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Sensitivity to thiamine deficiency in cultured human cells is dependent on cell type and is enhanced in cells from thiamine-responsive megaloblastic anemia patients

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To address tissue-specific variation in sensitivity to thiamine deficiency, three human cell types were grown in medium with various thiamine concentrations. The activity of a cytosolic and a mitochondrial thiamine diphosphate-dependent enzyme was examined. Each cell type displayed a unique response to thiamine depletion with respect to α-ketoglutarate dehydrogenase and transketolase activity and to inhibition of cell growth. Loss of α-ketoglutarate dehydrogenase activity was similar in lymphoblasts and fibroblasts, whereas loss of activity in neuroblastoma cells was significantly more resistant to thiamine depletion. Transketolase activity in neuroblastoma cells was only moderately resistant to thiamine depletion, with the activity in fibroblasts being the most and in lymphoblasts the least resistant. Total transketolase activity was 33% higher in fibroblasts than in lymphoblasts and neuroblastoma cells, indicating a differential requirement for production and maintenance of transketolase activity in this cell type. Compared with normal lymphoblasts, those derived from patients with thiamine-responsive megaloblastic anemia were 100 to 1000 times more sensitive to thiamine depletion. Although fibroblasts from these patients also demonstrated a 1000-fold increase in sensitivity with respect to transketolase activity, α-ketoglutarate dehydrogenase activity demonstrated no enhanced sensitivity. The results indicate a complex, cell-type dependent regulation of intracellular pools of thiamine and its phosphorylated derivatives in response to fluctuating extracellular thiamine levels. (J. Nutr. Biochem. 9:215–222, 1998) © *Elsevier Science Inc. 1998*

Keywords: thiamine deficiency; thiamine-responsive megaloblastic anemia; transketolase; α-ketoglutarate dehydrogenase

Introduction

Thiamine deficiency in humans results in severe complications of the central nervous system, which can manifest itself in region-specific damage in brain structures such as the thalamus, midbrain, and brainstem.[1,2] In determining the biochemical basis for neuronal damage, most investigators have focused on three enzymes that use thiamine diphosphate (ThDP) as a cofactor. Transketolase, α-ketoglutarate dehydrogenase (α-KGDH), and pyruvate dehydrogenase complex have all been suggested to play a role in the tissue damage produced when thiamine concentration is limited.[1,3–5] However, there are no definitive explanations for the tissue specific damage that occurs. Although a variety of mechanisms including specific enzymatic reductions, enzymatic alterations, and variable thiamine transporter expression have been proposed,[6–8] no consensus has been achieved as to why certain regions are damaged during thiamine deficiency while other regions remain clinically unaffected.

This work was supported by Grant AA10433 from the National Institute on Alcohol Abuse and Alcoholism and in part by the Vanderbilt Clinical Research Center (CRR-GCRC 5MO1RR00095) and the John F. Kennedy Center for Research on Human Development through a core grant (P30-HD15052) from NICHHD.
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Received July 25, 1997; accepted December 16, 1997.

655 Avenue of the Americas, New York, NY 10010
0955-2863/98/$19.00
PII S0955-2863(97)00187-3

To help address these questions, we used a cell culture system in which cells were grown in the presence of progressively decreasing thiamine concentrations. Neuroblastoma cells were chosen because of their neuronal derivation, while lymphoblasts and fibroblasts served as cell types easily obtainable from various individuals. To examine possible differences in intracellular pools of thiamine, we followed the activities of a cytosolic enzyme, transketolase, and a mitochondrial enzyme, α-KGDH. Additionally, potential effects on cell growth and viability were also examined, as clinically observed tissue damage is presumed to result from cell death.

Cellular uptake of thiamine at physiological concentrations occurs by a saturable, high-affinity transport system.[9,10] The driving force behind uptake in most systems that have been analyzed is the immediate diphosphorylation of intracellular thiamine to ThDP by thiamine diphosphokinase.[10] Thiamine-responsive megaloblastic anemia (TRMA) is a rare disease associated with diabetes mellitus and sensorineural deafness.[11,12] Erythrocytes from TRMA patients lack the saturable, high-affinity component of thiamine transport and have a small but reproducible decrease in thiamine-phosphorylation capability.[12–14] Thus, it was of interest to measure the activities of both transketolase and α-KGDH within TRMA cells under conditions of decreasing thiamine to gain insight into the contribution of the transport system to sensitivity to thiamine deficiency.

