Effects of maternal thiamine deficiencies on the pyramidal and granule cells of the hippocampus of rat pups

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Abstract. To understand the implication of thiamine deficiency in the neuronal atrophy and cell death we undertook to induce thiamine (B_1 vitamine) deficiency during three essential periods of the ontogenesis of rat central nervous system (CNS). Female rats were fed with a thiamine deprived diet during the gestation and lactation, and the fetuses and pups were alternately exposed to prenatal, perinatal or postnatal thiamine deficiencies. On the 45th postnatal day, histological studies were done on the brains of the pups and the structure of the hippocampus was analyzed. The effects of each treatment were assessed by measuring the size and the density of cell nuclei throughout the dentate gyrus and fields CA4, CA3 and CA1 of the hippocampal formation. The hippocampus showed a regional vulnerability in the pups exposed to maternal thiamine deficiencies. It appears that the thiamine deficiency decreased nuclear density (27.20%) more severely than nuclear size (10.56%) in the fetal hippocampus. Consequently, the major part of the teratogenic effects of thiamine deficiency was cellular death, rather than cellular atrophy.

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INTRODUCTION

General effects of alcohol on the developing brain are usually cellular atrophy and death (Bhave and Hoffman 1997, Bonthius and West 1991, Miller 1988, see however Miller 1995). Cellular atrophy and death could be the main causal factors of cerebral dysfunction in the fetal alcohol syndrome (Goodlett et al. 1992, Samson and Grant 1985). These factors contribute to mental retardation in children born from alcoholic mothers (Stoltenburg-Didinger and Spohr 1983). Another possible dependent factor may be the prenatal protein malnutrition (Lister et al. 2005), that is a frequent problem of children born to alcoholic mothers. Results of our previous studies suggest that in the fetal alcohol syndrome, alcohol provokes cellular atrophy and death by induction of B_1 vitamin (thiamine) deficiency (Bâ et al. 1999).

The studies carried out on the degenerative effects of alcohol on the fetal brain are legion (Bhave and Hoffman 1997, Jones and Smith 1973, Sokol et al. 1980). However, studies on the effects of thiamine deficiency on the fetal brain are few (Butterworth 1987, Haas 1988). In particular, there are no investigations of the effects of maternal thiamine deficiency on the developing brain. It has been reported that thiamine plays a crucial role in the cerebral metabolism (Greenwood et al. 1985, Héroux and Butterworth 1992) and that brain is especially vulnerable to the B_1 avitaminosis during its ontogenesis (Greenwood and Craig 1987, Greenwood et al. 1985). Also the maternal thiamine deficiency influences the fetus adversely (Roeckelein et al. 1985).

In the rat, neurogenesis of the hippocampal areas CA1–CA3 takes place in the prenatal period, while proliferation in the dentate gyrus continues throughout life (Bayer 1980, Miller 1995, Schlesinger et al. 1975, 1978). Shortly after neurogenesis, the process of developmental cell death (apoptosis) removes a large part of the newly generated cells (Cowan et al. 1984, Thomaidou et al. 1997). In the rat, the wave of physiological apoptosis in areas CA1–CA3 takes place prenatally, and in the dentate gyrus it accompanies neurogenesis throughout life (Biebl et al 2000, Ferrer et al. 1990). Therefore, the number of cells in the rat areas CA1–CA3 is constant in the postnatal life, while in the dentate gyrus it increases during the period of postnatal development (Boss et al. 1985, 1987). In the period of physiological cell death the population of newly generated cells is especially vulnerable (Castagne 1999, Thomaidou 1997), but also later hippocampal neurons may selectively die out in response to pathological factors (Tabuchi et al. 1992).

We undertook this work to get a quantitative assessment of cellular atrophy and death induced in the developing brain by the maternal thiamine deficiency. For this purpose, the cellular parameters such as density of cells and size of nuclei have been measured within the hippocampal formation that is a particularly vulnerable to thiamine deficiency (Iwata et al. 1985). The aim of this study was to understand how developmental thiamine deficiency was implicated in the processes of cellular atrophy and death.

METHODS

Subjects

Nulliparous female Wistar rats, weighing 180–200 g, were housed individually in plastic cages (27 cm × 37 cm × 18 cm) with the floor covered by wood-dust. A Wistar male was placed into each female's cage for 18 h daily. Presence of a vaginal plug indicated the day 1 of gestation. Starting from approximately 1 week prior to parturition, the dams were checked daily in the morning for pups. The colony was bred in an aerated noiseless vivarium room subjected to diurnal daylight/night cycles, humidity of 75% and an ambient temperature of $25 \pm 2°C$.

