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Regional Activation of L-Type Voltage-Sensitive Calcium Channels in Experimental Thiamine Deficiency

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During pyrithiamine-induced thiamine deficiency (PTD), specific regions of the brain develop histological damage. The basis of this selective vulnerability is unknown but the mechanism may involve a glutamate-mediated excitotoxic process in affected structures, leading to alterations in membrane potential and disturbances in calcium homeostasis. In this study, we have examined the volume of distribution of [^{3}H]nimodipine, an L-type voltage-sensitive calcium channel (VSCC) antagonist, in the brain of the PTD rat. An increase in specific binding of [^{3}H]nimodipine was detected only in the posterior thalamus at the symptomatic stage, immediately following the loss of righting reflexes ($P < 0.0001$). There was also an increase in nonspecific binding in the medial geniculate and inferior colliculi. Replenishment with thiamine at the symptomatic stage returned [^{3}H]nimodipine binding to normal levels. These findings provide evidence that depolarization and activation of L-type VSCCs occur in the posterior thalamus and may contribute to the appearance of histological lesions in this structure during experimental thiamine deficiency. J. Neurosci. Res. 52:742–749, 1998.

Key words: glutamate; calcium channels; dihydropyridine; cerebral vulnerability; autoradiography

INTRODUCTION

Experimental thiamine deficiency in the rat evolves gradually into a metabolic disorder. The deficiency is characterized in its advanced stages by the development of histological lesions which are symmetrical in distribution, and occur in a limited number of brain structures which include the thalamus, mamillary bodies, medial geniculate, and inferior colliculi (Troncoso et al., 1981).

Recently, extracellular glutamate concentration was shown to be increased in focal regions of the thiamine-deficient brain that subsequently develop neuropathological damage (Hazell et al., 1993; Langlais and Zhang, 1993). Also, the noncompetitive glutamate receptor antagonist MK-801 was shown to be effective in attenuating the rise in extracellular glutamate and reducing the degree of histological damage occurring in experimental thiamine deficiency (Langlais and Mair, 1990; Langlais and Zhang, 1993; Robinson and Mair, 1992). This suggests that *N*-methyl-D-aspartate (NMDA) receptors may be involved in the pathophysiology of this disorder. Increased extracellular glutamate may lead to membrane depolarization, opening of NMDA receptor-operated calcium channels, and activation of voltage-sensitive calcium channels (VSCCs). Resulting entry of calcium into the cell along a steep electrochemical gradient is a major step in the induction of excitotoxic cell death. Such a mechanism may also form the basis for the regionally selective lesions that develop in pyrithiamine-induced thiamine deficiency (PTD).

Studies have shown that nimodipine, a member of the 1,4-dihydropyridine (DHP) class of L-type VSCC antagonists, binds specifically to ischemic brain regions vulnerable to infarction (Hakim and Hogan, 1991). The binding of DHP calcium channel antagonists is known to be reversible, saturable, and specific in nature (Bellemann et al., 1982, 1983; Gould et al., 1982). It occurs in a state-dependent manner, with depolarization of the cell membrane resulting in greatly increased affinity of the VSCC for DHPs (Bean, 1984; Kokubun et al., 1986; Krol et al., 1987). We used [^{3}H]nimodipine as a tool to identify the occurrence of regional depolarization. We hypothesized that in vivo binding of this DHP calcium channel antagonist may be used to identify vulnerable brain

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regions in PTD. Accordingly, we examined the in vivo binding of [^{3}H]nimodipine in rat brain during the progression and following treatment of this disorder.

MATERIALS AND METHODS

These studies were reviewed and found to satisfy the criteria for humane treatment of animals established by the Canadian Council of Animal Care (CCAC) and were approved by the Animal Care Committees of both the Montreal Neurological Institute, McGill University and the University of Ottawa. Male Sprague Dawley rats (275–300 g) were used in all experiments. Animals were weighed daily and housed individually in separate cages under constant conditions of temperature, humidity and 12/12 hr day/night cycles. Assessments for neurological signs of thiamine deficiency (ataxia, opisthotonus, loss of righting reflexes, convulsions, nystagmus) and behavioral changes (rotation, backward movements) were made on a daily basis. We did not monitor the animals for seizure activity electrophysiologically.