Methods and materials

Cell culture

Normal lymphoblasts and fibroblasts were obtained as described.[6,15] The neuroblastoma cells were an SH-SY5Y cell line, a thrice-cloned subline of SK-N-SH.[16] TRMA patients P.F. and P.M.R. have been described.[12–14] All cell types were grown at 37°C in the presence of various concentrations of thiamine-HCl (Sigma Chemical, St. Louis, MO USA) in RPMI 1640 medium without thiamine (Gibco, Gaithersburg, MD USA) supplemented with 20% heat-inactivated fetal calf serum (Gibco), 2 mM L-glutamine (Gibco), and 1 g/L penicillin/streptomycin. The fetal calf serum was dialyzed against four changes of Hanks Balanced Salt Solution (Gibco) at a total volume 24 times that of the serum using dialysis tubing with a molecular weight cut off of 12,000 to 14,000 (Millipore, Bedford, MA USA). Based on the serum concentration of thiamine provided from the supplier, estimated thiamine concentrations in the growth media without any additional thiamine added was 0.1 nM, and this medium was referred to as TDM. Enzyme activity and cell growth were normal when thiamine was added back to TDM, indicating no essential growth factors were removed upon dialysis. The concentration of thiamine, or the presence of pyrithiamine, was the only parameter that was varied and which resulted in alterations in enzyme activity and in cell growth.

Cultures were started by adding about 10^6 cells, grown in control medium, to either 11 mL (lymphoblasts) or 33 mL (fibroblasts and neuroblastomas) of experimental medium. At 7 days in control medium, lymphoblasts (which do not attach) were at mid to late log phase, whereas neuroblastoma cells and fibroblasts had covered about 90% of the plate surface. Cells were harvested at this time as previous work[6] and data not shown indicated that enzyme activity levels had equilibrated. The growth medium was changed once after 3 to 4 days. Effects on cell growth were monitored by counting viable cells stained with Trypan Blue (Gibco).

Enzyme assays

The cells were washed three times with a cold isotonic buffer and lysed by resuspending and vortexing in a lysis buffer containing 20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1 mM potassium EDTA, 0.2 g/L Triton X-100, 0.2 g/L sodium deoxycholate, and 0.2 mM phenylmethylsulfonyl fluoride. The protein concentration of the clarified supernatant was determined using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA USA), and the appropriate amount was added to the assay mixes as described below.

Transketolase activity (EC 2.2.1.1) was determined using the enzyme-linked method[17] under conditions in which coupling enzymes were not limiting.[18] Reactions were initiated by the addition of 0.36 mg total protein per mL of reaction mix to an otherwise complete reaction mix of 100 mM Tris-HCl (pH 7.5), 10 mM ribose 5-phosphate, 2 mM xylulose 5-phosphate, 1.2 mM $MgCl_2$, 0.1 mM NADH, 2000 U/L glycerol-3-phosphate dehydrogenase, and triose phosphate isomerase. Reactions were carried out at 37°C. The oxidation of NADH, which is directly proportional to transketolase activity, was followed by monitoring the decrease in A_{340} using a Beckman DU-70 spectrophotometer (Beckman, Palo Alto, CA USA). Activity was expressed as nanomole per minute per milligram of total protein. One hundred percent transketolase activity (control medium containing 10 μM thiamine) in lymphoblasts and neuroblastomas averaged 16 to 18 nM/min/mg. For fibroblasts, 100% activity was 26–28 nM/min/mg.

α-KGDH activity (EC 1.2.4.2, EC 2.3.1.6, EC 1,6,4,3) was measured as described[19] with minor modifications. Reactions were typically initiated by the addition of 0.36 mg total protein to an otherwise complete reaction mix of 50 mM MOPS (pH 8.0), 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.16 mM coenzyme A, 1 mM α-ketoglutarate, 1 mM NAD, 0.5 g/L Triton X-100, and 0.04 mM rotenone. The reduction of NAD, which is directly proportional to α-KGDH activity, was monitored as for transketolase activity, only at 30°C. Only the initial portions of the progress curves were used to determine activity as the products of the reaction, NADH and succinyl-CoA, are strong inhibitors of α-KGDH.[20] Activity was expressed as nanomoles per minute per milligram of total protein, and 100% activity (control medium containing 10 μM thiamine) averaged of 6–8 nM/min/mg for each cell type examined.

Results

Response to thiamine deficiency in neuroblastoma cells and normal lymphoblasts and fibroblasts

Figure 1 depicts the relative activities of the cytosolic enzyme transketolase (A) and the mitochondrial enzyme α-KGDH (B) in response to thiamine depletion within neuroblastoma cells, lymphoblasts, and fibroblasts. To reduce intracellular thiamine concentrations below those attainable in TDM,[6] increasing concentrations of pyrithiamine were added. Pyrithiamine is a competitive inhibitor of the thiamine transporter as well as thiamine diphosphokinase once it enters the cell.[21–23] The response of transketolase activity to thiamine depletion was distinct for each cell type examined indicating differential sensitivities to thiamine deficiency.

