Increased Mast Cell Degranulation within Thalamus in Early Pre-lesion Stages of an Experimental Model of Wernicke's Encephalopathy

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Abstract. A large increase in the number and percentage of degranulating mast cells was observed within thalamus of rats after 6–7 days of thiamine deficiency (TD). No mast cells were detected in the inferior olivary and lateral vestibular nuclei, which are also severely damaged by TD. After 11–12 days of TD, the number of ED2 immunopositive macrophages increased in thalamus. In the brainstem nuclei, an increase in the number of macrophages occurred much earlier in treatment (i.e. day 6). An increase in GFAP-positive astrocytes within thalamus occurred after the changes in mast cells and prior to the increase in macrophages. In brainstem, reactive astrocytes appeared along with the increase in macrophages. These data suggest that mast cell degranulation is a very early response induced by TD, and the resultant release of cytokines and other chemical mediators may play critical roles in both the early vascular damage and eventual tissue destruction within thalamus, but not within brainstem. These results also suggest that macrophages and reactive astrocytes may play more direct roles in the pathogenesis of brainstem lesions.

Key Words: Macrophages; Mast cells; Thalamus; Thiamine deficiency; Wernicke’s encephalopathy.

INTRODUCTION

Wernicke’s encephalopathy (WE) is characterized by symmetrical lesions within the thalamus, mammillary bodies, periaqueductal and periventricular regions, brainstem, cerebellum, and diffuse cortical atrophy and white matter loss (1). Although WE is most prevalent among chronic alcoholics, it is thiamine deficiency rather than ethanol that is generally accepted as the primary cause of this disease (2). The primary of thiamine deficiency is supported by studies of experimental thiamine deficiency in animals and the presence of WE in vitamin deficient nonalcoholic populations, such as those with AIDS (3–5), hyperemesis gravidarum (6–8), gastric resection (9), chronic hemodialysis (10), and malignancies such as leukemia (11–13).

Several pathogenic mechanisms appear to be involved in WE (reviewed in 14, 15), and include glutamate-NMDA receptor mediated excitotoxicity (16–18), lactic acidosis (19), free radical production (20), apoptosis (21), and impaired energy production (22). These mechanisms are highly interrelated and appear to be immediate causes of tissue damage since biochemical and molecular events associated with these mechanisms are observed during, or just prior to, onset of pathological changes.

Perhaps the most intriguing and puzzling issue is the biochemical and physiological bases for the selective distribution of lesions in WE. Certain observations suggest a link to regional changes in vascular permeability. In acute cases of human WE, edematous changes and blood-brain barrier (BBB) disturbances have been observed in affected regions using magnetic resonance imaging (23) and postmortem histological examination (24). In experimental thiamine deficiency, early vascular changes such as edema, perivascular leakage, and dilated blood vessels occur prior to cell death (25, 26). Two recent animal studies reported that breakdown of the BBB occurred only in vulnerable brain regions and preceded cell death by several days (27, 28).

Increased vascular permeability and BBB breakdown may be related to thiamine deficiency-induced increases in histamine release (29). Histamine is a well-known regulator of blood vessel diameter and vascular permeability (30). Furthermore, inhibition of histamine synthesis has been reported to attenuate thiamine deficiency-induced lesions of the thalamus (29). However, the recent observations of increased histamine release (31) and perivascular leakage of albumin within hippocampus (Bernard & Langlais unpublished observations, 1998), a relatively unaffected brain region, of thiamine deficient rats suggests a more complicated picture. Histamine within the mammalian brain is contained in nerve terminals of the hypothalamic tuberomammillary nucleus and mast cells. Histamine terminals innervate widespread regions of the brain including the thalamus, hypothalamus, hippocampus, and cortex (32, 33). Mast cells, on the other hand, are located almost exclusively within the thalamus (34) and in addition to histamine release several cytokines and chemokines.

Although the role of mast cells in CNS damage is poorly understood (35), mast cells can participate in delayed injury to neurons in the CNS (36) and are suspected to contribute to several disorders including multiple sclerosis (MS) and Alzheimer disease (37). Mast cells reside
in close proximity to glia and blood vessels (37, 38), allowing them to play a regulatory role in the BBB, and their degranulation can seriously compromise its integrity (39).