Experimental Groups

Total [^{3}H]nimodipine binding to brain in vivo was studied in five groups of animals:

(A) Presymptomatic group (n = 7). Rats were fed a thiamine-deficient diet (Ralston Purina Inc., Richmond, VA) with administration of pyrithiamine (Sigma Chemical Co., St. Louis, MO; 0.5 mg/kg body weight in 0.5 ml saline, i.p.) weekdays. Animals that exhibited no behavioral changes on day 13 of thiamine deficiency were chosen at that time for study.

(B) Acute symptomatic group (n = 6). Rats were placed on a similar regimen to that of group A but allowed to progress to a later stage of thiamine deficiency characterized by a loss of righting reflex. Animals were studied within 6 hr following development of this condition, prior to the onset of generalized convulsions.

(C) Thiamine-reversed group (n = 7). Rats were treated in a similar way to that of group B but were administered thiamine hydrochloride (5.0 mg Betaxin, i.p.) within 6 hr following loss of righting reflex, and once daily for 2 successive days before study.

(D) Pair-fed control group (n = 6). Rats were placed on an identical diet to that of group B with adequate levels of thiamine added and limited in quantity to that consumed by their thiamine-deficient counterparts. Saline (0.5 ml, i.p.) was administered weekdays. Feeding schedules were adjusted on a daily basis.

(E) Normal control group (n = 5). Rats were allowed unrestricted access to the same thiamine-containing diet as that fed to group D (Ralston Purina Inc.).

In addition, to examine the specificity of [^{3}H]nimodipine binding to vulnerable brain structures in vivo, both total and nonspecific binding was determined in the following groups of animals:

(F) Acute symptomatic group. Rats (n = 6), treated exactly as in group B, were used for the total binding experiments. Additional rats (n = 6) were also treated in a similar manner to those in group B and were used for determination of nonspecific binding which was assessed by infusing unlabelled nimodipine after the tritiated ligand (see below).

(G) Pair-fed control group. Rats were placed on an identical regime as in group D, with diet limited in quantity to the previous day's consumption of its match from group F for both total (n = 6) and nonspecific (n = 6) binding measurements.

Measurement of [^{3}H]Nimodipine Binding

Rats were anesthetized with halothane (4% induction, 1% maintenance) for placement of arterial and venous femoral catheters. The rats were then immobilized in lower-body plaster casts and allowed to recover from anesthesia for at least 2 hours before being studied. Body temperature was maintained between 36°C and 37°C throughout the experiments and mean arterial blood pressure was constantly monitored. Total binding studies were performed by administering [^{3}H]nimodipine (200 µCi, 123 Ci/mmole, New England Nuclear, Boston, MA) intravenously over 3 minutes in 600 µl of carrier (Bay e-9736 Placebo, Miles Pharmaceuticals, Etobicoke, Ontario) 30 minutes prior to decapitation. Arterial blood samples were obtained at the time of sacrifice for determination of plasma [^{3}H]nimodipine concentration, blood gases, and plasma glucose.

In nonspecific binding studies, [^{3}H]nimodipine was administered in the same manner, followed 10 minutes later by administration of unlabelled nimodipine (Bay e-9736, Miles Pharmaceuticals, 2 µM in 1:1 PEG 400: ethanol) infused at a maximally tolerated rate of 72 nmole/min for 20 minutes. Blood for plasma [^{3}H]nimodipine determination was then obtained and the rats decapitated.

Autoradiography and Histology

Following sacrifice, brains were rapidly removed, frozen in isopentane maintained at −45°C with liquid nitrogen and subsequently cut into 20µm-thick sections. These were apposed to photographic film (Hyperfilm, Amersham, Arlington Heights, IL) for 90 days to obtain autoradiographs of ^{3}H distribution. Twenty-µm sections of brain paste standards from rat forebrain containing known ^{3}H content were also apposed to the film for calibration. The films were then digitized using a microcomputer-based image display system (Imaging Research Inc., St. Catharines, Ontario). Regional [^{3}H]nimodipine

