LIPOGENESIS IN THE BRAIN OF THIAMINE-DEFICIENT RAT PUPS

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Summary  (1) The effect of thiamine deficiency during pregnancy and lactation on lipogenesis in the brain of rat pups was determined.
(2) Acetate incorporation to brain lipids in thiamine-deficient rats in vivo was no less than in pair fed control rats, apart from slightly reduced fatty acid synthesis in the cerebrum.
(3) Glucose incorporation to brain lipids in vivo was considerably reduced in thiamine-deficient pups.
(4) The inducible NADP dependent malic enzyme activity was increased in thiamine-deficient pup brains.
(5) The synthesis of acetyl-CoA appears to be the rate limiting step in lipogenesis in thiamine-deficient pup brains.

In both experimental animals and men, thiamine deficiency produces clearly defined lesions in the central nervous system (1, 2) which have not been properly investigated as yet. Thiamine deficiency reduces the activity of brain transketolase [EC 2.2.1.1] (2-4), and pyruvate decarboxylase [EC 4.1.1.1] in animals (5). The pentose phosphate shunt (PPS) represents the major pathway of NADPH synthesis, and oxidation of pyruvate is the step leading to acetyl-CoA formation via the tricarboxylic acid cycle (TCA). Thus, lipogenesis, which requires both acetyl-CoA and NADPH may be reduced. DREYFUS (6) reported that the microscopic appearance of the central nervous system lesions in thiamine deprived animals revealed destruction of selective myelinated structures. However, GEEL and DREYFUS (7) were not able to detect changes in brain gross lipid composition that could be attributed to thiamine deficiency rather than to the accompanying malnutrition resulting from anorexia. Lipid composition would, however, only be expected to be altered in advanced deficiency. Under more severe thiamine deficiency than that induced by GEEL and DREYFUS (7), we have recently reported decreased brain lipid levels in rat pups reared by thiamine deficient dams as compared to a pair fed control (8). This study reports on changes in lipogenesis occurring in thiamine deficient rat pups.
MATERIALS AND METHODS

Female Charles River rats, weighing 180–200 g were mated with males of the same strain. Pregnancy was established when vaginal plugs or sperm were found. The day following the night of mating was considered day zero of pregnancy. Rats were housed individually in cages with galvanized mesh flooring. For the first 10 days of pregnancy rats had access to commercial chow and water ad lib. On the 10th day of pregnancy rats were divided into three groups of ten rats each, matching in weight and maintained on rations with the following composition, in percent: vitamin-free casein (NBC, Cleveland, Ohio), 30; dextrose, 55; soybean oil, 8; salt mix, 6 (9); vitamin mix (9) thiamine free, 1. Thiamine (Thiamine hydrochloride, Sigma Chem. Corp., St. Louis, Mo.) was added daily to each food cup as follows: control (C)—had free access to food and received 34 μg thiamine daily; thiamine deficient (TD)—had free access to food and received 4 μg thiamine daily; pair fed control (PFC)—each rat received the amount of food consumed the previous day by the matched TD rat and received 34 μg thiamine daily. Water was available ad lib. A light was kept on from 6 a.m. to 6 p.m., and the temperature in the room was 24°C to 26°C. Food intake was recorded daily, and body weight every seven days. Litters were weighed within 6 hr of birth and were reduced to 4 (2 female and 2 male pups). Pups were weighed at 3-day intervals and were left to nurse for 21 days. At the specified age matching pups from the 3 groups were weighed and killed by decapitation. The brain was rapidly excised, weighed and either immediately processed or quickly frozen in acetone dry ice mixture and stored in sealed, waterproof plastic bags at −20°C until tested.

Transketolase activity was determined by sedoheptulose accumulation by the method of BENNETT (10). Pyruvate levels were determined by a modified coupled enzymatic reaction of LONG (11) and HENRY (12), described in Sigma Technical Bulletin No. 726-UV/826-UV (1974).

Malic Enzyme (NADP) [EC 1.1.1.40] activity was measured by following the production of NADPH after addition of malic acid essentially according to the method of OCHOA (13) and SALGANICOFF and KÖEPPE (14). The above procedure was modified by addition of dialysis of the mitochondrial fraction in 0.15 M KCl for 12–16 hr in 4°C. Results are expressed as units per 4 min per 100 mg brain tissue.