The present study was conducted to test the hypothesis that thiamine deficiency is associated with an increased number and degranulation of mast cells and that these changes occur very early in a rat model of WE. Measurements of intact and degranulated mast cell number were obtained in separate groups of rats examined after 6–12 days of pyrithiamine-induced thiamine deficiency (PTD). This study also examined the hypothesis that mast cell degranulation is associated with sites of necrotic lesions (i.e., thalamus), and not with sites of selective neuronal loss such as inferior olives and lateral vestibular nuclei, or brain regions resistant to thiamine deficiency such as the hippocampus and facial nuclei. Temporal and regional changes in macrophage number were also examined since macrophages are potent producers of cytokines and cytotoxins (40), are activated by microglia (the main immune cell population of the brain [41]), and monocytes can cross over the BBB and mature into macrophages during conditions of inflammation (42, 43).

Finally, GFAP immunopositive astrocytes were qualitatively evaluated in these same brain regions to examine the temporal relationship between onset of astrocyte activation and changes in mast cells and macrophages. Astrocytes play a key role in regulating the BBB and swollen astrocyte processes are frequently observed in vulnerable brain regions of presymptomatic thiamine deficient animals (25, 26, 44).

MATERIALS AND METHODS

Experimental Animals

Fifty-three adult, male Sprague-Dawley rats, 270–310 g, were used in this experiment. Animals were housed in pairs and maintained under conditions of constant temperature, humidity, and light/dark (12 h/12 h) cycles. All animal procedures were carried out in strict accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the local Animal Care Committee (APF #97-009). Animals were randomly assigned to 1 of 3 treatment groups.

Treatments

Pyrithiamine-induced Thiamine Deficiency (PTD): Rats were fed thiamine-deficient chow (Teklad Diets, Madison, WI) and given daily injections of pyrithiamine hydrobromide (0.25mg/kg, i.p., Sigma Chemical Co., St. Louis, MO). Separate groups of animals were examined on days 6 and 7 (N = 5/group), and days 8, 9, 10, 11, and 12 (N = 7/group) of treatment.

Pair-fed Controls (PFC): Rats (N = 5) were fed an amount of thiamine-deficient chow equal to the average amount consumed by the PTD rats on the same day of treatment. In addition, they were given daily injections of pyrithiamine hydrobromide (0.4 mg/kg, i.p., Sigma Chemical Co.). These animals were killed after 12 days of treatment in order to control for the maximum effect associated with a reduction in food intake and the stress of daily handling and injections.

Ad-Lib Controls: An additional group of 3 rats were given ad lib access to regular chow and water. These animals were examined 12 days after initiation of the study. Only tissues from the diencephalon of these animals were examined to determine if pairfeeding alone stimulates degranulation of mast cells within the thalamus. This possibility was recently suggested by the observation of increased histamine release within thalamus of pair-fed animals (45).

Tissue Preparation

On the appropriate day of treatment, animals were anesthetized with an injection (0.25 ml/100 g body weight) of a solution containing 33.3 mg/ml of ketamine (Ketaject, 100 mg/ml, Bristol Laboratories, Syracuse, NY), 5 mg/ml of xylazine (Rompun, 20 mg/ml, Miles Laboratories, Shawnee, KY), and 0.3 mg/ml of acepromazine maleate (Techem Group Inc., Elwood, KY). The brain was perfused transcardially with 150–300 ml of 0.9% NaCl, followed by 500 ml of 4% phosphate-buffered paraformaldehyde. The brains were postfixed for 24 h in 4% paraformaldehyde, at which time they were switched into a 30% sucrose-4% paraformaldehyde solution. After sinking once in this sucrose solution, the brains were blocked and switched into a fresh mixture of 30% sucrose-4% paraformaldehyde to ensure complete saturation of the tissue and avoid ice crystal formation during frozen sectioning.