In lymphoblasts, transketolase activity began to decrease at an extracellular thiamine concentration below 10 nM (*Figure 1A*). At 0.1 nM thiamine, the decrease in transke-

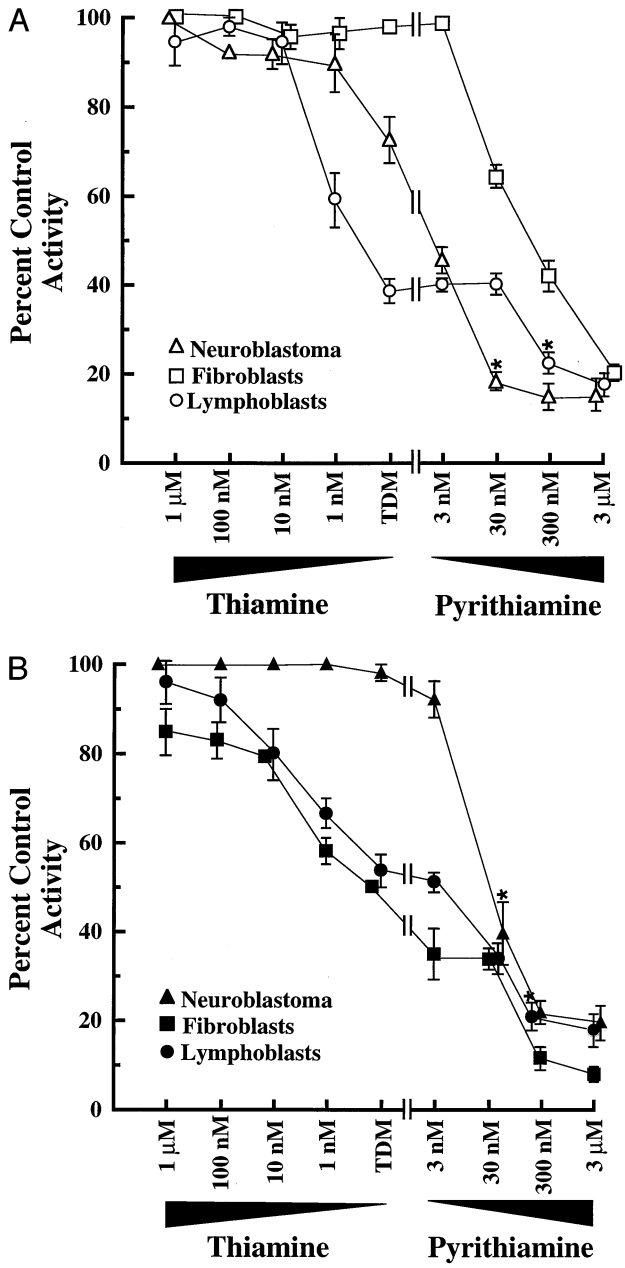

Figure 1 The decrease in activities of transketolase and α-ketoglutarate dehydrogenase (α-KGDH) in human lymphoblasts, fibroblasts, and neuroblastoma cells grown in thiamine deficient media (TDM), to which was added either increasing concentrations of thiamine or pyrithiamine. The data are presented as mean percentages ± SEM of control activity determined when 10 μM thiamine was present; for lymphoblasts $n = 8$ (four different cell lines; two independent experiments were performed on each cell line); for fibroblasts $n = 4$ (two different cell lines; two different experiments were performed on each cell line); for neuroblastoma cells $n = 2$ (one cell line; two independent experiments were performed). The asterisk indicates the medium condition at which an effect on cell growth was first observed; cell growth also was inhibited in all conditions to the right of the asterisk. (A) Transketolase activity for lymphoblasts, fibroblasts, and neuroblastoma cells in response to growth in increasingly thiamine-deficient medium. (B) α-KGDH activity for lymphoblasts, fibroblasts, and neuroblastoma cells in response to growth in increasingly thiamine deficient medium.

tolase activity leveled off at 40% of the control activity. As pyrithiamine was added to further deplete intracellular thiamine levels, the activity remained constant until the pyrithiamine concentration was greater than 30 nM, at which time transketolase activity resumed its decrease. Transketolase activity in the neuroblastoma cells began to decrease at 1 nM extracellular thiamine and continued to decrease until a level of about 15% that of control was reached. Transketolase activity in fibroblasts was the most resistant to thiamine depletion, with a decrease in activity not observed unless substantial pyrithiamine was added to TDM. The total activity (units per mg total cellular protein) for transketolase in cells grown with control medium was 33% higher in fibroblasts than in lymphoblasts and neuroblastoma cells. Thus, the 100% value in fibroblasts represents a greater amount of active transketolase than the concomitant value in the other two cell types.

Alterations in α-KGDH activity also demonstrated a dependency on cell type, but the relative order of sensitivity to thiamine depletion was distinct from that for transketolase (*Figure 1B*). The changes in α-KGDH activity in response to thiamine depletion in fibroblasts and lymphoblasts were very similar. Activity decreased steadily beginning at thiamine levels of 1 μM, well above the normal human plasma levels of 10 to 20 nM,[24] and activity continued to decrease monotonously as thiamine was reduced to 0.1 nM. The decrease in activity continued as pyrithiamine was added in increasing amounts. Activity in the neuroblastoma cells was maintained at control levels under more severely deficient conditions. Neuroblastoma α-KGDH activity showed no decrease even when the extracellular thiamine concentration was reduced to 0.1 nM (TDM), and a reduction in activity required the addition of 30 nM pyrithiamine to TDM.