Incorporation tests with 14C labelled compounds were carried out as follows: 12.5 μCi U-14C glucose or 1-14C acetate in physiological solution were injected I.P. into 21 day old rats and the animals were decapitated 30 min later, based on time course studies for both glucose and acetate on control brains from 21-day-old rats (Table 1). Brains without the olfactory bulbs were quickly excised and separated into cerebrum and cerebellum. In vitro incorporation of 1-14C acetate and U-14C glucose into rat brain lipids was determined after incubation for 2 hr in buffer (15, 16). The tissue was saponified with KOH and separated into un-
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Table 1. Incorporation of 1-14C acetate and U-14C glucose in brains of control 21 day old pups in vivo.

| Time   | Site       | Acetate Saponifiable lipids | Acetate Unsaponifiable lipids | Glucose Saponifiable lipids | Glucose Unsaponifiable lipids |
|--------|------------|-----------------------------|-------------------------------|-----------------------------|-------------------------------|
| 15 min | Cerebrum   | 0.51 ± 0.09                 | 1.00 ± 0.13                   | 0.11 ± 0.03                 | 0.14 ± 0.05                   |
|        | Cerebellum | 0.79 ± 0.18                 | 0.12 ± 0.05                   | 0.21 ± 0.10                 | 0.25 ± 0.11                   |
| 30 min | Cerebrum   | 1.40 ± 0.30                 | 4.10 ± 0.40                   | 0.31 ± 0.08                 | 0.38 ± 0.11                   |
|        | Cerebellum | 2.60 ± 0.50                 | 0.80 ± 0.60                   | 0.51 ± 0.11                 | 0.53 ± 0.14                   |
| 60 min | Cerebrum   | 2.10 ± 0.60                 | 7.60 ± 0.50                   | 0.46 ± 0.18                 | 0.71 ± 0.27                   |
|        | Cerebellum | 3.60 ± 0.70                 | 1.90 ± 0.40                   | 0.68 ± 0.34                 | 0.80 ± 0.13                   |

Values represent means ± S.D. of 4–6 rats at each time interval.

saponifiable and saponifiable fractions (15). The unsaponifiable material contained at least 90% cholesterol as determined by gas chromatography (15). Radioactivity was determined by liquid scintillation, and quench correction was applied using an external standard. Statistical treatments used were analysis of variance and multiple range tests (17).

RESULTS

Rat pups gained weight at normal rate for the first two weeks, after this pups from the TD and PFC groups showed reduced weight gain. Starting at this age the TD pups developed typical signs of thiamine deficiency. At first their spontaneous activity was reduced, their back became arched, and the hind legs extended. On a flat surface they moved in circles. At 18 days most of the TD pups could not stand but could only lay on their side in an arched posture, they also suffered from frequent quivering attacks. TD pups died by the 21st to 23rd day of life unless a large dose of thiamine was injected subcutaneously.

As shown in Fig. 1 transketolase activity in whole brain was reduced in TD pups from the first day of life and decreased further, reaching a minimum at approximately 30% of control levels at 14 days. Brain pyruvate levels began to increase from the 7th day, reaching almost 400% of the PFC and C levels. Malic enzyme activity was enhanced in the TD rats from the 14th day of life. Transketolase activity was slightly decreased and pyruvate levels and malic enzyme activity were somewhat increased in the PFC pups as compared to the C group, but these differences were not significant. Brain wet weights were similar (80–83%) in all groups.

Incorporation of acetate into the saponifiable lipids of the cerebrum was slightly decreased in TD pups relative to PFC, although increased incorporation into Unsaponifiable lipids was observed. Incorporation of acetate to lipids in the cerebellum of TD pups was above the levels found in PFC and C pups (Table
Fig. 1. Changes in whole brain transketolase (upper panel) and malic enzyme (lower panel) activities and in pyruvic acid levels (mid panel) with age in rat pups. Results are means of 10–12 pups and the bars represent 1 S.D., □ control, ■ TD, ■ PFC.

2) $^{14}\text{C}$-glucose incorporation in all lipids was considerably depressed in TD pups, both in the cerebrum and cerebellum (Table 3). In vitro incorporation (in parentheses) paralleled results obtained in vivo.

DISCUSSION

The appearance and development of clinical and biochemical symptoms of thiamine deficiency observed in this study parallel those in previous reports (1, 5, 6). In our study thiamine deficiency led directly to maternal malnutrition and secondary to pups' growth retardation due to its anorexic effect. We could also detect specific effects of thiamine deficiency in brains of TD pups. Transketolase activity was already decreased in TD pups at birth and after 7 days brain pyruvate was elevated.

Malnutrition has been shown to alter lipid composition in the developing rat brain (18, 19). We have noted this effect in brains of TD pups as has been reported elsewhere (8). Lipogenesis tended to be higher in the cerebrum than in the cerebellum; this may be connected with the fact that cerebrum neurones do not proliferate post-natally, whereas cerebellum neurone and glial proliferation, oc-
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Table 2. Incorporation of 1-14C acetate into brain lipids of 21 day old pups in vivo and in vitro.*

| Site        | C                  | TD                  | PFC                  |
|-------------|--------------------|---------------------|----------------------|
|             | (%) dose per 100 mg wet tissue | (%) dose per 100 mg wet tissue | (%) dose per 100 mg wet tissue |
|             | Saponifiable lipids | Unsaponifiable lipids |                     |
| Cerebrum    | 1.5 ± 0.2a         | 1.3 ± 0.6a          | 2.6 ± 0.3b          |
|             | (2.8 ± 0.4)        | (1.7 ± 0.3)         | (3.8 ± 0.4)         |
| Cerebellum  | 2.5 ± 0.4a         | 4.6 ± 0.8b          | 4.0 ± 0.6b          |
|             | (4.8 ± 0.8)        | (6.6 ± 0.7)         | (7.2 ± 1.1)         |

Values represent means ± S.D. of 11 pups and values in parentheses are results of 10 in vitro incorporation experiments and are expressed as percentage of injected dose incorporated per 100 mg wet brain tissue 30 min after injection. Results not followed by the same letter differ significantly (p<0.01).

