease in concentrations of TDP and TDPase together with increased concentrations of TMP, consistent with reactive gliosis (Rao *et al.* 1993; Héroux *et al.* 1996). Furthermore, both ethanol and the thiamin anti-metabolite pyriethiamin cause inhibition of thiamin pyrophosphokinase and neuronal cell damage and loss.

The precise physiological role of TTP has not yet been determined but it has been proposed that the triphosphate ester activates high-conductance chloride channels (Bettendorff, 1994). TTP also has regulatory properties on proteins involved in the clustering of acetylcholine receptors (Gautam *et al.* 1995), suggestive of a direct role in the regulation of neurotransmission in the brain. Interestingly, rats with a high sensitivity to the central nervous system effects of ethanol express low levels of TTPase (Zimatkina *et al.* 2000). The eventual cloning and characterization of the genes coding for thiamin phosphorylating–dephosphorylating enzymes will no doubt stimulate further research in this interesting area and help to shed light on the precise role of TTP in brain function.

### Neuronal cell death due to thiamin deficiency

Thiamin deficiency in man (WE) is characterized by a selective loss of neurons in the midbrain, thalamus and

cerebellum. It has been proposed that two distinct types of neuropathological damage occur in WE (Torvik, 1985), namely:

neuronal disintegration, mild endothelial swelling and sparing of the neuropil, generally confined to the thalamus and inferior olives;

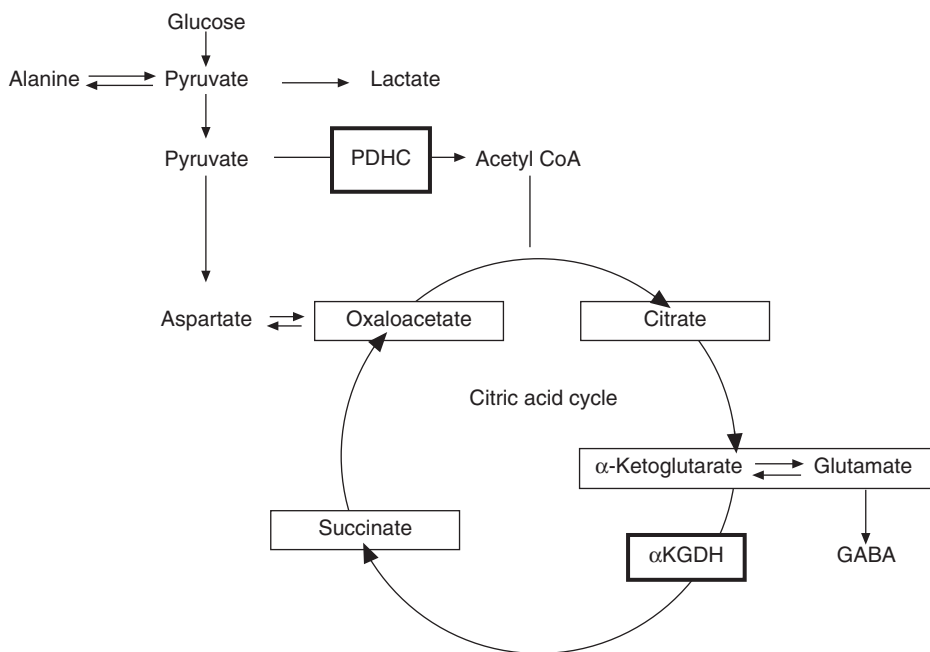
destruction of the neuropil, severe endothelial swelling and neuronal sparing in mammillary bodies and periventricular brainstem nuclei.

Further insights into the nature and cause of these neuropathological features have been (and continue to be) provided by studies in experimental animal models of WE, the most popular and well characterized of which is the rat treated for 12–15 d with the central thiamin antagonist pyrithiamin. Such treatment reproduces both the metabolic and neuropathological features of WE. Using this experimental model, evidence of both apoptosis (Matsushima *et al.* 1997) and necrosis has been described. The vascular endothelium is one of the sites of early changes in the brains of pyrithiamin-treated rats (Gibson & Zhang, 2002) followed by microglial activation (Todd & Butterworth, 1999), breakdown of the blood–brain barrier, neuronal cell death and ensuing proliferation of reactive astrocytes. Several mechanisms have been proposed to explain the phenomenon of selective neuronal cell damage and death due to thiamin deficiency.