Treatment of the dams

Experimental dams were fed a synthetic thiamine-deficient diet n° 211 B_1, manufactured by UAR (Usine d'Alimentation Rationnelle, Epinay-Sur-Orge, France). Thiamine deficiency was induced during three main periods of CNS development: during fetal life (prenatal thiamine deficiency), from the end of fetal life to the 10th postnatal day (perinatal thiamine deficiency) and from birth to the 25th postnatal day (postnatal thiamine deficiency). At the start of the experiment, 3 or 4 dams were randomly assigned to one of the following experimental groups.

INDUCTION OF PRENATAL THIAMINE DEFICIENCY

A 10 day thiamine deprivation is sufficient to induce anoestrus in the female rat (Greenwood et al. 1983). In order to obtain maximum deficiency days before parturition (20 to 21 days after copulation), dams were fed the

thiamine-deficient diet 3 days before introducing a male into the female's cage. Females were then given a thiamine-deficient diet from conception to parturition. At the day of parturition, females were returned to normal synthetic diet (UAR, n° 210) which was given to pups after weaning until the 45th postnatal day. The average length of this thiamine deficiency induction period was 26 ± 2 days.

INDUCTION OF PERINATAL THIAMINE DEFICIENCY

Females were fed the thiamine-deficient diet 7 days after copulation, so that the real thiamine deprivation started around the Gestation Day 17. The diet was maintained during gestation and for the first 10 days of lactation. On the 10th postnatal day, females were given a normal diet and pups received the maternal regimen after weaning until the 45th postnatal day. This period of thiamine deficiency induction averaged 26 ± 1 day.

INDUCTION OF POSTNATAL THIAMINE DEFICIENCY

Females received the thiamine-deficient diet from birth till weaning, corresponding to the 25th postnatal day. At the weaning, the pups were treated identically to their mothers, being fed with a normal diet until the age of 45 days. During experimental induction of thiamine deficiency and the subsequent dietary reversion, all the groups were allowed access *ad libitum* to the different diets and water.

AD LIB CONTROL

Dams were fed a normal regimen (UAR, n° 210) *ad libitum* through gestation and lactation. After weaning, pups received the same treatment until 45 days of age. We did not intend to assess any specific effects of thiamine deficiency through the study of pair-fed groups, but we attempted to quantify grossly effects of different patterns of developmental thiamine deficiency on cellular size and density. In all the treatments, 8 to 10 pups were assigned to each nursing dam.

Histology of pups' brains

Histological studies were done on the brains of experimentally thiamine-deficient pups and controls to analyze the structure of the hippocampus. Ethical rules concerning *in vivo* experiments were observed in accordance with the Guidelines of the U.S. Public Health Service and NIH regarding the care and use of animals for experimentation.

TISSUE PREPARATION

On the postnatal day 45 pups born from at least three dams were pooled within each experimental group in order to minimize the litter influence. In every experimental group, six pups were killed randomly by carotid transection. The brains were carefully removed, weighed and fixed in Bouin's fluid. Then the brains were dehydrated through successive washes of alcohol solutions of increasing concentrations and embedded in paraffin. Parasagittal cuts, 10 μm thick, started with the left hemisphere and traversed the hippocampal formation. When the dentate gyrus of the ventral hippocampus exhibited a well-rounded crest (Bayer 1980), a sample of 20 consecutive cuts was taken for analysis. The cuts were stained with a combination of haematoxylin-eosin (nucleus and cytoplasm staining respectively) and indigo carmine (nucleus staining in particular). The slides were observed under oil immersion (× 1000).

REGIONS ANALYZED

In the 45-day-old rats, the cells were scanned, and then counted per 1000 μm^2 area throughout the hippocampal formation, from the fields CA1, CA2/CA3 and CA4 of Lorente de Nò (1934), to the granular layer of the dentate gyrus (Fig. 1). Here, reference to the hippocampal field CA3 overlaps fields CA2 and CA3 of Lorente de Nò (1934). An illustration of the effects of experimental thiamine deficiencies was shown on photomicrographs of field CA3.