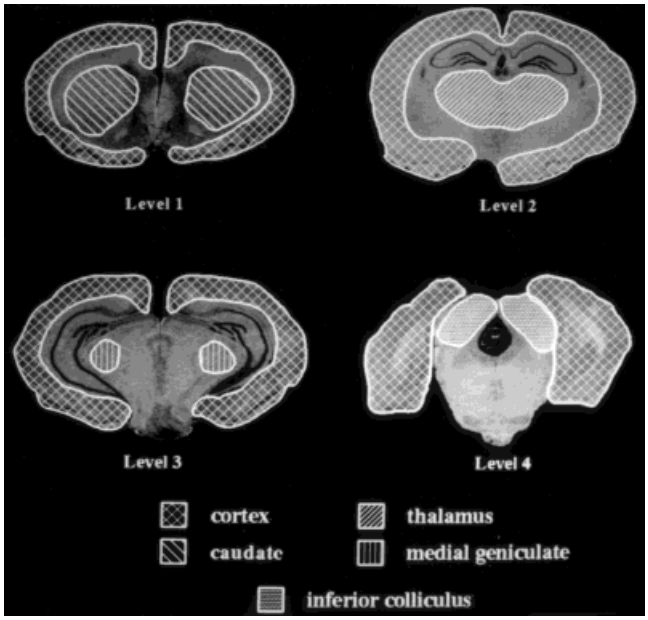

Fig. 1. The standardized template of regions of interest (ROI) used at the four coronal levels studied. The five grouped ROIs are identified.

content was measured at four coronal levels using a standardized region of interest template (Fig. 1). Template regions of interest were selected to include regions known to have varying degrees of vulnerability to injury in thiamine deficiency. Unmetabolized plasma and brain [^{3}H]nimodipine concentrations were determined from plasma and tissue radioactivity using known metabolite corrections previously reported (Hogan et al., 1991). Regional volumes of distribution of [^{3}H]nimodipine were calculated as the ratio of [^{3}H]nimodipine in brain over the plasma [^{3}H]nimodipine content in normal and thiamine-deficient studies. In each experiment, regional binding values were normalized against the binding observed in anterior frontal cortex, a region which remains histologically and metabolically normal at the acute symptomatic stage of thiamine deficiency (Hakim, 1984; Hakim et al., 1983). This normalization minimizes between-rat variation in the subsequent statistical analysis. For each region of interest, the normalized pairs of binding ratios, one for each hemisphere, were then averaged. Binding was also averaged over the pairs of cortical regions of interest (excluding the anterior frontal cortex). This resulted in five grouped regions: cerebral cortex, caudate, thalamus (posterior), medial geniculate, and inferior colliculi (Fig. 1). All subsequent analyses were performed on data obtained from these grouped regions of interest.

Several sections at the level of the thalamus, inferior colliculi, and medial geniculate bodies were obtained in each rat, mounted and stained with cresyl violet and submitted for histological examination. This permitted [^{3}H]nimodipine binding measurements and the histological assessment to be performed in the same rats. The tissue was assessed by a pathologist (M.K.S.) who was blinded to the identity of the experimental groups.

Statistical Analysis

Comparisons of normalized regional volumes of distribution of [^{3}H]nimodipine between control, presymptomatic, acute symptomatic, and thiamine-reversed groups were made using analysis of variance with Bonferroni correction. In the specificity studies, the data were analyzed using the Mann-Whitney U test and subjected to Bonferroni correction for multiple comparisons.

RESULTS

General Observations

Rats treated with pyrithiamine developed anorexia during days 8–10 of thiamine deprivation, resulting in weight loss. This was followed, starting on day 13, by changes in behavior of the animal consisting of rotational and/or backward movements. Ataxia and opisthotonic episodes became evident 48–72 hr later and this was followed in 12–18 hr by a loss of the righting reflex. Two rats showed clinical seizure-like activity at this stage and were eliminated from the study. Administration of thiamine following loss of the righting reflex completely reversed this condition within 3 hr.

Physiological Parameters

The results of physiological measurements are not shown, as they were identical to previous findings we reported in Hazell et al. (1993). Symptomatic and thiamine-replenished rats showed a significant elevation in plasma glucose concentration compared with pair-fed controls ($P < 0.0001$). This increase in plasma glucose concentration has been observed previously in this condition (Hakim, 1986; Hazell et al., 1993). These physiologic differences are assumed inconsequential to the nimodipine binding measurements and will not be discussed further.