*Cellular energy failure*

Both WE in man and experimental thiamin deficiency are characterized by decreases in brain concentrations of TDP and a reduction in activities of TDP-dependent enzymes (Butterworth & Héroux, 1989; Butterworth *et al.* 1993). A great deal of attention has been focused particularly on the role of decreased  $\alpha$ KGDH in the pathogenesis of neuronal cell death due to thiamin deficiency since it is well established that  $\alpha$ KGDH is a rate-limiting enzyme in the citric acid cycle, which is responsible for the maintenance of cellular energy metabolism (Fig. 3).

Prolonged reductions in activity of  $\alpha$ KGDH due to thiamin deficiency result in decreased glucose (pyruvate) oxidation and increased brain concentrations of alanine and lactate. Studies of oxidative metabolism in mitochondria isolated from the brains of thiamin-deficient animals reveal decreased respiration using  $\alpha$ -ketoglutarate as substrate but no such changes in respiration using succinate (Parker *et al.* 1984), findings that are consistent with decreased activities of  $\alpha$ KGDH (Fig. 3). Direct measurements of high-energy phosphates in the brain in experimental thiamin deficiency have been made and decreased levels of ATP described in brainstems of deficient animals (Aikawa *et al.* 1984). Decreased activity of  $\alpha$ KGDH due to thiamin deficiency also results in decreased synthesis of the important amino acid neurotransmitters glutamate and  $\gamma$  amino butyric acid (Butterworth & Héroux, 1989). Increased brain lactate con-



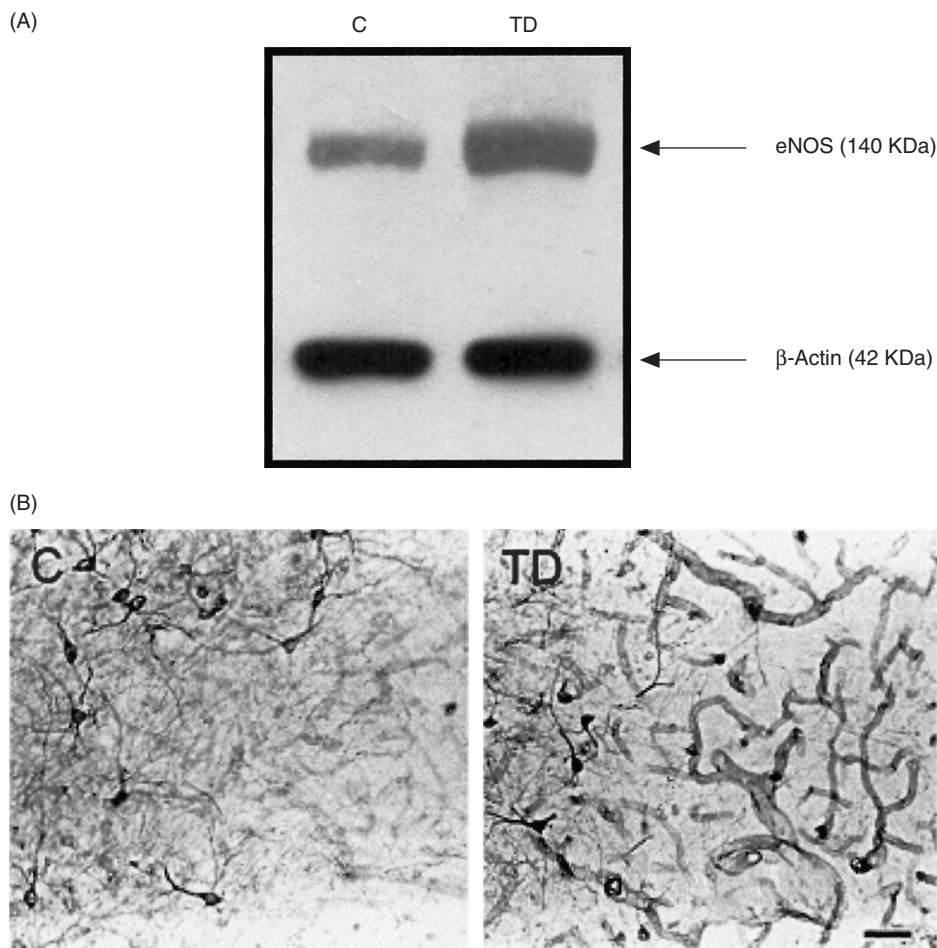
**Fig. 3.** Thiamin diphosphate-dependent enzymes include the pyruvate dehydrogenase complex (PDHC) and  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ KGDH) both of which are implicated in glucose oxidation and in the synthesis of the neurotransmitters glutamate and  $\gamma$  amino butyric acid (GABA). Thiamin deficiency results in decreased  $\alpha$ KGDH, and in lactate and alanine accumulation in brain; the synthesis of glutamate and GABA is concomitantly decreased.