CELL MEASUREMENT

Nuclei sizes from individual cells were measured on brain slides using an Olympus Bx 40 light microscope equipped with a camera lucida which projects a virtual image on a digitizing tablet. A cursor, connected to a microcomputer and equipped with a laser, allowed outlining the morphology of the cells seen through the ocular on the virtual image projected on the digitizing tablet. The distance run by the cursor on the virtual image was directly translated into real dimensions (μm) by the
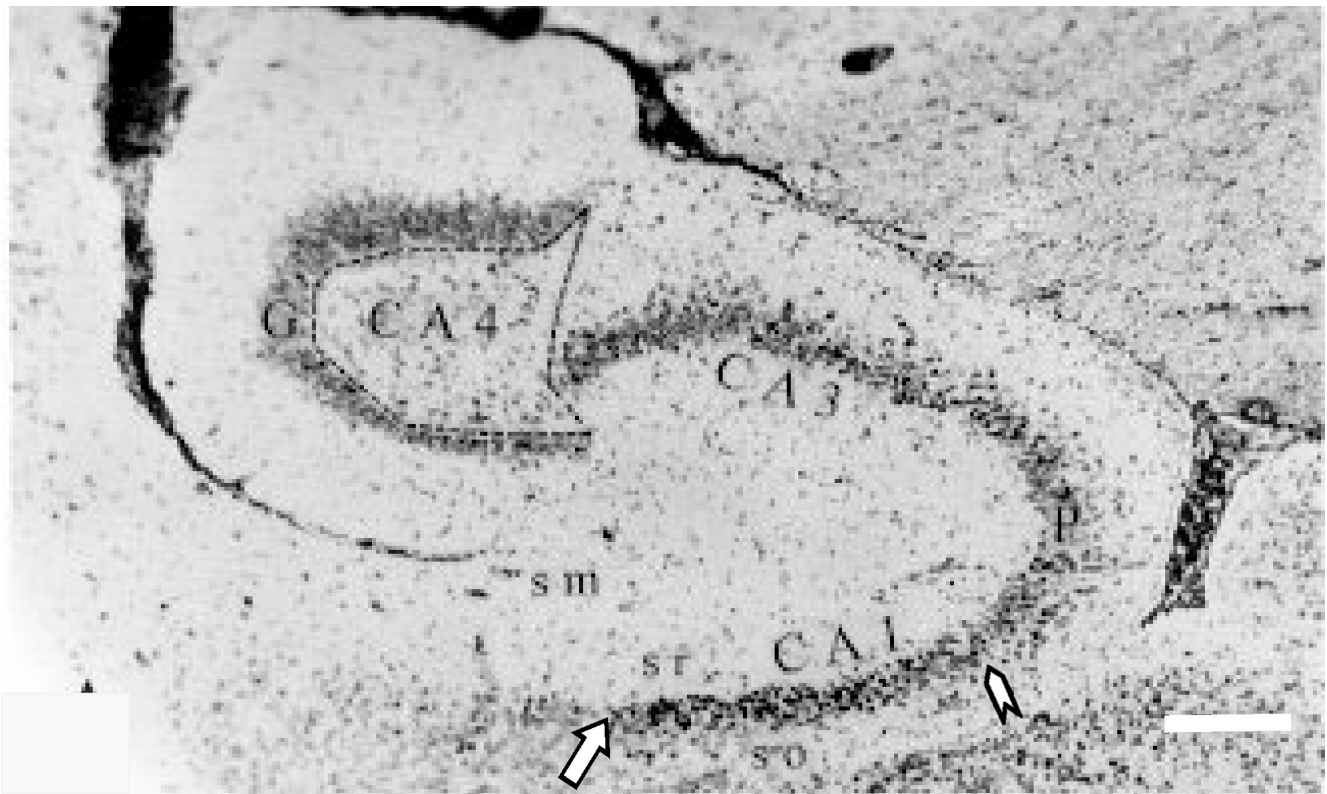
The percentages of reduction of the nuclear size and density variables *via* the 3 patterns of thiamine deficiencies were compared using the Kruskal-Walis one-way analysis of variance (Wayne 1987).

RESULTS

Effects of developmental thiamine deficiencies on the brain weight of the pups

ANOVA on brain weight (Table I) yielded a main treatment effect, $F_{3,24}=7.941$, $P<0.005$. *Post hoc* comparisons, using Scheffé's test ($P>0.1$) showed that the mean brain weight in the controls did not differ significantly from that exhibited by prenatal, $F_{1,12}=0.039$, and

Table I

Effects of developmental thiamine deficiencies on brain weight

Treatment	Brain weight (g) (n=7)
Control	1.54 ± 0.03
Prenatal thiamine deficiency	1.55 ± 0.01
Perinatal thiamine deficiency	1.39 ± 0.02*
Postnatal thiamine deficiency	1.50 ± 0.01

Brain weight in 45-day-old rats from different treatment groups. Values represent means ± SEM. The number of brains sampled is shown in parentheses. Scheffé test: (*) significantly different from all other groups ($P<0.01$).

postnatal thiamine-deficient pups, $F_{1,12}=1.594$. The only significant reduction of mean brain weight was exhibited by perinatal thiamine-deficient pups compared to the controls, $F_{1,12}=11.23$, $P<0.01$, prenatal, $F_{1,12}=11.17$, $P<0.01$, or postnatal thiamine-deficient pups, $F_{1,12}=10.4$, $P<0.01$.