Potential effects on cell viability were also examined. For lymphoblasts no effect was seen until pyrithiamine was added to give 300 nM. Under these medium conditions, a decrease in the rate of growth of the lymphoblasts was observed by 5 days (*Figure 2*). Higher pyrithiamine concentrations resulted in a greater inhibition of growth. This effect was presumed to be due to the further reduction of intracellular thiamine brought about by pyrithiamine, as pyrithiamine in concentrations as high as 200 μM had no effect on lymphoblast growth as long as high levels of thiamine (10 μM) were present.[6] The effect on cell growth occurred under medium conditions that resulted in reductions of both transketolase and α-KGDH activities to below 20% of their control activities.

Cell growth was inhibited in neuroblastoma cells under less severe thiamine depletion (TDM plus 30 nM pyrithiamine; data not shown). At the medium conditions under which inhibition of neuroblastoma cell growth were first observed, α-KGDH activity was reduced to about 40% of control, whereas transketolase was reduced to less than 20% of control. In contrast to thiamine depletion altering cell viability for lymphoblasts and neuroblastoma cells, an inhibition of growth was not observed for fibroblasts even when up to 150 μM pyrithiamine was added to TDM (data not shown). Under these conditions, no α-KGDH activity could be detected yet transketolase activity remained at 20 to 25% of control.

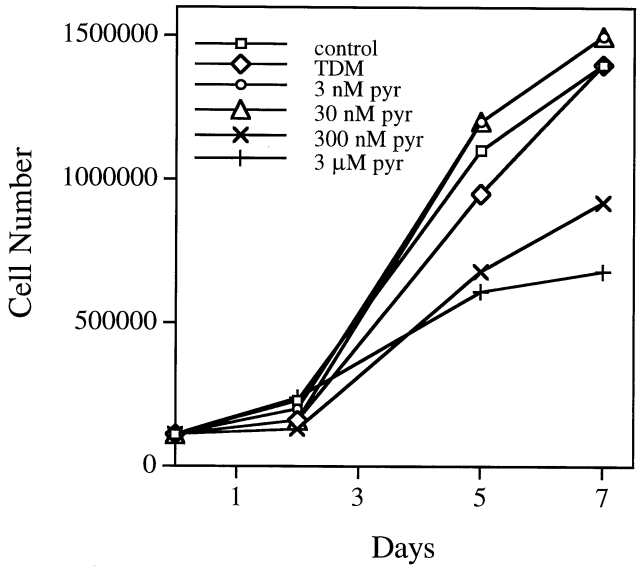

Figure 2 Cell count at various times during growth of normal lymphoblasts in thiamine deficient medium (TDM) with 10 μM added thiamine or varying amounts of added pyrithiamine. Trypan blue was used to indicate cell viability, and in all instances greater than 95% of all the cells were viable. Two independent determinations were performed at each medium condition for a single cell line.

Response to thiamine deficiency in lymphoblasts derived from patients with thiamine-responsive megaloblastic anemia

Figure 3 shows the transketolase and α-KGDH activity in response to thiamine depletion in lymphoblasts derived from patients with thiamine-responsive megaloblastic anemia compared with the activity in lymphoblasts from normal individuals. Transketolase activity in the TRMA lymphoblasts is reported as a percent of control; however, maximum activity (100%) of both enzymes for the TRMA cells was obtained in medium containing 1 mM thiamine instead of 10 μM as for normal lymphoblasts. Nonetheless the total activity of both enzymes was the same for the TRMA and normal cells grown in the respective control media, and thus the 100% values represent the same enzyme levels.

Transketolase activity in TRMA lymphoblast cell lines was about three orders of magnitude more sensitive to thiamine depletion than it was in normal lymphoblasts (*Figure 3A*). For TRMA lymphoblasts, the thiamine concentration corresponding to that normally found in human plasma (10 to 20 nM) was able to support a transketolase activity of only 10% of control. Activity in normal lymphoblasts was barely affected (94% of control) at this concentration of thiamine. α-KGDH activity in TRMA lymphoblasts (*Figure 3B*) was also more sensitive to thiamine depletion, but not to the extent found for transketolase. At normal plasma thiamine concentrations, α-KGDH was reduced to about 40% of control activity, whereas 80% of control activity was observed in normal lymphoblasts.

For TRMA lymphoblasts, enhanced sensitivity to thiamine depletion was also manifest by effects on cell growth. Growth was affected even at an extracellular thiamine