centrations in the brain due to thiamin deficiency were first described by Peters in the 1930s (Peters, 1936). More recently, focal accumulation of lactate leading to a reduction of tissue pH has been described in the brains of thiamin-deficient animals (Hakim, 1984). Moreover, cultured rat cerebellar granule cells exposed to a thiamin-deficient medium manifest decreased synthesis of TDP, decreased activities of  $\alpha$ KGDH, increased lactate production and a concomitant reduction of cellular pH leading to significant cell death (Pannunzio *et al.* 2000). Similarly, exposure of neuroblastoma cells to the thiamin transport inhibitor amprolium results in decreased activities of  $\alpha$ KGDH, uncoupling of mitochondria and disorganization of the cristae, all of which are restored by the addition of either thiamin or succinate (Bettendorff *et al.* 1995). A more prolonged exposure of these cells to amprolium led to energy compromise, depolarization and cell death. Disintegration of mitochondria has also been described in degenerating diencephalic neurons of thiamin-deficient animals (Gibson & Zhang, 2002).

#### *Oxidative and nitrosative stress*

Tau-positive granular and fibrillary inclusions, consistent with oxidative damage, have been described in the brain in WE (Cullen & Halliday, 1995) and increased production of reactive oxygen species has been reported in the brains of pyrithiamin-treated rats (Langlais *et al.* 1997). Other markers of oxidative stress in the brain in experimental thiamin deficiency include microglial activation (Todd & Butterworth, 1999) and increased expression and activity of inducible NO synthase in these cells (Calingasan *et al.* 1998). Induction of inducible NO synthase results in increased nitrotyrosine immunoreactivity in regions of the brain shown ultimately to manifest neuronal cell death; nitrotyrosine is a specific nitration product of peroxynitrite, a highly potent oxidant generated by the reaction of superoxide with NO.

Vascular factors also contribute to oxidative damage to neurons in thiamin deficiency. For example, endothelial NO synthase is increased in thiamin deficiency (Fig. 4) and targeted disruption (knockout) of the endothelial NO synthase



**Fig. 4.** Increased endothelial NO synthase (eNOS) expression in brain in experimental thiamin deficiency. (A), Increased eNOS protein in medial thalamus of a thiamin-deficient (TD) rat compared with control (C). (B), Photomicrograph showing NADPH-diaphorase staining of the inferior colliculus of a thiamin-deficient rat (TD) compared with control (C). Note the increased NADPH-immuno-staining of microvessels in TD indicative of increased eNOS activity (scale bar = 10  $\mu$ m). (From Calingasan *et al.* 1998, with permission.)

gene significantly attenuates the neuronal cell death due to thiamin deficiency in mice (Gibson & Zhang, 2002). Antioxidants are neuroprotective in thiamin deficiency. For example, the cell death that accompanies the exposure of cerebellar granule cells to thiamin deficiency is significantly reduced by vitamin E or butylated hydroxyanisole (Pannunzio *et al.* 2000), both of which are powerful antioxidants. In other studies, neuronal cell loss in the thalamus, inferior colliculus and inferior olive was found to be attenuated by the free radical scavenger L-deprenyl (Todd & Butterworth, 1998a).

Of particular importance to an understanding of cell death mechanisms implicated in the pathogenesis of neuronal cell loss due to thiamin deficiency is the report that TDP-dependent enzymes are themselves susceptible to oxidative stress. Both NO and peroxynitrite inactivate  $\alpha$ KGDH (Park *et al.* 1999). Thiamin deficiency also results in accumulation in the brain of amyloid precursor protein (Calingasan *et al.* 1995) and exposure of isolated brain mitochondria to amyloid precursor protein has been shown to result in decreased activity of  $\alpha$ KGDH and diminished mitochondrial respiration, which were further reduced by the addition of NO (Fig. 3) (Casley *et al.* 2002).