Effects of developmental thiamine deficiencies on the nuclear size

All 3 patterns of pre-, peri-, and postnatal thiamine deficiencies, compared to normal diet, significantly decreased the mean nuclear size in the dentate gyrus significantly ($F_{3,222}=39.74$, $P<0.0001$) and in the fields CA4 ($F_{3,222}=13.97$, $P<0.0001$), CA3 ($F_{3,222}=21.39$, $P<0.0001$) and CA1 ($F_{3,222}=22.26$, $P<0.0001$), (Table II).

An exclusive comparison between thiamine deficiency patterns only, using the Scheffé F-test indicated that:

In the dentate gyrus, perinatal thiamine deficiency decreased mean nuclear size more significantly compared with either prenatal ($F_{1,48}=7.11$, $P<0.01$), or postnatal ($F_{1,48}=10.38$, $P<0.005$) thiamine deficiency. However, pre- and postnatal thiamine deficiencies did not differ significantly from one another ($F_{1,48}=0.31$, $P>0.1$).

In CA4, as in the dentate gyrus, thiamine deficiencies exhibited similar features. Indeed, while pre- and postnatal thiamine deficiencies did not differ from each other ($F_{1,48}=2.03$, $P>0.1$), they did significantly differ from the perinatal group that showed a more drastic reduction of mean nuclear size ($F_{1,48}=10.12$, $P<0.005$; $F_{1,48}=3.94$, $P=0.05$, respectively).

Table II

Effects of developmental thiamine deficiencies on nuclear size

Nuclear Size (μm)	Thiamine deficiency			
	Control	Prenatal	Perinatal	Postnatal
Dentate Gyrus (n=75)	26.84 ± 0.36 *	24.04 ± 0.28	22.79 ± 0.19 †	24.28 ± 0.24
CA4 (n=75)	30.58 ± 0.58 ‡	29.09 ± 0.58	26.54 ± 0.29 †	27.95 ± 0.31
CA3 (n=75)	34.41 ± 0.41 *	30.84 ± 0.62	30.76 ± 0.33	29.91 ± 0.28
CA1 (n=75)	28.83 ± 0.45 *	27.04 ± 0.47 ‡	25.11 ± 0.23	25.41 ± 0.24

Nuclei circumferences (sizes in μm) from individual neurons were scanned within the dentate gyrus, CA4, CA3 and CA1 to assess the effects of pre-, peri- and postnatal thiamine deficiencies. Values are given as means ± SEM. Values in parentheses (n) represent the number of nuclei scanned in every hippocampal field. (*) $P\leq0.01$ vs. all other groups; (‡) $P\leq0.01$ vs. peri- and postnatal thiamine deficiencies; (†) $P\leq0.05$ vs. pre- and postnatal thiamine deficiencies.

In CA3, pre-, peri- and postnatal thiamine deficiencies reduced the mean nuclear size with the same intensity, since comparison among the three treatments showed no significant difference ($F_{2,48}$=1.34, P=0.265).

In CA1, peri- and postnatal thiamine deficiencies, compared to the prenatal group, decreased mean nuclear size significantly ($F_{1,48}$=8.91, P<0.01; $F_{1,48}$=6.71, P<0.01, respectively). However, there was no significant difference between the effects of peri- and postnatal thiamine deficiencies ($F_{1,48}$=0.22, P>0.1).

Effects of developmental thiamine deficiencies on the nuclear density

Effects of the pre-, peri- and postnatal thiamine deficiencies found expression in a significant alteration of the average nuclear density in the dentate gyrus ($F_{3,27}$=7.34, P=0.0009), in fields CA4 ($F_{3,33}$=2.92, P=0.05), CA3 ($F_{3,33}$=6.24, P=0.0018), and CA1 ($F_{3,33}$=14.89, P<0.0001), (Table III).

The Scheffé F-test (P=0.05), used to identify the effects resulting from each pattern of thiamine deficiency indicated that:

In the dentate gyrus, in comparison with the normal diet, only prenatal thiamine deficiency decreased the average nuclear density significantly ($F_{1,18}$=5.59, P<0.05), whereas peri- and postnatal deficiencies had no significant effects ($F_{1,18}$=3.86 and $F_{1,18}$=0.43, respectively, P>0.05 in both).

In CA4, only prenatal thiamine deficiency, compared to the normal diet, decreased the average nuclear density significantly ($F_{1,22}$=4.39, P=0.05), while peri- and postnatal thiamine deficiencies showed no significant effects ($F_{1,22}$=2.08 and $F_{1,22}$=0.33, respectively).