Tissues from the diencephalon and brainstem were cut on a frozen microtome into 40-micron-thick sections. One step series (200 μm between sections) containing the entire thalamus and hippocampus of every animal in the study was stained with cresyl violet and used to identify the anterior-posterior level and evaluation of neuronal damage. A second step series (120 μm between sections) containing the lateral vestibular nucleus, inferior olivary complex, and facial nucleus for N = 5/group (days 6 through 12) were also stained with cresyl violet to identify the level of the nuclei within the series and evaluate neuronal damage. A second set of sections containing these same structures was double-stained with toluidine blue and an ED2 antibody for quantitative measurement of mast cell number, percentage of degranulated mast cells, and number of macrophages. A select number of sections from the thalamus was also stained with a histamine-toluidine blue double stain to further characterize the toluidine blue positive cells as typical mast cells and a potential source of histamine. A limited number of sections from the anterior, middle, and posterior limits of each brain structure were immunostained with a GFAP antibody and qualitatively examined for changes in reactive astrocytes.

Staining Procedures

Cresyl Violet (CV): Sections were dry mounted onto slides, rinsed in deionized water and then dehydrated and rehydrated through graded alcohol solutions, rinsed in deionized water, and stained with 0.2% cresyl violet acetate (Sigma Chemical Co.) for 5 min. After staining, the slides were rinsed in deionized water, cleared in a 6% acetic acid (differential) solution, rinsed again in deionized water, and finally dehydrated with 95% and 100% alcohol (all steps 1 min except for the differential solution which involved only a 3–5 s agitation). Then the slides
were dried overnight before coverslipping with cytoseal (Stephen Scientific, Riverdale, NJ).

**Immunochemical Staining for Histamine with Toluidine Blue Counterstain:** A few sections adjacent to tissues containing a large number of toluidine blue-positive cells were immunostained for histamine and then stained with toluidine blue. This was done to determine if the metachromatic (toluidine blue-positive) cells also contained histamine and thus could be characterized as mast cells. The immunohistochemistry procedures used were similar to those used for ED2 immunostaining of macrophages, except for the primary antibody (1:500, rat anti-rabbit histamine antibody, Chemicon, Inc.), secondary antibody (1:250, biotinylated anti-rabbit IgG, made in goat, Vector), and blocking serum (1% goat serum in TBS). The counterstaining procedure with toluidine blue was also carried out in the same manner as with the ED2 stained sections.

**GFAP Immunostaining:** Select sections from the anterior, middle, and posterior levels of each brain region were stained immunohistochemically for GFAP (N = 4/group). This was done to qualitatively determine the reactivity of astrocytes. The staining procedures used were similar to that used for the ED2 immunostaining except for the primary antibody (1:100, mouse anti-rat GFAP antibody), secondary antibody (1:166, biotinylated anti-mouse IgG, made in horse, Vector), and blocking serum (5% horse serum). After defatting with 50% chloroform-50% alcohol, the sections were taken through graded alcohols and xylene before being coverslipped with DPX (BDH Laboratory Supplies).

**Quantitative and Qualitative Histological Measurements**

The number of intact and degranulated mast cells and macrophages were counted in every section of each step series through the thalamus, lateral vestibular nucleus, and inferior olivary complex as brain regions that are vulnerable to thiamine deficiency and undergo significant neuronal and tissue loss in this PTD rat model of WE. The hippocampus and facial nucleus were also quantitatively analyzed as negative controls since these nuclei are relatively resistant to thiamine deficiency. Mast cells were counted as the number of toluidine blue-positive cells and macrophages were counted as the number of ED2-positive cells. Mast cells were considered degranulated if granules were seen extruding out of the cell boundaries, or there was a >50% decrease in the number of granules present within the mast cell. A step series of sections (200 µm apart) extending from the anterior thalamic nuclei (i.e. anterodorsal, anteroventral [AD and AV]), and paratalal nuclei to the posterior limits of the parafascicular and posterior nuclear group (Bregma -0.92 to -4.52 mm, according to 46) were used for the quantitative measurements within thalamus. Numbers of cells were counted separately for the medial and lateral regions of the thalamus as shown in Figure 1. Included in the analysis of the lateral thalamus were most of the lateral geniculate nucleus and the anterior one third of the medial geniculate nucleus. Both dorsal and ventral regions of the hippocampus were examined in the same step series used for thalamic measurements. The entire vestibular nucleus (superior, lateral, medial, and inferior nuclei), facial nuclei, and most of the inferior olivary complex (dorsal, medial, and principal nuclei) were examined in a second step series (160 µm apart) extending from Bregma -10.04 to -13.30 mm (46). The mediodorsal nuclei, lateral dorsal, and ventral posteromedial/posterolateral of the thalamus and vestibular nuclei, inferior olives, and facial nuclei of the brainstem were evaluated for changes in astrocyte reactivity. Changes in...
GFAP immunostained astrocytes were rated as 0 = unchanged from control; 1+ = mild increase in density; and 2+ = moderate to heavy increase in density and size. Cresyl violet stained sections were examined for neuronal damage as evidenced by a greater than normal number of dark pyknotic neurons, swollen and vacuolated neurons, or a reduction in cell density. All slides were coded so that the rater was blind to the treatment category of each specimen examined for quantitative and qualitative measurements.