Total Binding Studies

Autoradiographs of [^{3}H]nimodipine binding. At the presymptomatic stage, the volume of distribution of [^{3}H]nimodipine was relatively uniform throughout all regions with no difference in binding compared to pair-fed controls. Figure 2 shows autoradiographs of [^{3}H]nimodipine binding in pair-fed control, acute symptomatic, and thiamine-reversed animals. Symptomatic animals showed increased total binding of [^{3}H]nimodipine in the posterior thalamus, medial geniculate, and

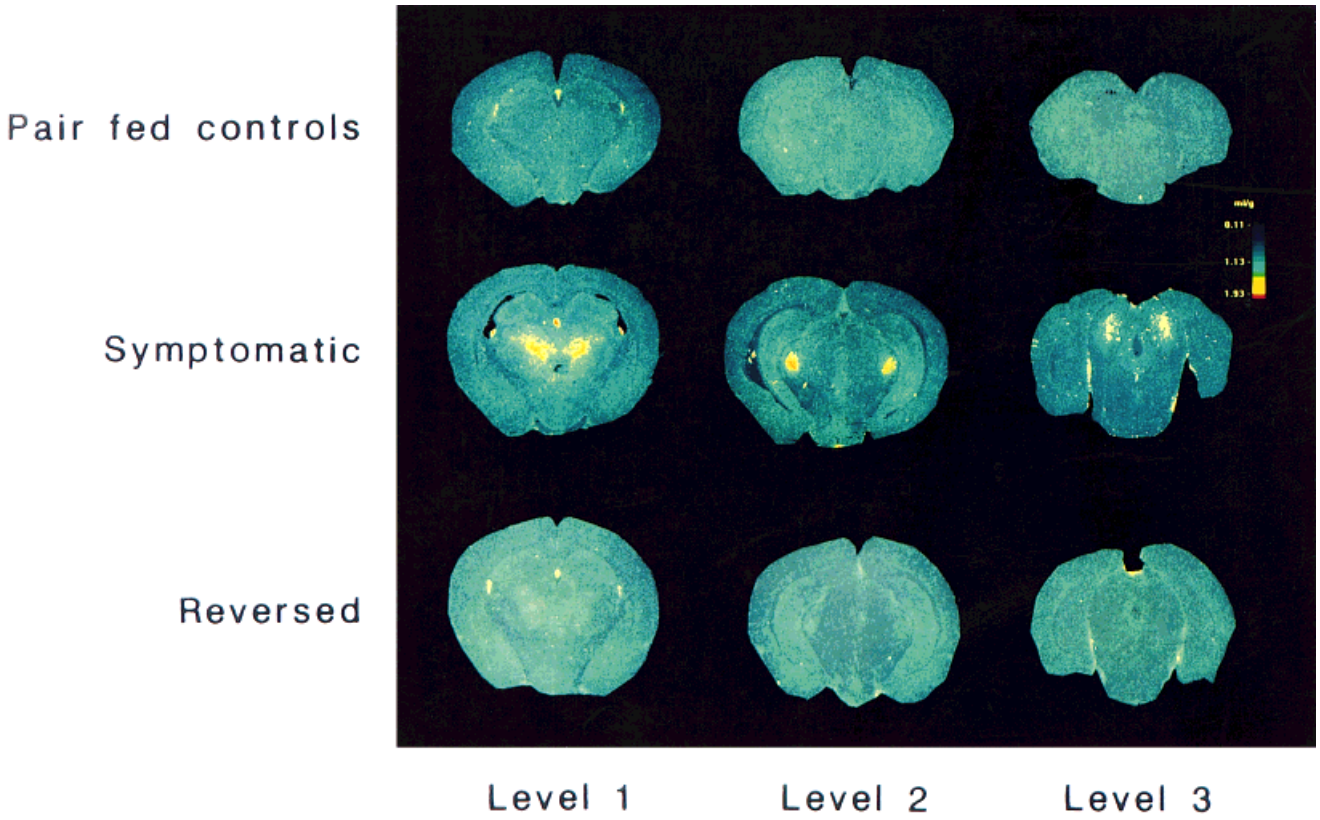

Fig. 2. Autoradiographs of total binding of [^{3}H]nimodipine to coronal sections of rat brain in pair-fed controls, symptomatic thiamine-deficient animals, and those reversed with thiamine at the level of the posterior thalamus (Level 1), medial geniculate (Level 2), and inferior colliculi (Level 3). Images have been normalized to the calibration bar.

inferior colliculi. Thiamine replenishment lowered nimodipine binding intensity in these regions to normal levels.

