Regional Changes in Brain Histamine Levels Following Dietary-Induced Thiamine Deficiency in Rats

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Abstract—Histamine levels in thiamine deficient rats were significantly lower in the hippocampus, amygdala, olfactory bulb, thalamus and pons-medulla oblongata than those of normal and pair-fed groups. In the case of the hypothalamus, thiamine deficiency produced a significant increase in histamine levels. These changes observed in the thiamine deficient group were reversed to the normal levels by supplying the normal diet. These data present a new finding that thiamine deficiency affects the central histaminergic neuron system as well as other monoaminergic systems.

Although earlier studies on the physiological roles of thiamine have shown that thiamine pyrophosphate (cocarboxylase) functions as a coenzyme in the metabolism of pyruvate and α-ketoglutarate (1), many other direct or indirect biochemical disturbances are known to occur in thiamine deficient animals (2, 3). For example, thiamine deficiency affects a number of the putative neurotransmitter systems such as serotonin (4, 5), catecholamines (6, 7), acetylcholine (8), glutamate (9), γ-aminobutyric acid (GABA) and so on (3, 10). Abnormalities in the uptake, binding, synthesis and metabolism of such neurotransmitters in the brain have also been found in thiamine deficient rats (3, 11). However, there are very few reports on the effect of thiamine deficiency on the central histaminergic system, since it has only recently been recognized that histamine is a neurotransmitter and/or neuromodulator in the mammalian central nervous system (12, 13). As the levels of histamine itself provide an index of the distribution of histaminergic nerve terminals and might be expected to be sensitive to impairment of interneuronal histamine storage or metabolic functions, we have therefore tried to study the regional changes in brain histamine contents in thiamine deficient rats.

Male Wistar rats weighing 60–80 g at the beginning of the experiments were obtained from Funabashi Farm Co. The animals were housed at a constant temperature (22±2°C) with a constant relative humidity (55±5%), and the light cycle was automatically controlled (7:30–19:30 hr). The rats were housed individually in mesh cages (17×25×37 cm) and divided into the following three dietary treatment groups: 1) The thiamine deficient group was provided with a powdered thiamine deficient diet (Funabashi Farm Co.) consisting of a basic ration, including 67.6% carbohydrate, 18% protein and 8% lipids, and supplemented with vitamins (except thiamine) and minerals. 2) The pair-fed group was given a complete diet during the experimental period in the amount consumed by its thiamine deficient littermates on the preceding day. The complete diet was identical to the thiamine deficient group diet except that it contained 0.5 mg/kg of thiamine hydrochloride per 100 g of diet. 3) The normal group was supplied ad lib. with the same complete diet. Water was available continuously to all groups. On the 30th day of experimental feeding, the thiamine deficient rats whose heart rate was less than 70% that

of normal rats were selected as the criteria of acute thiamine deficiency, using a programmable sphygmomanometer (PS-100, Riken Kaihatu and Tokai Irika). To obtain animals that have recovered from thiamine deficiency, some of the rats were again housed in mesh cages, but provided with thiamine-added normal diet ad lib. for the successive 30 days. We labeled them as the "reversal group".

The animals of all four groups were sacrificed by decapitation, and their brains were quickly removed and dissected on ice into regional parts by the method of Glowinski and Iversen with a slight modification (14). After homogenation of brains in 3% perchloric acid containing 5 mM sodium ethylenediaminetetraacetate by a Polytron homogenizer (Kinematica, Switzerland) for 10 sec in an ice-bath, the homogenate was centrifuged at 10,000 g for 30 min at 4°C, and then stored at −80°C until use. Fifty μl of the clear supernatant were injected into an HPLC column, and histamine was measured fluorometrically by the *o*-phthalaldehyde method as described by Yamatodani et al. (15). Statistical significance was assessed by Student's *t*-test.

In general observations, the rats maintained on a thiamine deficient diet for 30 days showed significant decreases in food intake, body weight, heart rate and rectal temperature. Thus, the thiamine deficient rats used in our experiments are in a state of acute thiamine deficiency based on criteria as described by Iwata et al. (6). Circling movements and muricide were also evident in this stage of acute thiamine deficiency in rats. These changes except muricide were normalized in the reversal group.

It is noteworthy that a state of acute thiamine deficiency affected the brain histamine levels in rats, as shown in Table 1. Histamine levels in the thiamine deficient group were significantly lower in the hippocampus, amygdala, olfactory bulb, thalamus and pons-medulla oblongata than those of the normal and pair-fed groups in our experiment ($P<0.05$). In the case of the hypothalamus, thiamine deficiency significantly increased histamine levels, which were 1.5 times higher than those of the pair-fed and normal groups ($P<0.01$).