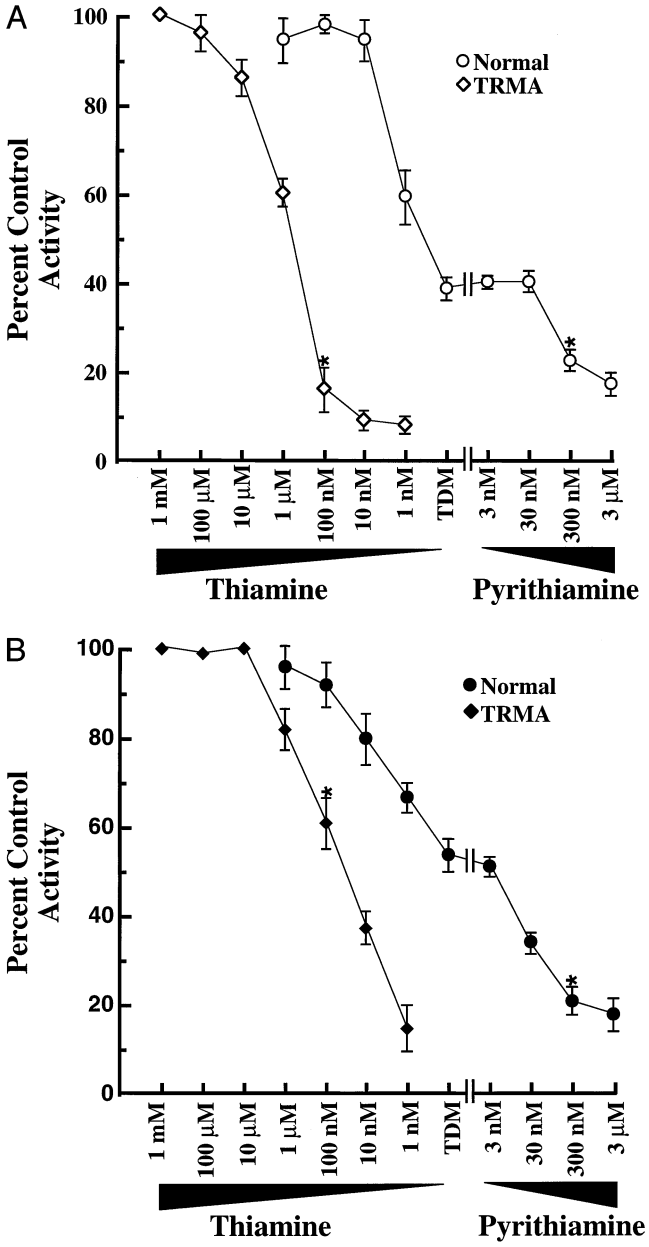

Figure 3 The decrease in activities of transketolase and α-ketoglutarate dehydrogenase (α-KGDH) in normal human lymphoblasts and in lymphoblasts derived from patients with thiamine responsive megaloblastic anemia (TRMA) grown in a thiamine-deficient media (TDM), to which was added either increasing concentrations of thiamine or pyrithiamine. The data are presented as mean percentages ± SEM of control activity determined when either 10 μM thiamine (for normal lymphoblasts) or 1 mM thiamine (for TRMA lymphoblasts) was present; for normal lymphoblasts $n = 8$ (four different cell lines; two independent experiments were performed on each cell line); for TRMA lymphoblasts $n = 4$ (two different cell lines; two different experiments were performed on each cell line). The asterisk indicates the medium condition at which an effect on cell growth was first observed; cell growth also was inhibited in all conditions to the right of the asterisk. (A) Transketolase activity for normal and TRMA lymphoblasts in response to growth in increasingly thiamine deficient medium. (B) α-KGDH activity for normal and TRMA lymphoblasts in response to growth in increasingly thiamine-deficient medium.

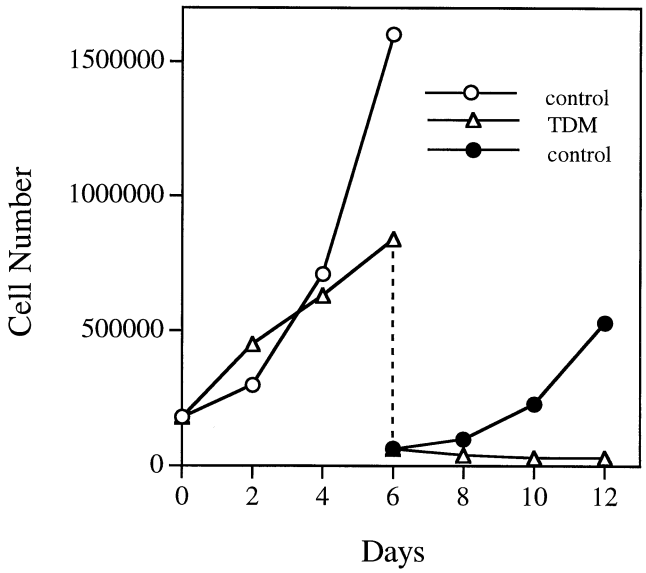

Figure 4 Cell count for TRMA lymphoblasts at various times during growth in thiamine deficient medium (TDM) containing 1 mM (control) or no added thiamine. After 6 days, cells growing in the TDM media were transferred to fresh TDM containing 1 mM (control) or no added thiamine, at 1×10^5 cells per mL, and were allowed to grow for 6 more days. Trypan blue was used to indicate cell viability, and in all instances greater than 95% of all the cells were viable. Two independent determinations were carried out at each condition for a single cell line.