In CA3, although the 3 patterns of thiamine deficiency tended to reduce the average nuclear density, it was the perinatal thiamine deficiency that decreased this density significantly, in comparison to the normal diet ($F_{1,22}$=5.01, P=0.05), unlike the pre- and postnatal groups that showed no effects ($F_{1,22}$=1.76 and $F_{1,22}$=2.24, respectively).

In CA1, in contrast, all the 3 patterns of pre-, peri- and postnatal thiamine deficiencies, in comparison with the normal diet decreased the average nuclear density very significantly (P's<0.01; $F_{1,22}$=8.51; $F_{1,22}$=12.99 ; $F_{1,22}$=6.63, respectively).

Quantitative assessment of the effects of developmental thiamine deficiencies

The effects of each pattern of developmental thiamine deficiency on the reduction of nuclear size (Table IV) and density (Table V) were expressed in percentages compared to the control, within fields CA4, CA3, CA1 and the dentate gyrus of the hippocampus. The Kruskal-Wallis one-way analysis of variance by ranks showed the effects of the 3 patterns of thiamine deficiencies to be different significantly

Table III

Effects of developmental thiamine deficiencies on nuclear density

Nuclear density	Thiamine deficiency			
	Control	Prenatal	Perinatal	Postnatal
Dentate Gyrus (n=10)	13.17 ± 1.36	6.71 ± 0.61 §	7.80 ± 0.51	11.40 ± 1.51
CA4 (n=12)	1.83 ± 0.44	0.92 ± 0.23 §	2.17 ± 0.42	2.25 ± 0.52
CA3 (n=12)	5.92 ± 0.36	4.08 ± 0.29	3.70 ± 0.52 §	3.83 ± 0.56
CA1 (n=12)	7.42 ± 0.42 *	4.58 ± 0.38	3.92 ± 0.38	4.92 ± 0.5

Density of nuclei within each hippocampal field was assessed following pre-, peri- and postnatal thiamine deficiencies. The microscopic images of dentate gyrus, CA4, CA3 and CA1 projected on a digitizing tablet, *via* a camera lucida, were compartmentalized into 1000 μm^2 areas within which nuclear counting was carried out. Cellular density was expressed as the number of nuclei per 1000 μm^2 of histological area. Values represent means ± SEM, with the number (n) of 1000 μm^2 areas sampled in each field in parentheses. (*) $P \leq 0.01$ vs. all other groups; (§) $P \leq 0.05$ vs. control.

from one another, for reductions of both nuclear density (H=741.23; P<0.008) and size (H=67.86; P<0.008), throughout the hippocampus.

Table IV

Average percentage of reduction on nuclear size by each type of thiamine deficiency

	% Reduction on nuclear size		
	Prenatal deficiency	Perinatal deficiency	Postnatal deficiency
Dentate Gyrus	10.43	15.11	9.54
CA4	4.87	13.21	8.60
CA3	10.37	10.61	13.08
CA1	6.21	12.90	11.86
Averaged percentage	7.97	12.95	10.77

The partial percentages of reduction of the nuclear size variable were calculated through different fields of the hippocampus, i.e., dentate gyrus, CA4, CA3, CA1 and averaged by type of thiamine deficiency. Percentages were calculated on mean values by the following formula: [100 – ((td*100)/c)] where td = thiamine deficiency; c = control.

Table V

Average percentage of reduction on nuclear density by each type of thiamine deficiency

	% Reduction on nuclear density		
	Prenatal deficiency	Perinatal deficiency	Postnatal deficiency
Dentate Gyrus	49.24	40.90	13.63
CA4	49.73	-18.58	-22.95
CA3	22.63	37.49	35.30
CA1	38.27	47.17	33.69
Averaged percentage	39.97	26.75	14.92

The different percentages of reduction on the variable nuclear density were calculated in dentate gyrus, CA4, CA3, CA1 and averaged by type of thiamine deficiency.

The general feature of cellular density alteration, caused by different types of pre- (Fig. 2B), peri- (Fig. 2C) and postnatal (Fig. 2D) thiamine deficiencies,

compared to the control (Fig. 2A), are shown on photomicrographs of CA3 pyramidal cells (Fig. 2). Pre- and perinatal thiamine deficiencies had 2.7 and 1.8 times greater effects on the reduction of nuclear density than the postnatal group, respectively. So, calculation of the average percentages of reduction indicated that the greatest effects on the reduction of nuclear density were exhibited by pre- (39.67%) and perinatal (26.75%) thiamine deficiencies, *versus* only 14.92% for the postnatal group (Table V). On the other hand, peri- and postnatal thiamine deficiencies reduced nuclear size more severely (12.95 and 10.77% respectively) than the prenatal group (7.97%), (Table IV).