Regional binding of [^{3}H]nimodipine. A comparison of the volumes of distribution of [^{3}H]nimodipine in the anterior frontal cortex between all thiamine-deficient and control groups of rats in the total binding studies revealed no significant differences, thus justifying the choice of this region for normalization of the binding data in each study. Table I shows normalized volumes of distribution of [^{3}H]nimodipine (in five brain regions) in groups of animals at different stages of thiamine deficiency compared with controls. [^{3}H]nimodipine binding in normal and pair-fed groups of control animals revealed no significant regional differences. Presymptomatic animals also failed to show enhanced binding of nimodipine in any of the structures examined. Rats in the acute symptomatic group showed significantly increased normalized volumes of distribution of [^{3}H]nimodipine in posterior thalamus ($P < 0.0001$), medial geniculate ($P < 0.0001$), and inferior colliculi ($P < 0.005$) compared with pair-fed controls. In the caudate and cerebral cortex, the normalized volume of distribution of [^{3}H]nimodipine at the symptomatic stage was not statistically different from controls. Thiamine replenishment was found to reverse the increased [^{3}H]nimodipine binding observed in symptomatic animals.

Specificity of [^{3}H]Nimodipine Binding

Table II summarizes the normalized [^{3}H]nimodipine volume of distribution data for the specificity of binding studies. It can be seen that total binding of [^{3}H]nimodipine in the thalamus of the acute symptomatic rats were the highest of all the structures examined. Infusion of [^{3}H]nimodipine followed by administration of unlabelled ligand in acute symptomatic animals resulted in a decrease in binding in the posterior thalamus ($P < 0.01$). This was not observed in the medial geniculate or inferior colliculi; similar treatment in pair-fed controls did not alter the relatively low [^{3}H]nimodipine binding in the thalamus. Normalized [^{3}H]nimodipine volumes of distribution within the caudate were lowered by unlabelled nimodipine in both pair-fed and PTD rats. These findings indicate that a major component of total binding seen in the thalamus of symptomatic animals was displaceable and therefore representative of specific binding to

TABLE I. Normalized Volumes of Distribution of [^{3}H]nimodipine in Total Binding Study[†]

Region	Normal controls (n = 5)	Pair-fed controls (n = 6)	Pre-symptomatic (n = 7)	Acute symptomatic (n = 6)	Reversed (n = 7)
Cerebral cortex	1.16 ± 0.01	1.11 ± 0.05	0.99 ± 0.05	1.06 ± 0.05	0.91 ± 0.06
Caudate	1.20 ± 0.04	1.13 ± 0.05	1.19 ± 0.06	1.08 ± 0.01	1.03 ± 0.01
Thalamus	1.08 ± 0.03	1.02 ± 0.06	0.94 ± 0.07	2.04 ± 0.23*	1.18 ± 0.07
Medial geniculate	1.04 ± 0.05	1.03 ± 0.04	0.95 ± 0.05	1.83 ± 0.22*	0.97 ± 0.07
Inferior colliculi	0.94 ± 0.02	0.94 ± 0.07	1.16 ± 0.12	1.38 ± 0.13**	0.77 ± 0.05

[†]Values are mean ± S.E.M.
*$P < 0.0001$.
**$P < 0.005$ compared with pair-fed control group (ANOVA with Bonferroni correction for multiple comparisons).

TABLE II. Normalized Volumes of Distribution of [^{3}H]nimodipine in Specificity of Binding Study[†]

	Pair-fed control		Acute symptomatic	
Region	Total (n = 6)	Nonspecific (n = 6)	Total (n = 6)	Nonspecific (n = 6)
Cerebral cortex	1.12 ± 0.06	1.11 ± 0.04	1.08 ± 0.08	1.02 ± 0.02
Caudate	1.39 ± 0.05	1.07 ± 0.01*	1.27 ± 0.05	1.08 ± 0.02**
Thalamus	1.17 ± 0.05	1.00 ± 0.03	1.63 ± 0.14	1.23 ± 0.02**
Medial geniculate	1.15 ± 0.07	1.04 ± 0.07	1.35 ± 0.10	1.13 ± 0.03
Inferior colliculi	0.87 ± 0.07	0.94 ± 0.04	1.33 ± 0.16	1.21 ± 0.08