concentration of 100 nM, a concentration 5 to 10 times greater than the normal concentration of thiamine in human plasma. Inhibition of growth of normal lymphoblasts required a further depletion of extracellular thiamine of at least 1000 times that which affected TRMA lymphoblasts. Nonetheless as was true for the normal cells, growth was inhibited only when thiamine depletion resulted in a drop in transketolase activity below 20%. *Figure 4* illustrates the growth behavior of TRMA lymphoblasts in control and TDM media. After 6 days, growth ceased in TDM medium, even though the cells remained viable at this point as demonstrated by transfer to control medium after 6 days. At 10 to 12 days, cell numbers began to decrease and by 16 days less than 2% of the cells remained (data not shown; medium was changed every 4 to 6 days). The initial decrease in growth rate was accompanied by rapid acidification of the medium, as also was found when thiamine depletion began inhibiting growth of normal lymphoblasts and neuroblastoma cells. However, when growth had ceased and the cells were transferred to fresh thiamine depleted medium, further acidification was not apparent. Thus, whereas acidification is a likely consequence of thiamine deficiency[25] and may have contributed to the effect on cell growth, it was not the primary cause of growth inhibition.

Response to thiamine deficiency in fibroblasts derived from patients with thiamine-responsive megaloblastic anemia

Transketolase activity in TRMA fibroblasts as compared with normal fibroblasts also was approximately 1,000 times more sensitive to thiamine depletion through 0.1 nM thiamine (TDM) (*Figure 5A*). Upon addition of increasing pyrithiamine to TDM, no further decrease in transketolase activity occurred, as might be expected, because the initial site of pyrithiamine action is the high-affinity thiamine

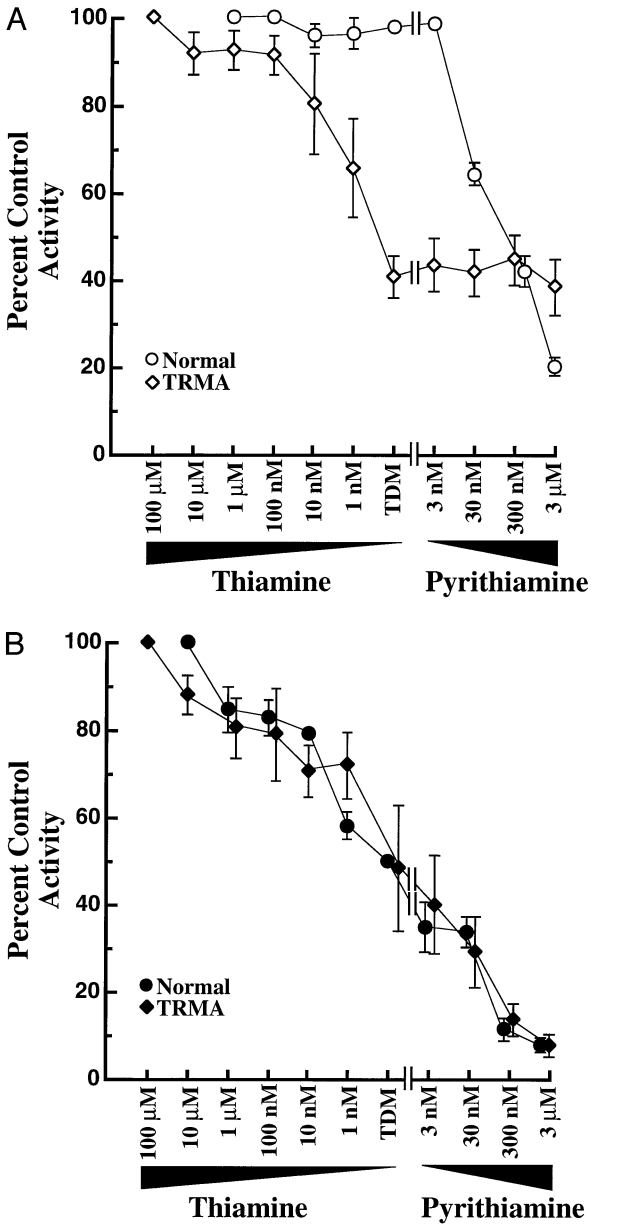

Figure 5 The decrease in activities of transketolase and α-ketoglutarate dehydrogenase (α-KGDH) in normal human fibroblasts and in fibroblasts derived from patients with thiamine responsive megaloblastic anemia (TRMA) grown in a thiamine-deficient media (TDM), to which was added either increasing concentrations of thiamine or pyrithiamine. The data are presented as mean percentages ± SEM of control activity determined when either 10 μM thiamine (for normal fibroblasts) or 1 mM thiamine was present (for TRMA fibroblasts); for normal fibroblasts $n = 4$ (two different cell lines; two independent experiments were performed on each cell line); for TRMA fibroblasts $n = 4$ (two different cell lines; two different experiments were performed on each cell line). (A) Transketolase activity for normal and TRMA fibroblasts in response to growth in increasingly thiamine-deficient medium. (B) α-KGDH activity for normal and TRMA fibroblasts in response to growth in increasingly thiamine-deficient medium.

transporter that is defective in TRMA cells. Also, growth out to 14 days as opposed to the standard 7 days revealed no further decrease in activity. In contrast to the response by transketolase, the decrease in α-KGDH activity in both normal and TRMA fibroblasts was essentially identical at each thiamine concentration (*Figure 5B*). Somewhat surprisingly, α-KGDH activity of TRMA fibroblasts continued to decrease in line with that of normal fibroblasts even when pyrithiamine additions were made. This indicates that at least some pyrithiamine was able to enter the cell in the absence of the high-affinity thiamine transport system. Thiamine depletion did not affect growth of TRMA fibroblasts, consistent with the fact that transketolase activity could not be reduced below 35 to 40% in these cells.