The mean of averaged percentages of reduction following the three patterns of developmental thiamine deficiencies, showing the general intensity of maternal thiamine deficiency, indicated a mean reduction rate of 27.20% on nuclear density (Table V) and only 10.56% on nuclear size (Table IV), (P<0.008).

DISCUSSION

Induction of experimental thiamine deficiency at different stages of the hippocampal development showed that only the prenatal thiamine deficiency induced a clear deficit of cell number in the dentate gyrus and CA4, while only the perinatal thiamine deficiency caused a significant cellular deficit in CA3. On the other hand, the 3 patterns of pre-, peri- and postnatal thiamine deficiencies caused significant deficits of cells in CA1. In addition, the three patterns of thiamine deficiency significantly decreased the mean nuclear size in the fields throughout the hippocampus: perinatal thiamine deficiency was the more severe in the dentate gyrus and CA4, while peri- and post-natal thiamine deficiencies had the greatest effects in CA1. The 3 types of thiamine deficiencies affected the mean nuclear size in CA3 with similar intensity.

It appears that hippocampus shows regional vulnerability to thiamine deficiencies. While the dentate gyrus and CA4 were more vulnerable to pre- and perinatal deficiencies than the postnatal one, CA3 and CA1, i.e. the pyramidal layer exhibited a long-lasting vulnerability from embryonic to post-natal stage, since both structures were affected by the 3 types of thiamine deficiency. Consequently, induction of thiamine deficiency at any time of the brain ontogenesis could hinder the hippocampal functions.
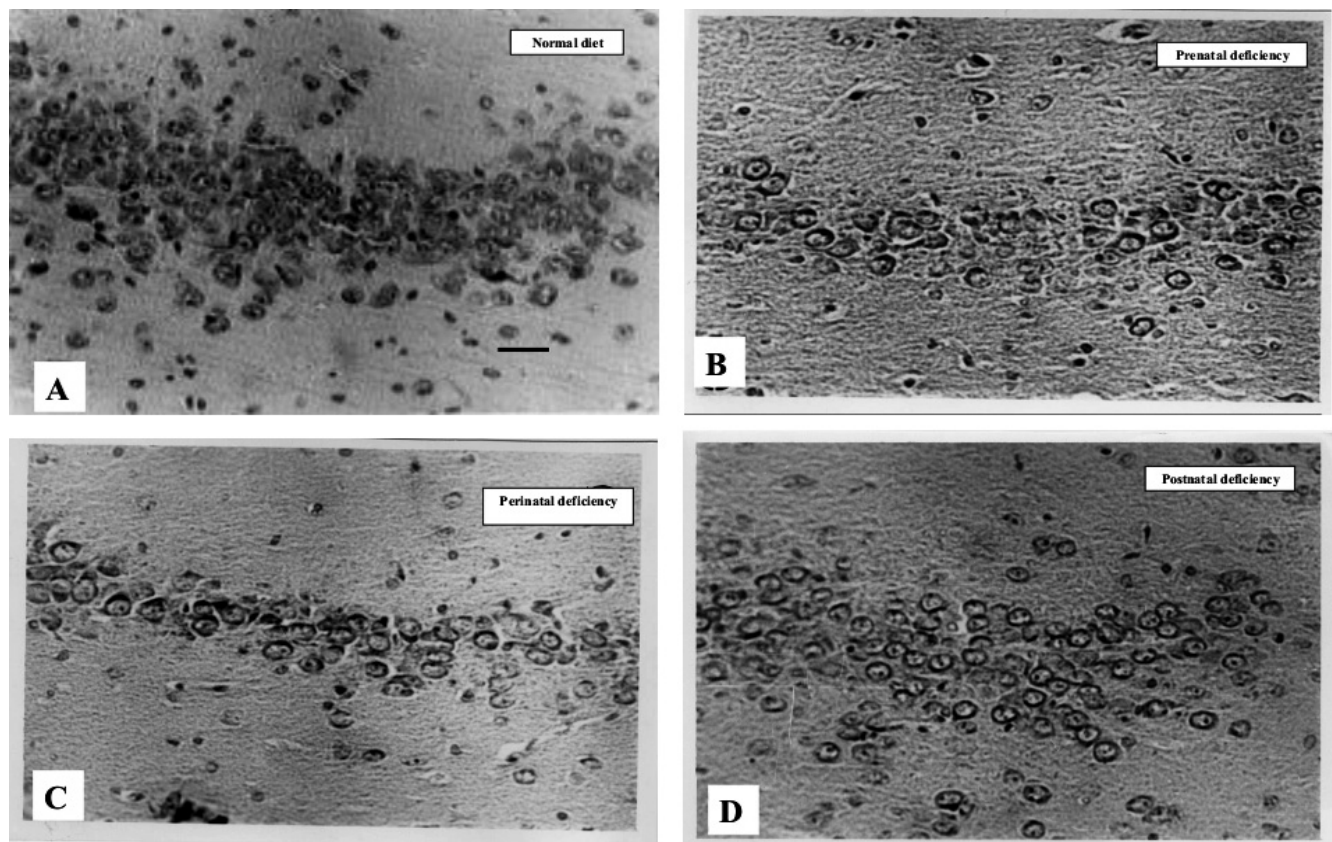
Fig. 2. Effects of developmental thiamine deficiencies on the CA3 pyramidal cells of the hippocampus. (A) controls; (B) prenatal exposed pups; (C) perinatal exposed; (D) postnatal exposed. The bar equals 20 μm on all pictures.