### Statistical Analyses

Initial analysis of each dependent variable demonstrated no significant differences between the pair-fed and ad lib fed controls (t-test, all ps > 0.27) with the exception of a larger number of mast cells in medial thalamus of the pair-fed controls compared with ad lib controls (p = 0.033). However, this difference was very small (<10% increase), and these measures of mast cell number in medial thalamus and the remaining data from these 2 groups were combined into 1 control group and used for all subsequent analyses. The data were log transformed and analyzed using one- and/or two-way analysis of variance (ANOVA) on SPSS, with an alpha level of 0.05 used for all statistical tests. When justified by significant main effects, a Newman-Keuls post hoc test was used to compare specific treatment groups or regions.

### RESULTS

#### Qualitative Description of Mast Cells and Macrophages

Mast cells, whose granules stained deep blue-violet against a faint background, were readily distinguishable from macrophages, which stained brown (Fig. 2A). Degranulated mast cells (Fig. 2B) were observed in both the control and PTD treated groups. The cells that metachromatically stained with toluidine blue also stained positively for histamine. The characteristic brownish-black, DAB-peroxidase immunoprecipitant was observed in the typically blue-violet granules of the toluidine blue stained cells, suggesting that these metachromatic cells do contain histamine and were presumably mast cells. Although most of the toluidine blue-positive cells stained with the histamine antibody, not every mast cell displayed the typical immunoprecipitant reaction. Astrocytes were identified as star-shaped cells that stained the characteristic brownish-black from the GFAP immunohistochemistry.

The majority of mast cells and macrophages in the thalamus and macrophages in the hippocampus and brainstem were associated with blood vessels, as shown in Figure 2A. In the thalamus, mast cells were seen not only on blood vessels that appeared to be venules, but were also found on arterioles. Occasionally a mast cell or a macrophage appeared to be located out in the parenchyma; however, at the light microscopic level the exact perivascular versus extravascular location of these cells could not be determined.

Although the majority of mast cells and macrophages were associated with and in proximity to blood vessels, most of the mast cells were not found adjacent to macrophages. Most mast cells were frequently seen in large clusters within or next to a single blood vessel. Often the mast cells within these aggregations appeared to be attached to each other and to the blood vessel. Occasionally, a macrophage and mast cell appeared to be attached to each other or the same part of the endothelial cell (Fig. 2A). The distribution of these 2 cell types within the thalamus was also quite different; while mast cells were found distributed throughout the thalamus, in most animals macrophages were localized along the ventral and lateral edges of the thalamus. In the brainstem nuclei, macrophages also appeared to be located on or in the vicinity of blood vessels. In the vestibular nucleus macrophages were often most numerous in the lateral region. Macrophages were also observed (but not counted) in vessels close to the ventricular surface.