It is well-known that thiamine pyrophosphate (cocarboxylase) functions as a coenzyme in the metabolism of pyruvate and α-ketoglutarate (1). However, it is very hard to explain the changes in regional histamine levels solely in terms of the coenzymatic action of thiamine. Thiamine deficiency has also been reported to inhibit net protein synthesis, namely in the cortex, cerebellum, and brain stem of rat brain (16). Enwonwu and Worthington reported that the protein-deficiency produced the elevation of brain histamine levels in rats (17). There are no significant increases in histamine levels in most

Table 1. Regional changes in rat brain histamine levels following dietary-induced thiamine deficiency

Regions	Histamine levels (ng/g)			
	Normal group	Pair-fed group	Thiamine deficient group	Reversal group
Cortex	32.2± 5.3	44.9± 2.6	26.6± 3.6	50.9± 9.6
Hippocampus	25.0± 4.5	21.5± 2.3	11.6± 1.5*	15.2± 3.7
Amygdala[a]	31.9± 2.0	37.2± 2.2	22.0± 3.6*	56.5± 9.4#
Striatum	31.6± 5.5	42.7± 5.9	47.3± 6.6	35.1± 5.5
Olfactory bulb	66.0±10.6	43.8± 7.5	10.5± 1.1**	38.4± 3.0#
Thalamus	104.9±14.4	101.3± 8.2	60.4± 8.5*	73.1±15.1
Hypothalamus	228.2±19.3	316.9±39.9	485.9±25.9**	209.3±28.0##
Midbrain	33.6± 7.6	54.3± 5.8	46.6± 6.1	53.8± 8.9
Pons-medulla oblongata	42.1± 8.8	46.3± 3.1	14.8± 3.2*	44.3± 6.3##
Cerebellum	16.1± 2.4	16.0± 2.7	10.1± 2.0	17.4± 2.1#

Each value represents the mean±S.E.M. of 6–8 animals. [a]Including pyriform and enthorhinal cortex. Statistical difference from the normal and pair-fed groups (*$P<0.05$ and **$P<0.01$) or from the thiamine deficient group (#$P<0.05$ and ##$P<0.01$).

brain regions of the thiamine deficient group, and the pair-fed rats showed the same histamine levels as those of the normal rats. Thus, it is likely that changes in histamine levels induced by thiamine deficiency in rats do not directly result from the concomitant decrease in food intake. The changes in histamine levels observed in the thiamine deficient group were reversed to those of the normal group by supplying a normal diet, as seen in the reversal group. Therefore, it is evident that the changes in regional histamine levels can be a consequence of the thiamine deficient feeding.

In addition, the participation of non-neuronal cells (mast cells) in the results obtained here should be also considered, since both histochemical and biochemical studies identified the presence of mast cells in the brain regions of several animal species (18). It has also been reported that histamine in mast cells might regulate the vasomotor tone or permeability of cerebral blood vessels (18). Thiamine deficiency resulted in abnormalities of glial metabolism followed by histopathological damage; and ultrastructural studies showed that in the early stages of thiamine deficiency, the lesion was intracellular edema involving glial cells, especially perivascular glial foot processes, and a ballooning and disintegration of myelin sheaths in the later stages (19). Thus, necrosis and hemorrhage may occur in the brain of thiamine deficient rats (19). These results suggest that the changes in histamine levels of thiamine deficient rats might reflect an increase in the number of mast cells during the course of the inflammatory processes developed in thiamine deficiency as described above. However, results on brain histamine levels, except for that in the hypothalamus, are difficult to reconcile with this hypothesis of the increase in mast cells. The decreased levels of histamine in most regional brains of thiamine deficient rats may be associated with an acceleration of histamine turnover (20, 21) and/or disturbance of axonal transport (22). This in turn requires a supply of histamine from cell bodies in the hypothalamus (23), where the synthesis of histamine is increased.

Anyhow, the changes in brain neurotransmitter systems may be responsible for the neurological manifestations observed in thiamine deficient animals. Recent immunohistochemical data, which indicated that histaminergic nerve fibers with clear appearance of varicosity are widely distributed in rat brain emanating from cell bodies in the mammillary body and posterior hypothalamus (23), suggest a variety of physiological roles of histamine in the hypothalamus such as thermoregulation, feeding behavior, sexual activity, sleep-wakefulness cycle, hormonal regulation and so on (12, 13, 24, 25). For example, a relationship between hypothermia and increasing histamine levels in the hypothalamus of thiamine deficient rats was suggested, because the intracerebroventricular injection of histamine leads to hypothermia in the rostral hypothalamic thermostat of the rats (25, 26).

Further investigation will be needed to clarify these mechanisms, but the results obtained here present a new finding that thiamine deficiency affects the central histaminergic neuron system as well as other monoaminergic systems (3–7).

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