[†]Values are mean ± S.E.M.
*$P < 0.005$, pair-fed control nonspecific compared to pair-fed control total binding.
**$P < 0.01$, acute symptomatic nonspecific compared to acute symptomatic total binding.

activated L-type VSCCs. The same conclusion regarding specificity of [^{3}H]nimodipine binding in the thalamus was reached if the data were not normalized. In the pair-fed controls, total binding did not decrease in response to unlabelled ligand in the same region. The absence of any changes in binding in the medial geniculate and inferior colliculi in symptomatic animals following infusion of unlabelled nimodipine suggests that the total binding increase noted in the medial geniculate and inferior colliculi (Table I) was mainly nonspecific in nature. In summary, thiamine deficiency caused an increase in specific binding of [^{3}H]nimodipine that was localized to the posterior thalamus.

Histologic Studies

Histological evaluation of brain sections stained with cresyl violet by light microscopy showed normal tissue in the control animals. Presymptomatic rats showed evidence of mild edema in posterior thalamic nuclei and inferior colliculi. In the acute symptomatic rats, neuronal cell numbers were moderately decreased at the level of the thalamus, medial geniculate and inferior colliculi in a patchy distribution, with some gliosis and areas of edema. The center of the geniculate body was affected before the periphery. Figure 3A shows a region of the medial thalamus in a pair-fed control animal, while Figure 3B shows the evident neuronal cell loss in this region during the acute symptomatic phase. Thiamine-replenished brains showed a similar appearance to the acute symptomatic group, but with reduced edema. In the inferior colliculi, compared to pair-fed controls (Fig. 3C), the most notable response to thiamine deficiency appeared to be increased gliosis at the symptomatic stage (Fig. 3D). No hemorrhages or pannecrosis were noted in any of the brain regions examined.

DISCUSSION

In this study, we report two important new observations in thiamine deficiency. Firstly, the volume of distribution of [^{3}H]nimodipine increases in a limited number of cerebral structures in the thiamine-deficient rat, and secondly, the increased binding of [^{3}H]nimodipine in the thalamus is specific. In resting polarized cells, L-type VSCCs exhibit low affinity for DHPs such as nimodipine (Boland and Dingledine, 1990). The VSCCs respond to depolarization by increasing the binding of DHPs such as nimodipine, and reports have indicated the occurrence of this phenomenon in both cerebral ischemia (Hakim and Hogan, 1991) and in nonpathological conditions involving cortical depolarization (Osuga et al., 1997).

Our data suggest that the thalamus is depolarized during thiamine deficiency. This depolarization is probably multifactorial in origin. The formulation of the excitotoxic theory was based on an increase in the release of glutamate following an ischemic or hypoxic insult (Olney, 1978). Binding of this amino acid to glutamate receptors leads to ligand- and voltage-gated calcium