Various shapes of macrophages were found throughout the thalamus, hippocampus, and brainstem of control and PTD rats. The shapes of the ED2-positive cells in all of the treatment groups varied from the ramified shape of a resting microglia to the ameboid shape of a brain macrophage or fully activated microglia. However, within the thalamus there did appear to be an increased number of ameboid-shaped cells and a concomitant decrease in the number of ramified shaped cells as the duration of thiamine deficiency increased. In the brainstem, most macrophages were ramified and only a few ameboid-shaped cells were observed throughout the entire period of thiamine deficiency. Since activated microglia that express ED2 antigen are usually ameboid, it would appear that most of the ED2 positive cells observed in brainstem were macrophages and not microglia.

#### Number of Mast Cells

As expected, intact and degranulated mast cells were observed within thalamus of control and thiamine-deficient animals. There were no mast cells detected in hippocampus, lateral vestibular nucleus, inferior olivary complex, or facial nucleus in any of the PTD-treated or control animals. As a consequence, these latter nuclei were not included in the statistical analysis of the total number of mast cells. A two-way ANOVA demonstrated a significant main effect of treatment (F [7, 90] = 3.34, p = 0.003) and region (F [1,90] = 74.55, p < 0.0001). Compared with controls, the number of mast cells was increased within the medial and lateral thalamus on several of the PTD treatment days (Fig. 3). The analysis also demonstrated that the lateral thalamus contained significantly more mast cells than the medial thalamus. There was, however, no significant interaction (treatment × region, p > 0.05), indicating that the effect of thiamine...
deficiency on mast cell number was similar in the medial and lateral regions of thalamus.

Separate one-way ANOVAs of the number of mast cells in the medial and lateral thalamus failed to demonstrate a significant treatment effect. However, it is important to note that there was a definite trend for increased mast cell numbers in the thiamine deficient groups (medial thalamus and lateral thalamus, p = 0.09). A biphasic increase in the number of mast cells was seen in both the medial and lateral thalamus of PTD treated animals. This increase attained a maximum at days 6 and 9 of treatment. On the remaining days of treatment the number of mast cells was reduced compared with days 6 and 9, but remained elevated compared with controls (Fig. 3).
Fig. 3. The total number of granulated and partially granulated mast cells (mean ± SEM) within the medial and lateral thalamus of groups of rats examined on days 6 and 7 (N = 5/treatment day) and days 8–12 (N = 7/treatment day) of pyrithiamine-induced thiamine deficiency (PTD) or control (N = 8) treatment.

Percentage of Degranulating Mast Cells in Thalamus

The percentage of degranulating mast cells within thalamus of PTD animals was also significantly increased from controls, (F[7, 90] = 28.93, p < 0.0001). Post hoc tests demonstrated that the percentage of degranulating mast cells, averaged across the medial and lateral thalamus, was increased significantly in PTD 7–12 animals as compared to controls. There was also a significant increase in the percentage of degranulating mast cells in PTD 8–12 animals as compared with PTD 6 animals, and PTD 9–12 as compared with PTD 7 and 8 (Fig. 4). The analysis also indicated a main effect of brain region, with the percentage of degranulating mast cells being greater in the lateral thalamus as compared with the medial thalamus, (F[1, 90] = 39.36, p < 0.0001).

Further analyses with separate one-way ANOVAs demonstrated that the percentage of degranulating mast cells in both the medial (F[7, 45] = 10.30) and lateral thalamus (F[7, 45] = 28.08) of the PTD treated animals was significantly greater than controls (p < 0.0001). In addition, the temporal pattern of change in mast cell degranulation was slightly different for the medial and lateral thalamus. The increase in the percentage of degranulating mast cells in the lateral thalamus occurred on day 7, whereas in the medial thalamus a significant increase was observed 1 day later, i.e. PTD 8 (Fig. 4).

Number of Macrophages

Five animals had 2–8 times more macrophages in cortex, hippocampus, and thalamus of 1 hemisphere compared with the other. All other animals had a relatively equal number of macrophages in these regions of the right and left hemispheres. This unilaterally large increase in macrophages was observed in 1 ad lib control, 2 PTD 8, 1 PTD 9, and 1 PTD 10. In these same 5 animals, the number of mast cells was relatively similar in both hemispheres and comparable to data obtained in the remaining animals in the appropriate group, and thus appeared unaffected by this unilateral increase in number of macrophages. The number of macrophages within the brainstem of these same animals corresponded to similar treatment animals and were evenly distributed between right and left hemispheres. The data from these anomalous animals were eliminated from the analysis of macrophage number within the thalamus and hippocampus, but were included in the analysis of the brainstem nuclei since these brain regions were not affected.

