Activities of Thiamine-Dependent Enzymes in Two Experimental Models of Thiamine Deficiency Encephalopathy: 3. Transketolase

Jean-François Giguère¹ and Roger F. Butterworth¹,²

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Chronic thiamine deprivation in the rat leads to ataxia, loss of righting reflex and neuropathological damage to lateral vestibular nucleus. Before onset of neurological symptoms, transketolase (TK) activities were found to be selectively reduced by 25% in lateral vestibular nucleus and surrounding pons. Further progression of thiamine deprivation resulted in a generalized reduction in TK activity. Measurement of enzyme activity in the presence of added TPP cofactor in vitro did not lead to normalisation of enzyme activities suggesting loss of apoenzyme. Administration of thiamine to symptomatic thiamine-deprived rats resulted in reversal of neurological symptoms and to normalisation of defective TK activities in less vulnerable structures such as cerebral cortex, striatum and hippocampus; reduction of TK activity, however, persisted in brainstem and cerebellar regions. Pyrithiamine treatment results, within 3 weeks, in loss of righting reflex, convulsions and more widespread neuropathological damage compared to that observed following thiamine deprivation. TK activity was found to be significantly decreased before the onset of neurological symptoms in all brain regions and appearance of symptoms was accompanied by more severe reductions of TK. In contrast to chronic thiamine deprivation, TK activities following pyrithiamine treatment were: (i) equally reduced in magnitude in vulnerable and non-vulnerable brain structures, (ii) unchanged following reversal of neurological abnormalities by thiamine administration.

KEY WORDS: Thiamine deficiency; thiamine deprivation; pyrithiamine; thiamine pyrophosphate; transketolase; Wernicke’s encephalopathy.

INTRODUCTION

Thiamine deficiency in man and experimental animals results in characteristic neurological symptoms, most of which are reversed by thiamine administration. This had led to the general acceptance of the concept of ‘‘the biochemical lesion’’ first postulated by Peters in the 1930’s to describe biochemical findings in thiamine-deprived pigeons (1). Since that time, many studies have attempted to define the biochemical lesion in thiamine-deficiency encephalopathy. Unfortunately, while such studies have achieved a great deal in better defining the role of thiamine in cerebral function, a consensus on the nature of the biochemical lesion has so far eluded us. One reason for this lack of consensus is, undoubtedly the lack of uniformity of the experimental approach to the problem. Not only have various species of experimental animal been used; in ad-
dation, animals were sacrificed at different stages of thiamine deficiency and essential control groups of animals “pair fed” to deficient groups were often omitted. In addition, while some investigators made use of thiamine-deficient diets to induce neurological signs of deficiency, others administered the central thiamine antagonist pyrithiamine. Earlier reports suggested that the deficiency state induced by pyrithiamine resembled that caused by dietary deprivation of the vitamin. More recent studies, however, have revealed substantial differences of a neurochemical nature between thiamine deficiency caused by these two approaches. Examples of such neurochemical differences include altered metabolism of glucose (2), amino acids (3) and acetylcholine (4). The basis for such differences has not been clearly established.

The study now described is part of an ongoing study to attempt to elucidate the metabolic basis for the regional vulnerability of certain brain structures to lack of thiamine. Three important enzyme systems of carbohydrate metabolism are known to be thiamine dependent, namely the pyruvate dehydrogenase complex (EC 1.2.4.1), α-ketoglutarate dehydrogenase (αKGDH, EC 1.2.4.2.) and transketolase (EC 2.2.1.1). In the study now described, transketolase activity was measured in the brain of thiamine-deficient rats. Pair fed control groups of animals were included where appropriate and TK activities were measured as follows:

(i) in groups of presymptomatic, symptomatic and thiamine-treated (reversed) animals
(ii) in 9 discrete brain regions, including structures known ultimately to show signs of neuropathological damage
(iii) in the absence and presence of added thiamine pyrophosphate (TPP) cofactor in vitro.

Results obtained from rats made thiamine deficient by dietary deprivation were compared to those obtained following treatment with pyrithiamine.

**EXPERIMENTAL PROCEDURE**

**Experimental Animals, Treatment Groups.** Adult, male, Sprague-Dawley rats weighing 150–200 g. were used for the experiments described. All animals were housed individually in animal quarters under constant conditions of temperature, humidity and light cycles. The following treatment groups were used for the studies described:

1. **Chronically Thiamine-Deprived (TD) Rats.** Rats were fed a thiamine-deficient diet (ICN, Nutritional biochemicals, Cleveland, Ohio) ad libitum.

2. **Pair Fed Controls for TD Group.** Rats were fed a thiamine-enriched diet (Vitamin B Complex Test Diet, ICN Nutritional Biochemicals) pair fed to equal food consumption to that of rats in group 1. Weights of animals were recorded and feeding schedules adjusted daily.

3. **Pyrithiamine-Treated (PT) Rats.** Rats were fed a thiamine-deficient diet and administered pyrithiamine s.c. (10 µg in 0.2 ml saline per 100 g. body weight per day) as previously described (5).

4. **Pair Fed Controls for PT Group.** Rats were fed a thiamine deficient diet pair fed to equal food consumption to that of rats in group 3. In addition, all animals received thiamine s.c. (10 µg per 100 g. body weight per day).

**Neurological assessment**

Analysis of righting reflex was evaluated daily (starting from day 8 in pyrithiamine-treated rats and from day 15 in thiaminedeprived rats) as previously described by us (6). Neurochemical measurements were performed at three stages during the treatments described:

(a) **Presymptomatic Stage** at which rats showed no evidence of neurological abnormalities of gait or righting reflex. Studies were performed on day 15 in the case of thiamine-deprived rats and day 10 in the case of pyrithiamine-treated rats.

(b) **Symptomatic Stage,** defined in the present study as the day upon which an animal from either the TD or PT group first lost its righting reflex. This stage appeared between days 40 and 50 in the case of TD rats and after day 18 in the case of PT rats.

(c) **Thiamine-Reversed Stage.** Thiamine-deprived or pyrithiamine-treated rats were fed, on the day each rat first lost its righting reflex, a thiamine-supplemented diet (Vitamin B Complex Test Diet) in addition to being administered thiamine S.C. (1 mg per 100 g. body weight per day) for 3 consecutive days. Thiamine-reversed animals so treated were found to have normal righting reflexes in all cases.

**Sacrifice-Dissection Technique.** Presymptomatic, symptomatic and thiamine-reversed rats from either deprived or pyrithiamine-treated groups were sacrificed by decapitation along with their appropriate pair fed controls. Brains were rapidly removed on ice, pons-medulla and cerebellum were dissected from remainder of brain and the latter was then dissected according to the parameters of Glowinski and Iversen (7) to afford the following brain regions: cerebral cortex, hippocampus, striatum, midbrain, hypothalamus. Pons-medulla was then sliced in a freezing microtome at –10°C into serial 0.5 mm sections. Lateral vestibular nucleus was microdissected using a 0.5 mm micropunch under a dissecting microscope, according to the parameters described in the atlas of Paxinos and Watson (8).

**Measurement of Transketolase in Rat Brain Regions.** Transketolase activity was measured by a modification of the technique of Dreyfus and Moniz (20) as follows: weighed tissue was homogenised in 50 vol. glycylglycine buffer 40 mM, pH 7.6. 0.5 ml of homogenate was added to 0.3 ml ribose-5-phosphate, 9 mM and the mixture was incubated at 37.5°C, 30 min. Incubations were carried out either in the presence or absence of TPP 9 µM. The reaction was stopped by the addition of 0.4 ml trichloroacetic