The average percentage of reduction, induced *via* every type of thiamine deficiency, was calculated on nuclear size and density from all hippocampal fields. The results showed that the greatest effects on the reduction of nuclear density were exhibited by pre- (39.67%) and perinatal (26.75%) thiamine deficiencies, *versus* only 14.92% for the postnatal deficiencies.

Which stages of cellular development would be particularly affected by developmental thiamine deficiency induced cellular death? Our previous studies showed that administration of an overdose of thiamine during developmental alcohol exposure results in irregular and sparse pyramidal cells within the hippocampal field CA3. Neither the developmental alcohol exposure alone nor the pair-fed saccharose produced such effects (Bâ et al. 1999). These results suggest that thiamine should interfere with the processes of proliferation, migration and apoptosis during development of the fields CA1–CA4 (Angevine and Sidman 1961, Bisconte and Marty 1975, Cowan et al. 1984, Schlesinger et al. 1978); the perinatal thiamine defi-

ciency would cover the cellular differentiation period (Hattori and Mcgeer 1973, Miller 1986) and the postnatal thiamine deficiency would interfere with the stages of axonal and dendrites' growth, synapses formation and myelinogenesis (Aghajanian and Bloom 1967, Hattori and Mcgeer 1973). However, neurogenesis in the dentate gyrus is very active also in the perinatal and postnatal periods and continues throughout life (Bayer 1980, Djavadian 2004, Schlesinger et al. 1975).

Our results showed that the prenatal thiamine deficiency may disturb those developmental processes that are active at that time: neuronal proliferation and migration. It is also very probable that it enhances the developmental apoptosis that is active shortly after that (Cowan et al. 1984, Ferrer et al. 1995). As a result, hippocampal cellular density was 39.67% lower than normal. Likewise, disturbances of cellular differentiation, axonal growth and synapses formation dependent on thiamine deficiency during the perinatal and postnatal periods could have induced cellular deficit (26.75% and 14.92%, respectively) because of

the diminished availability of trophic factors due to poor development of axons and synapses (Catapano et al. 2004).

Peri- and postnatal thiamine deficiencies reduced more severely nuclear size (12.95 and 10.77%, respectively) than the prenatal one (7.97%). These deficiencies would act directly or indirectly on cellular growth that begins during the perinatal period and continue through the postnatal period (Miller 1986, 1995). By what possible mechanisms can the developmental thiamine deficiency induce cellular death and atrophy? Thiamine (vitamin B_1) plays a crucial role in the cerebral metabolism (Greenwood and Craig 1987, Héroux and Butterworth 1992). In addition to its metabolic function, this vitamin was considered to exert a specific role on the central nervous tissues (Itokawa et al. 1972, Matsuda and Cooper 1981).

The massive cellular death, induced by thiamine deficiency during cellular proliferation and migration, could be attributed to the catabolic role of thiamine. During proliferation and migration, cellular movements would require a great deal of energy (Jacobson 1991) and thiamine is strongly implied in the cerebral synthesis of ATP (Butterworth 1986). In the brain of mice, activity of the thiamine-dependent enzymes, e.g. α-ketoglutarate and pyruvate dehydrogenases complexes, as well as the activity of the thiamine-independent enzymes, e.g. succinate and malate dehydrogenases of the tricarboxylic acid cycle was reduced following thiamine deficiency in the brains of mice (Bubber and Gibson 2004). When thiamine was removed from the culture of rat heart cells, thiamine-deprived cells showed a rapid decline of TPP concentration after 5 days, a decrease of ATP level by 50% after 8 days, an intensive degeneration and cellular death after 16 days of deprivation (Zangen and Shainberg 1997). Consequently, the failure of cellular metabolic energy could influence the cellular functions of proliferation, migration and apoptosis, resulting in massive cellular death. Another possibility is that the multiple changes due to the thiamine deficiency influence the redox state of neurons, which may either enhance or reduce both neurogenesis and apoptosis (Castagne et al. 1999).