A two-way ANOVA demonstrated a significant effect of treatment (F[7, 216] = 2.27, p < 0.05). Post hoc analysis did not find any significant difference between the individual treatment groups, most likely due to the large amount of variance seen in the data. There was also a significant main effect of brain region (F[5, 216] = 248.88, p < 0.0001). The medial and lateral thalamus and hippocampus had significantly greater numbers of macrophages than were found in the vestibular, inferior olivary, and facial nuclei of the brainstem. In addition both the inferior olivary and vestibular nuclei of the brainstem had greater numbers of macrophages than were seen in the facial nucleus, and the vestibular nuclei had a greater number of macrophages than the inferior olives.

The analysis also demonstrated a significant interaction (treatment × brain region, F[35, 216] = 2.14, p <
the number of macrophages within hippocampus. However, post hoc tests did demonstrate that the pattern for the proliferation of macrophages within the thalamus was slightly different in the medial and lateral thalamus. In the medial thalamus, the increase in the number of macrophages occurred earlier with both PTD 11 and 12 animals having a larger number of macrophages than day 8 animals, while in the lateral thalamus, PTD 12 animals had a significantly larger number of macrophages than day 6 and day 8 animals (Fig. 5A). It is also important to note that the effect of PTD treatment on the number of macrophages within nuclei damaged by thiamine deficiency (i.e. vestibular nuclei and the inferior olives) approached significance ($p = 0.06$ and $0.08$, respectively). In contrast, there was no effect of PTD treatment on macrophage number within the facial nucleus ($p = 0.65$), which is not damaged by thiamine deficiency.

**Qualitative Analysis of Reactive Astrocytes**

Within thalamus there was an increase in both the number and intensity of GFAP immunoreactive astrocytes of PTD in animals treated on days 9 and 10. In PTD day 11 and 12 animals, GFAP immunoreactivity declined to levels similar to controls. These changes preceded the increase in macrophages and followed the increase in mast cell number and percentage of degranulating mast cells.

In the brainstem the astrogliosis appeared to follow a different pattern, with the density of GFAP immunostaining increasing in the earlier stages of thiamine deficiency (PTD 6 and 7) and then decreasing in the later stages coinciding with the pattern seen in the macrophage numbers. The largest increase in the GFAP immunoreactivity in the brainstem (PTD 7) occurred after the peak in the number of macrophages (PTD 5).

**Neuronal Damage**

Neuronal damage was determined by the presence of dark, pyknotic neurons, pale cells, a lack of cellular organization, and/or cell loss. As observed in the changes in the number of ED2 immunopositive macrophages and GFAP immunoreactive astrocytes, there appeared to be a differential pattern in the neuronal damage seen in the thalamus and brainstem.

Within the thalamus, neuronal damage was not evident until 11 or 12 days of treatment. On these treatment days, several dark pyknotic neurons were observed in nuclei that are the most vulnerable in thiamine deficiency, i.e. the submedius nucleus (also known as the gelatinous nucleus), anterior nuclei, and ventrolateral region of the mediodorsal nucleus. Occasional pyknotic neurons were also observed in the posterior and ventral posteromedia/ posterolateral nuclei of the thalamus of PTD day 11 and 12 animals. In these thalamic nuclei there was no evidence of marked neuronal loss, even in the PTD day 12.
animals. In the brainstem, however, almost all of the PTD day 11 and 12 animals had marked neuronal loss. In the inferior olivary complex, numerous dark pyknotic neurons were seen as early as PTD day 8, and in 1 PTD day 8 animal there was clear evidence of marked neuronal loss. Within the vestibular nuclei, PTD day 9 was the first day on which a majority of the animals exhibited neuronal damage (i.e., pyknotic, spindle-shaped neurons). By day 11 and 12 of PTD treatment, extensive loss of neurons was evident in vestibular and inferior olivary nuclei. These patterns suggest that in thiamine deficient animals, neuronal changes and loss occur in the brainstem several days prior to their onset in the thalamus.