Discussion

To examine cell type-specific susceptibility to thiamine deficiency, three different cell types were grown under identical conditions and with a progressive depletion of medium and intracellular thiamine. The resulting effects on the activities of a cytosolic ThDP-dependent enzyme, transketolase, and a mitochondrial enzyme, α-KGDH, were monitored along with potential effects on cell growth. Differential sensitivities to thiamine deficiency were manifest in two ways. Within a given cell type, the activities of the two enzymes responded differently. Additionally, the response of transketolase and α-KGDH activities to thiamine depletion was different among the three cell types, with the relative order of cell-type sensitivity being different for the two enzymes.

Several explanations may account for the different behavior of the two enzymes in response to increasing depletion of available thiamine. In vitro, it is more difficult to remove ThDP from holo-transketolase than holo-α-KGDH. However, as transketolase has a very short half-life in lymphoblasts[6] and as the in vivo rate of cofactor loss is not known for either enzyme, it is unclear if this may contribute to differential loss of activity in response to extracellular thiamine depletion. Another explanation, elaborated on below, may relate to the different cellular localization of the two enzymes.

There was a distinct difference in the sensitivity to thiamine depletion among the three cell types. Transketolase activity in fibroblasts was maintained at control levels under much more severe thiamine depletion than that required to reduce activity in lymphoblasts or neuroblastoma cells. In contrast, fibroblast α-KGDH activity decreased in the same manner as did activity in lymphoblasts, yet neuroblastoma cells were highly resistant to loss of α-KGDH activity.

In comparing fibroblasts and lymphoblasts, the present findings represent the third difference that we have seen between these two cell types concerning transketolase. Not only was fibroblast transketolase less sensitive to thiamine depletion, the total activity expressed as units per mg total cellular protein was 33% higher than lymphoblast transketolase activity. Additionally, we previously found that, in vitro, fibroblast transketolase formed a functional holoenzyme much more readily than lymphoblast transketolase.[15] Together, these results suggest that fibroblasts have a specific requirement for the production and maintenance of a higher level of transketolase activity than the level of activity needed in lymphoblasts. It is of interest to note that adipocytes are among the cell types that fibroblasts can differentiate into in vivo. The pentose phosphate pathway, of which transketolase is a part, is highly active in adipose tissue, presumably because large amounts of NADPH are required for the reductive biosynthesis of fatty acids from acetyl CoA.[20] This may account, at least in part, for the high level and maintenance of transketolase activity in fibroblasts. In this regard, the areas of the brain that are heavily damaged during the progression of Wernicke's encephalopathy, a disease resulting from thiamine deficiency, are areas high in white matter.[26] White matter contains myelinated axons whose high lipid content gives the white matter its appearance, and these areas have been shown to possess higher transketolase activity than other regions of the human brain.[27]

Neuroblastoma cells were also unique in their response to depletion of thiamine. Transketolase activity responded moderately to the thiamine depletion, being more sensitive than fibroblast transketolase but less sensitive, at least initially, than lymphoblast transketolase. However, the most striking aspect of the response to thiamine depletion in neuroblastoma cells was that α-KGDH activity was over three orders of magnitude more resistant than it was in either fibroblasts or lymphoblasts. One possible explanation for this observation is that α-KGDH activity is required for the production of glucose-derived neurotransmitters.[28] Thus, maintenance of this enzyme may be an important metabolic requirement for cells of neuronal origin. Consonant with these findings in culture, Lavoie and Butterworth[29] recently found that thiamine deficiency in alcoholics without Wernicke's encephalopathy had region specific decreases in brain transketolase activity from 18 to 33%, yet no decreases in α-KGDH activity.