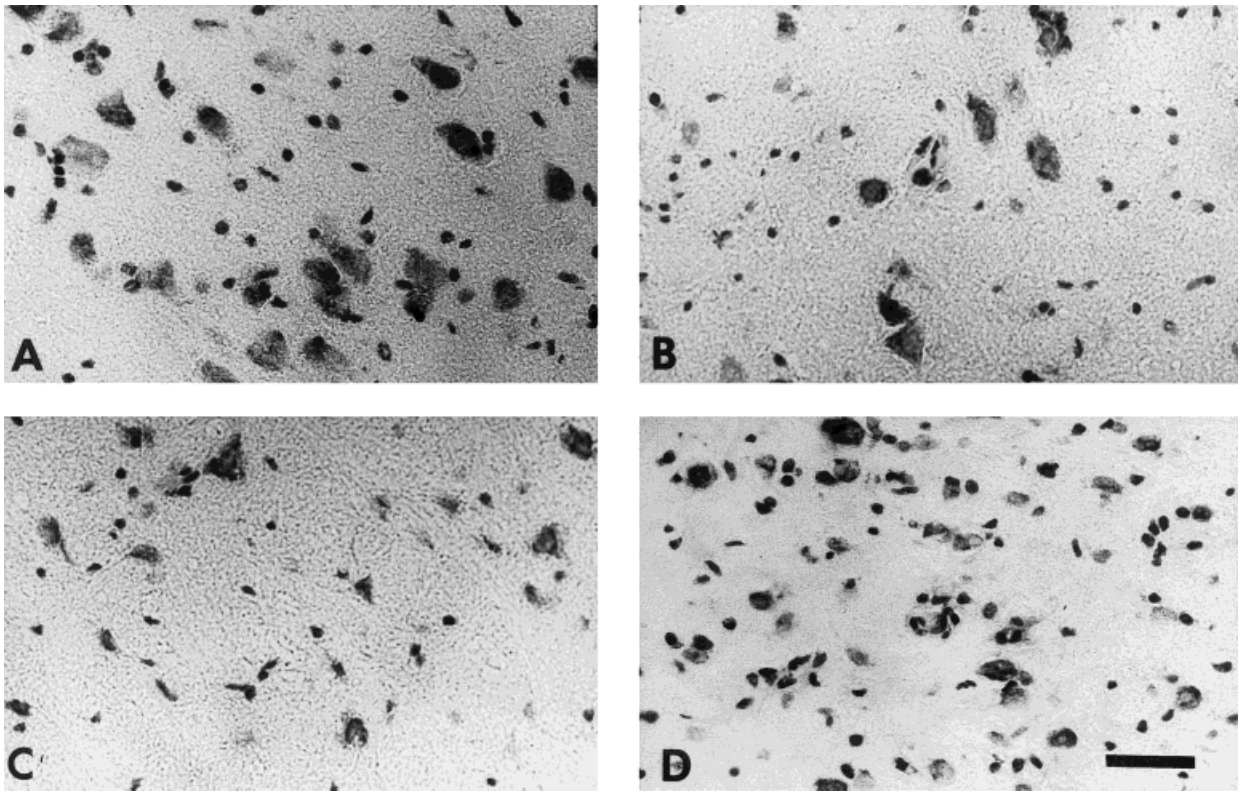

Fig. 3. Light microscopy of cresyl violet-stained sections from a pair-fed control (**A, C**) and acute symptomatic animal (**B, D**), showing areas of the medial thalamus (A, B) and inferior colliculus (C, D). Loss of neurons is particularly evident in the thalamus and reactive gliosis in the inferior colliculus when compared to controls. Bar = 50 μm.

channel activation which may result in calcium-mediated necrosis of the postsynaptic cell. Osuga and Hakim (1994) have shown, at least in ischemia, that vulnerability is proportional to the intensity-duration product of exposure to glutamate. Thus, the rise in tritiated nimodipine binding is likely related to the rise in glutamate concentration. On the other hand, when changes in [^{3}H]nimodipine binding and extracellular glutamate concentration were compared simultaneously in vivo in the setting of ischemia, VSCC activation occurred at more moderate levels of ischemia than those needed to raise glutamate (Osuga and Hakim, 1996), suggesting that [^{3}H]nimodipine binding may respond to additional depolarizing influences such as a change in ionic homeostasis.

While [^{3}H]nimodipine binding increases in the medial geniculate, posterior thalamus, and inferior colliculi, all known to be histologically vulnerable to thiamine deficiency, our data suggest that only the posterior thalamus shows a significant increase in specific binding. This observation suggests that this region responds to thiamine deprivation differently or progresses at a different pace from the other vulnerable structures. In setting the thalamus apart as having a unique response to thiamine deficiency, our study confirms other reports in the literature. It is possible that the increase in specific binding in the thalamus is the reflection of an earlier and more intense rise in glutamate concentration in this structure. Studies using in vivo microdialysis in the thiamine-deficient animal have indicated that glutamate concentrations were higher in the thalamus than in structures resistant to histological damage (Hazell et al., 1993; Langlais and Zhang, 1993). Langlais and Zhang (1993) showed that both the hippocampus and the thalamus exhibited increases in extracellular glutamate concentration at the symptomatic stage, but only the thalamus was affected histologically. We have recently reported that apoptosis occurs primarily in the thalamus of PTD rats (Matsushima et al., 1997) and the current study showing focal depolarization in the thalamus, with the implied increase in the intracellular calcium concentration, which can trigger this mode of cell death, fits well with this finding. Thus, the current data support the accumulating evidence that the vulnerability of the thalamus to thiamine deficiency exhibits unique characteristics.