**DISCUSSION**

The present findings demonstrate that thiamine deficiency produces a dramatic increase in the number of mast cells within thalamus. Within other vulnerable regions such as the vestibular and inferior olivary nuclei, no mast cells were detected even in animals with marked neuronal loss. Since a significant increase in mast cell number within thalamus was observed on PTD day 6, the earliest day examined, the onset of this cellular response is unknown. Nevertheless, this observation clearly demonstrates that changes in mast cell number precede even the earliest changes reported in vascular permeability and astrocyte endfoot swelling within the PTD rat model. Failure to demonstrate a significant effect of thiamine deficiency in the number of mast cells within specific regions of the thalamus (i.e., medial and lateral) was most likely due to the large variance in mast cell number within the thalamus, an observation reported in other animal models (47) and untreated animals (34).

The overall increase in mast cell number within thalamus is due, at least in part, to blood born granulated cells migrating across endothelial tight junctions. A recent ultrastructural study conducted in our laboratories has observed granulated cells resembling mast cells within the smooth muscle layers of venules within thalamus of PTD day 8 animals (48). While mast cells do have the capability to divide by mitosis (49), our ultrastructural study found no evidence of dividing mast cells within thalamus of thiamine deficient animals. Furthermore, when mast cells degranulate they release IL-1 and TNF-α (50, 51), which have been shown to induce endothelial cells to produce adhesion molecules (52) such as ICAM-1 or VCAM. The recent report of increased endothelial ICAM expression within thalamus of thiamine deficient mice (53) may explain why mast cells or granulated cells are selectively attracted to blood vessels within the thalamus and not the hippocampus. In addition, thiamine deficiency may directly trigger endothelial cells to produce adhesion molecules since endothelial cells in culture are especially sensitive to thiamine deficiency (54).

Associated with an increase in cell number was a significant increase in proportion of mast cell that contained reduced numbers of toluidine-blue metachromatic granules, which was interpreted as evidence of mast cell degranulation and not fixation or staining artifact. Our recent electron microscopic study of thalamus of presymptomatic rats has detected mast cells containing partially empty vacuoles, many of which contained single or multiple swollen granules, as well as discharged granules containing fine punctate densities characteristic of a degranulating state, are present within thalamus (48). The present observations also demonstrate that the temporal onset of mast cell degranulation had a slightly different course within medial versus lateral thalamus. An increase in the percentage of degranulating mast cells occurred in the lateral thalamus on PTD day 7, and 1 day later in the medial thalamus. The pattern of change in the percentage of degranulating mast cells was also slightly different between the 2 thalamic regions. In the lateral thalamus, the percentage of degranulating mast cells steadily increased from days 7–12, while in the medial thalamus the percentage of mast cells degranulating reached a maximum on day 9 of treatment and remained relatively constant over days 10–12 of treatment.

The percentage of degranulating mast cells was also significantly greater in the lateral thalamus than the medial thalamus. This finding combined with the fact that the lateral thalamus also had a significantly larger number of mast cells than the medial thalamus suggests that a greater quantity of cytokines, chemokines, histamine, and other mast cell mediators were released into the lateral versus medial thalamus. This feature, together with the fact that mast cells begin to degranulate first in the lateral thalamus, may explain why the onset of lesions occurs earlier in lateral compared with medial thalamus in this PTD rat model (26).

The significant increase in the percentage of degranulating mast cells in PTD animals further suggests that thiamine deficiency is triggering the release of neurochemicals from mast cells, which may be the direct mediators of thalamic lesions in this model. Mast cells contain a variety of vasoactive substances (e.g., histamine, serotonin, heparin, and cytokines) that can alter the integrity of the BBB and potentially lead to the early vascular changes (24, 25) and BBB breakdown (27, 28) observed in experimental thiamine deficiency. Certain cytokines present in mast cells are also known to increase free radical production, elevate nitric oxide and extracellular glutamate levels, and decrease pH, all mechanisms that are known to cause neuronal destruction. Several studies have demonstrated an increase in reactive oxygen species (20), increased extracellular glutamate (17, 18), and acidosis (19) within the thalamus of the PTD rat.
model. In addition, mast cell degranulation may also contribute to the increase of extracellular histamine observed within the thalamus of PTD day 9 rats (31).