* In vitro values as in parenthesis.

Table 3. Incorporation of U-14C glucose into brain lipids in 21 day old rats in vivo and in vitro.

| Site       | C                  | TD                  | PFC                  |
|------------|--------------------|---------------------|----------------------|
|            | (%) dose per 100 mg wet tissue | (%) dose per 100 mg wet tissue | (%) dose per 100 mg wet tissue |
|            | Saponifiable lipids | Unsaponifiable lipids |                     |
| Cerebrum   | 0.29 ± 0.03a*      | 0.12 ± 0.03b        | 0.46 ± 0.01c        |
|            | (0.41 ± 0.04)      | (0.20 ± 0.03)       | (0.52 ± 0.05)       |
| Cerebellum | 0.47 ± 0.08a       | 0.16 ± 0.04b        | 0.61 ± 0.02c        |
|            | (0.61 ± 0.07)      | (0.26 ± 0.05)       | (0.84 ± 0.06)       |
|            |                    |                     |                     |
| Cerebrum   | 0.32 ± 0.08a       | 0.25 ± 0.06b        | 0.63 ± 0.05c        |
|            | (0.48 ± 0.05)      | (0.34 ± 0.04)       | (0.76 ± 0.09)       |
| Cerebellum | 0.49 ± 0.09a       | 0.21 ± 0.04b        | 0.70 ± 0.07c        |
|            | (0.59 ± 0.04)      | (0.31 ± 0.05)       | (0.83 ± 0.07)       |

* See footnote Table 1.

curring post-natally, might be more sensitive to nutritional deprivation (20). In TD pups fatty acid synthesis from acetate in the cerebrum was slightly reduced, but otherwise lipogenesis from acetate proceeded normally, with cholesterol synthesis in the cerebrum even enhanced. Further studies with this model are required in order to elucidate the reasons for the increased incorporation of acetate into cerebellar lipids of the TD group. Lipogenesis from glucose, however, was depressed in TD pup brains under all conditions. The in vivo incorporation into brain lipids is influenced both by the rate of precursor supply to the brain, and by the actual synthetic activity. Thus, while it is possible that glucose was utilized specifically for purposes other than brain lipogenesis, such as amino acids, the
in vitro incubations, in which lipogenesis from glucose was also depressed, indicate that less acetyl-CoA formed from glucose was available for lipogenesis. The decreased incorporation of glucose into lipids in the TD brains is probably the result of decreased glycolytic activity as indicated by elevated levels of pyruvate and sedoheptulose (Fig. 1) rather than a dilution effect. Excess glucose in brain cells would cause an osmotic shift, a phenomena which has not been observed. Reduced glucose incorporation into lipids of TD pups may be affected by the levels of ketone bodies present in the brain as a result of relative malnutrition. Above physiological levels of ketone bodies have been recently reported by Patel and Owen (23) to reduce glucose oxidation and incorporation into brain lipids. The same holds true for pyruvate. The development of increased levels of ketone bodies and pyruvate in TD and PFC brains is expected to follow the same course as they lactate from dams fed similar amounts of diet and as similar growth rates are shown (8). The ability of the TD brain to utilize glucose differs markedly from that of the PFC brain since the latter consumes an adequate amount of thiamine and has close to control level of transketolase activity and pyruvate. The PFC brain can therefore generate enough NADPH via the PPS to thereby utilize the ketone bodies and pyruvate. Young et al. (24) and Wise and Ball (25) have shown that transhydrogenation of NADH to NADPH was an inducible alternative source for required NADPH. Under the present circumstances the amount of NADPH generated via this system is apparently not capable of generating all the NADPH necessary for proper brain lipid synthesis. Oxidation of free fatty acids may provide an additional source of acetyl-CoA in the brain, and may possibly be the source of the “thiamine sparing” effect of fat described in thiamine deficiency (21). In the canine puppy oxidation of free fatty acids can account for up to 25% of the total oxidative metabolism in the brain (22). However, under conditions of thiamine deficiency it is unlikely that the supply of free fatty acids to the brain is increased.

The findings of this study may suggest that though under various conditions of substrate availability the brain can utilize ketone bodies and pyruvate as carbon sources for lipid synthesis; under severe thiamine deficiency this was not the case. Neither glucose nor pyruvate have been utilized to any appreciable amount. Transhydrogenation which was stimulated in thiamine deficiency could not fully compensate for the needed NADPH. As has been shown in a previous paper (8) this resulted in permanent changes in myelin lipids.

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