Relatively modest cellular atrophy induced by peri- and postnatal thiamine deficiencies may depend on the anabolic role of thiamine. Indeed, thiamine participates also in the synthesis of proteins and nucleotides (Haas 1988).Thiamine deficiency was suggested to induce cellular atrophy by weakening of anabolism and reduction of nuclear proteins and RNA synthesis (Bâ et al. 1999, Henderson et al. 1976). It seems probable that the peri- and postnatal thiamine deficiencies hinder the synthesis of proteins and RNA that is very active during the postnatal stage (Balasz et al. 1971, Fish and Winick 1969). At present, it is not possible to state if the observed changes are due to thiamine deprivation *per se* or to secondary nutritional effects of thiamine deficiency.

Calculation of the mean from averaged percentages of reduction obtained, following the three patterns of developmental thiamine deficiencies, allowed for quantification of the general intensity of maternal thiamine deficiency on each cellular parameter studied in fetal brain. It appears that maternal thiamine deficiency, in its general expression, decreases more severely nuclear density (27.20%) than nuclear size (10.56%) in the fetal brain. These observations show that maternal thiamine deficiency reduces the nuclear density three times more than the nuclear size.

Why does the maternal thiamine deficiency alter the nuclear density more than the nuclear size? The massive cellular death may be mediated by pre-and perinatal thiamine deficiencies interfering with cellular proliferation, apoptosis, migration and differentiation. If it is plausible to explain massive cellular death during cellular proliferation and migration by a failure of energy metabolism caused by the lack of thiamine, how to explain the high rate of cellular death lasting on cellular differentiation which should be less vulnerable to this energy metabolism failure of thiamine?

The lack of thiamine and absence of its structuring influence on the developing biological membranes could explain the high rate of cellular death recorded during cellular differentiation. The interference of thiamine with the biological membranes is ubiquitous. Some previous studies reported that thiamine is an active component of the axoplasmic, mitochondrial (Itokawa et al. 1972, Tanaka and Cooper 1968) and synaptosomal membranes (Matsuda and Cooper 1981). Thiamine acts against the effects of ethanol induced cellular membrane fluidity, and therefore it increase membrane stability (Bâ et al. 1996). Thiamine exerts a non-specific stabilizing interaction on the axonal membrane (Goldberg et al. 2004). A recent study has reported that vitamin B_1 protects against the cytotoxic effects of the main alcohol metabolite, acetaldehyde, through abolition of the

acetaldehyde-induced protein damage and apoptotic cell death in cardiac ventricular myocytes (Aberle et al. 2004). The last effects were reported to be relatively selective and specific, since neither the vitamin B_6 nor B_{12} were effective in abolishing the acetaldehyde-induced cytotoxicity (Aberle et al. 2004). From these observations we can assume that thiamine should be also able to stabilize the membranes of the newly generated neurons. Indeed, cells in the terminal phase of migration might need thiamine to stabilize their cell membrane and to begin differentiation. The stabilization of the cellular membrane could be the starting factor of cellular recognition and incorporation of the new cells into structures. The lack of vitamin B_1 should result in the lack of recognition of cells by its neighbors within the structure of destination, what could cause either cellular loss or ectopic placement of the cells.

Moreover, a significant cellular deficit and microencephaly was found exclusively in the perinatal thiamine deficiency, while cellular deficit without any microencephaly were noted in the pre- and postnatal thiamine deficiencies. Theses observations indicate that the cellular deficit and loss of brain weight is induced only at some developmental stages, possibly in relation to their metabolic or trophic regulation of the developing neurons at that time (Castagne 1999). The microencephaly induced uniquely by the perinatal thiamine deficiency shows evidently that the effects of the developmental thiamine deficiency may be also indirect, and their nature remains to be determined. Thus, further studies are required to elucidate the mechanisms underlying the developmental thiamine deficiency-induced cellular atrophy and death.

To conclude, our results showed that a major part of the teratogenic effects of thiamine deficiency was exerted by induction or enhancement of cellular death rather than by cellular atrophy. Consequently, the fetus exposed to the B_1 avitaminosis is at a real risk of developing a mental retardation.

CONCLUSIONS

In the rat pups hippocampus shows a region-dependent vulnerability for the maternal thiamine deficiency. In the fetal hippocampus this deficiency reduces cell density three times more than the size of nuclei (27.20% and 10.56%, respectively). Thus, the main effect of the thiamine deficiency was induction of massive cell death, rather than cell atrophy. Consequently, maternal thiamine deficiency is a major problem of public health.

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