An important, but unanswered question is the mechanism by which thiamine deficiency triggers mast cells to degranulate. Neuropeptides, growth factors, neurotransmitters, and cytokines stimulate mast cells to degranulate (37–39). Both macrophages and astrocytes can release cytokines and thus thiamine deficiency-induced activation of astrocytes and macrophages may be responsible for mast cell degranulation within thalamus. However, the increase of both ED2 positive macrophages and GFAP-positive astrocytes within thalamus occurred after the onset of mast cell degranulation. Alternatively, signals generated by endothelial cells in response to thiamine deficiency may trigger mast cell degranulation directly or by stimulating the release of cytokines from microglia and macrophages. This possibility is consistent with the observation of enhanced expression of endothelial nitric oxide synthase and nicotinamide adenine dinucleotide phosphate diaphorase within microvessels in thalamus of preleision stage thiamine deficient mice (54). Furthermore, exposure of rat brain endothelial cells in culture to thiamine deficiency produced increased glucose consumption and lactate production, cytotoxic effects, and increased permeability of a monolayer of these endothelial cells (55). Additional studies are obviously needed to determine the cellular origin and specific substance(s) responsible for thiamine deficiency-induced mast cell degranulation.

While not a focus of the present study, interesting and unexpected regional differences were observed in the onset of macrophage proliferation within the diencephalon and the brainstem. It is important to recognize that ED2 is an immunohistochemical stain for a mature tissue macrophage antigen that is not found on monocytes (56–58). Most of the ED2 positive cells counted in this study appeared to be on or near blood vessels. Within the thalamus, ED2 macrophage proliferation occurred on day 12 of thiamine deficiency whereas in brainstem nuclei of the same animals, macrophage proliferation occurred much earlier (i.e. PTD day 6). These differences suggest that these cells may be playing different roles in the pathogenesis of thiamine deficiency-induced lesions within these areas. The onset of increased numbers of ED2 macrophages coincided with the appearance of neuronal damage within thalamus at a relatively late stage of thiamine deficiency. This finding suggests that these macrophages are not playing a direct role in the pathogenesis of lesions, but rather are responding to ongoing neuronal injury. In contrast, macrophage proliferation within brainstem nuclei prior to the onset of any behavioral symptoms or neuronal damage suggests that macrophages within the vestibular nuclei and inferior olivary complex may play a critical role in initiating a cascade leading to cellular damage and destruction. This hypothesis is supported by the observation that in the facial nuclei, a region relatively unaffected by thiamine deficiency, there was no change in macrophage number.

Finally, activation and proliferation of astrocytes occurred earlier in the brainstem compared with the thalamus, but in neither vulnerable region was this the initial cellular response to thiamine deficiency. The present findings suggest that activation of astrocytes is triggered by mast cell activity within thalamus and by macrophage activity within brainstem. Activated astrocytes may contribute significantly to the progression of thiamine deficiency-induced excitotoxic and free radical associated cell damage by increasing extracellular glutamate levels and releasing nitric oxide.

In conclusion, the present study provides evidence that the unique distribution of mast cells within thalamus may help to explain the selective vulnerability of this brain region to thiamine deficiency-induced lesions. Extensive degranulation of these mast cells would be expected to result in the release of large quantities of highly cytotoxic compounds, which may explain the fulminant and necrotic nature of TD to induce damage to thalamus as opposed to the selective neuronal loss observed in other brain regions devoid of mast cells. This proposed critical and novel role of mast cell activity in WE is further suggested by the finding that the increase in number and degranulation of mast cells occurs as early as day 6 of thiamine deficiency. To our knowledge this is the earliest cellular and potential molecular event to be described in the PTD rat model of WE. Chemical mediators such as the cytokines released from mast cells may also prove to be critical factors responsible for the increased vascular permeability and eventual breakdown of the BBB that precede neuronal loss and tissue destruction within this brain region.

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