Transketolase and α-KGDH are localized to different intracellular compartments. The fact that the differences in response to thiamine deficiency of the two enzymes was very different in all three cell types examined suggests the existence of a complex, cell-type-dependent regulation of intracellular pools of ThDP in response to fluctuating extracellular thiamine levels. Such compartmentalization and regulation of the distribution of ThDP between the pools is consistent with recent work on the intracellular distribution and homeostasis of thiamine in rat neuroblastoma cells[10,30] and with findings of regional-specific loss of ThDP-requiring enzymes in the brains of alcoholics at risk for thiamine deficiency.[8,29] Differential distribution of intracellular ThDP would be brought about by regulation of intracellular transport and compartmental uptake of thiamine. Our findings of a reversal of which enzyme is most sensitivity to thiamine depletion in TRMA cells as compared with normal cells supports these suggestions. The distribution of ThDP between the different pools would vary among cell types as thiamine becomes limiting, presumably based on the metabolic needs of the particular cell type. Our interpretation of the data assumes that the levels of enzyme activity reflect the compartmental ThDP concentrations, an assumption strongly supported by the work of Bettendorff which demonstrates that the ThDP cofactor

enters a high turnover pool and is rapidly hydrolyzed when unbound to enzymes.[10] Similar regulation of the intracellular availability of another water soluble vitamin, riboflavin, has been documented for which changes in the cellular distribution of FMN and FAD occur in a cell-type-dependent manner during riboflavin deficiency.[31]

The importance of the high affinity thiamine uptake system in maintaining the activities of ThDP-requiring enzymes as well as cell viability was demonstrated by using cells derived from TRMA patients. Erythrocytes from these individuals have previously been shown to be devoid of any high affinity thiamine transport and to have only 70% of the thiamine diphosphokinase activity of normal erythrocytes.[12–14] We find that lymphoblasts from TRMA patients are more sensitive to thiamine depletion than normal lymphoblasts. Both transketolase and α-KGDH activities decreased, respectively, at 1,000-fold and 100-fold higher concentrations of thiamine in the medium. At normal human plasma thiamine concentrations, enzyme activity was severely depressed, with transketolase activity at 5 to 10% of control and α-KGDH activity at 40 to 50% of control. Growth of TRMA lymphoblasts was impaired at thiamine concentrations 5 to 10 fold higher than normal plasma levels.

Fibroblasts from the TRMA patients were also more sensitive to thiamine deficiency with respect to transketolase activity, with reductions in activity occurring at thiamine concentrations 1,000-fold higher than that required to reduce activity in normal fibroblasts. Yet even with the three orders of magnitude increase in sensitivity, transketolase activity in TRMA fibroblasts was still as resistant to thiamine depletion as that in normal lymphoblasts. Thus, the maintenance of the relatively high level of transketolase activity in normal fibroblasts involves not only the high-affinity transporter but also another mechanism which is independent of the transporter that is defective in TRMA cells. Possibilities include the existence of another transport mechanism in fibroblasts, a relatively slower turnover or loss of cytosolic thiamine from fibroblasts,[10] or a pool of stored thiamine in fibroblasts that can be drawn upon as extracellular thiamine decreases.[32]

α-KGDH activity unexpectedly demonstrated no difference in sensitivity between normal and TRMA fibroblasts over the entire range of thiamine depletion, including the addition of increasing pyrithiamine concentrations to TDM. The reason for this is unclear, but may have to do with the fact that TRMA cells have been shown to have a decreased cytosolic thiamine pyrophosphokinase activity.[12,13] It is likely that mitochondrial uptake of thiamine[33] remains unaffected in TRMA cells and results in a preferential accumulation, at the expense of the cytosolic thiamine pool, of the thiamine that diffuses into the cell or is generated by turnover of the cytosolic ThDP.

Such an interpretation is consistent with our findings that increasing concentrations of pyrithiamine added to TDM resulted in a continued decrease in α-KGDH activity but did not result in further decreases of transketolase activity. The amounts of pyrithiamine which might enter the cell in the absence of the high affinity, plasma membrane thiamine transport system would in all likelihood be insufficient to significantly inhibit cytosolic thiamine diphosphokinase (K_i in the micromolar range[34,35]). If so, pyrithiamine would be expected to have little or no effect on the cytosolic pool of ThDP in TRMA cells and thus on transketolase activity. In contrast, assuming mitochondrial uptake of thiamine is as sensitive to inhibition by pyrithiamine as all other thiamine transport systems which have been examined (K_i in the nanomolar range[9,30,36]), it is likely that the intracellular pyrithiamine concentrations reached here could significantly inhibit mitochondrial thiamine uptake. This would lead to further reductions in α-KGDH activity as pyrithiamine concentrations were increased.

Finally, we found that thiamine deficiency in culture could impair cell growth and viability in two of the three cell types examined. The extent of depletion of the medium and/or the intracellular levels of thiamine which were required to inhibit cell growth varied for each cell type, with TRMA lymphoblasts being the most sensitive. In all cases, cell growth was inhibited when transketolase activity fell to about 20% or below of its control activity, and no effect on growth was seen when transketolase activity was above this value. In contrast, α-KGDH activity was relatively high (40% for neuroblastoma) or undetectable (TRMA fibroblasts) at thiamine levels that affected growth. Although the effect on growth seemed to be related to a drop in transketolase below 20% of control, it is not possible to draw firm conclusions on cause and effect as other ThDP-dependent enzymes were not examined.

Acknowledgments

This work was supported by Grant AA10433 from the National Institute on Alcohol Abuse and Alcoholism and in part by the Vanderbilt Clinical Research Center (CRR-GCRC 5MO1RR00095) and the John F. Kennedy Center for Research on Human Development through a core grant (P30-HD15052) from NICHHD.

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