Thiamine deficiency is a disorder that evolves over a period of weeks, thus offering a significant advantage

over conditions such as ischemia that can evolve over minutes. This has prompted an extension of the [^{3}H]nimodipine binding model developed in focal ischemia (Hogan et al., 1991), to thiamine deficiency. To do so, it has been assumed that an approximate equilibrium of distribution of [^{3}H]nimodipine is established between all compartments by 30 minutes following infusion of the ligand. This has been demonstrated in a model of focal cerebral ischemia where blood supply is severely limited (Hogan et al., 1991), and therefore is presumed to hold in thiamine deficiency, where patchy changes in cerebral perfusion but no net decline in blood flow have been reported at the symptomatic stage (Hakim, 1986). While it is possible that thiamine deficiency may alter the metabolism of [^{3}H]nimodipine, normalization of binding in each region of interest by that in the anterior frontal cortex, as done here, will greatly reduce the impact of any peripheral variations in [^{3}H]nimodipine metabolism on the observed changes in binding. As well, since hypothermia has been described in thiamine deficiency (Hazell et al., 1993; Plaitakis et al., 1978; Vortmeyer and Colmant, 1988; Zimmerman and Burack, 1932), we maintained the animals at normal body temperature to eliminate any influence brain temperature changes may have on nimodipine binding. Thus, we believe the nimodipine binding model we described for ischemic brain can be applied in this model.

Additional considerations must be discussed in evaluating our binding studies. Permeability of the blood-brain barrier is known to be increased in the thiamine-deficient rat (Calingasan et al., 1995; Phillips and Cragg, 1984), and mouse (Harata and Iwasaki, 1995). If breakdown of the blood-brain barrier had occurred in our animals, this would have allowed exudation and trapping of the protein-bound component of [^{3}H]nimodipine in serum, directly affecting our results. We do not believe that the blood-brain barrier to nimodipine was affected at the stage of thiamine deficiency at which we studied our animals. There was no histologic evidence for this, but more importantly, replenishment of the animals with thiamine returned the volumes of distribution of [^{3}H]nimodipine to normal. This would not have occurred if [^{3}H]nimodipine had leaked across the blood-brain barrier. Also, even though we eliminated two animals with overt seizures, it is possible that seizures which are not clinically evident may have influenced our results, particularly if they were thalamic in origin. The latter, however, have been extremely difficult to prove in thiamine deficiency, and indeed seizure models in general. Finally, even though replenishment with thiamine decreases the edema seen histologically in the deficient animals, cell loss persists, in keeping with other studies indicating the incomplete reversibility of the metabolic (Hakim et al., 1983), and cognitive deficits (Victor et al., 1971) of thiamine deficiency.

Our data showing the activation of VSCCs in thiamine deficiency are compatible with previous studies showing that calcium channel blockers are effective in protecting the brain from histological damage in this disorder. Munujos et al. (1993) showed that in the presence of nicardipine, a calcium channel blocker, lesions associated with thiamine deficiency did not appear and there was no induction of c-fos mRNA. This beneficial effect of a VSCC antagonist may be due to the activation of the receptor we are reporting, but may also arise from the reestablishment of pH homeostais, which is known to be disturbed in thiamine deficiency (Hakim, 1984), and which can be corrected by the administration of a blocker of the VSCC (Vogel and Hakim, 1988). As well, activation of VSCCs has been linked to the induction of immediate-early genes (Morgan and Curran, 1986; Murphy et al., 1991), and the production of trophic factors (Zafra et al., 1990), suggesting that these molecular responses may play a role in the selective cerebral vulnerability in this model. Immediate-early genes are involved in the induction of programmed cell death (Jorgensen et al., 1989; Smeyne et al., 1993; Wessel et al., 1991), and may produce long-term changes in gene expression in this disorder. Indeed, our finding of a predominance of programmed cell death in the thalamus of thiamine-deficient rats, as opposed to the other vulnerable structures (Matsushima et al., 1997), is consistent with an involvement of immediate-early genes in this structure (Hazell et al., 1998). Thus, this study, by suggesting that the thalamus behaves differently than the other vulnerable structures in thiamine deficiency, opens new avenues of research in the quest to explain the causes of selective vulnerability in the brain.

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