#### *N*-methyl-D-aspartate receptor-mediated excitotoxicity

It has been proposed that the nature of the neuropathological damage due to thiamin deficiency resembles that encountered in excitotoxic brain injury (i.e. brain injury resulting from excessive stimulation of N-methyl-D-aspartate receptors by glutamate, a process known as excitotoxicity and shown to result in excessive accumulation of intracellular Ca leading to the activation of cell death mechanisms). Evidence consistent with a role for excitotoxicity in relation to neuronal cell loss due to thiamin deficiency includes the consistent finding of increased extracellular concentrations of glutamate in brain regions known to be selectively lesioned in thiamin deficiency (Hazell *et al.* 2001) as well as the report that pre-treatment of thiamin-deficient animals with the competitive N-methyl-D-aspartate receptor antagonist MK801 was neuroprotective (Langlais & Mair, 1990). However, a subsequent study found that the neuroprotective effect of MK801 was largely due to the anticonvulsant properties of the drug (Todd & Butterworth, 1998b).

Probably the most plausible explanation for the increases of extracellular brain glutamate in thiamin deficiency relates to the finding of a selective downregulation of astrocytic glutamate transporters in brain structures vulnerable to thiamin deficiency (Hazell *et al.* 2001). Loss of these transporters could be related to the increase of reactive oxygen species and NO (described earlier, p. 281) since such entities are known to lead to dysfunction of these transporters (Trotti *et al.* 1998).

#### Thiamin-dependent enzymes and neurodegenerative diseases

Brain tissue from patients with AD contains decreased concentrations of TDP (Héroux *et al.* 1996) and TDPase activities are reduced by up to 60 % in this material (Rao *et al.*

1993). Furthermore, activities of TDP-dependent enzymes have consistently been found to be decreased in AD brain (Gibson *et al.* 1988; Butterworth & Besnard, 1990) with activities of  $\alpha$ KGDH showing particularly low levels in patients with both genetic and sporadic forms of the disease. However, in patients bearing the epsilon 4 allele of the apolipoprotein E gene (Apo E4), the correlation between  $\alpha$ KGDH activity and clinical dementia rating is 0.7 (Gibson *et al.* 1988). Reduced activities of the pyruvate dehydrogenase complex and transketolase have also been reported in AD (Gibson *et al.* 1988; Butterworth & Besnard, 1990). Amyloid- $\beta$  peptide is an important component of senile plaques in AD. There is increasing evidence to suggest that excess amyloid- $\beta$  peptide production is the cause of AD and a recent study showed that exposure of isolated brain mitochondria to amyloid- $\beta$  peptide caused a significant reduction in activities of both  $\alpha$ KGDH and the pyruvate dehydrogenase complex (Casley *et al.* 2002) suggesting that these changes contribute to the cerebral energy deficit and neuronal cell death in AD. Reduced activities of  $\alpha$ KGDH have also been described in other neurodegenerative diseases including Parkinson's disease (Mizuno *et al.* 1994) and progressive subnuclear palsy (Albers *et al.* 2000). As was the case in AD, it is probable that oxidative stress also plays a role in the pathogenesis of  $\alpha$ KGDH deficiencies in these disorders (Humphries & Szewda, 1998).

In conclusion, WE remains a serious neuropsychiatric complication of chronic alcoholism, HIV-AIDS and severe gastrointestinal disease. The diagnosis of WE is frequently missed by current diagnostic criteria. If untreated, irreversible brain lesions in both thalamic, brainstem and cerebellar structures may occur in WE, the extent of which can be effectively assessed by magnetic resonance imaging. Studies in experimental thiamin deficiency reveal a series of probable mechanisms responsible for thiamin deficiency-related neuronal cell loss. Such mechanisms include cellular energy compromise, lactic acidosis, oxidative and nitrosative stress as well as N-methyl-D-aspartate receptor-mediated excitotoxicity. Decreased activities of thiamin-dependent enzymes have also been reported in the brain in neurodegenerative diseases such as AD and Parkinson's disease, findings that have been attributed to the toxic effects of oxidative stress and  $\beta$ -amyloid deposition rather than systemic thiamin deficiency in these patients.

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