Influence of Neonatal Hypothyroidism on the Development of Ketone-Body-Metabolizing Enzymes in Rat Brain

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Neonatal hypothyroidism markedly retarded the postnatal development of three ketone-body-metabolizing enzymes in rat brain during the first 4 weeks after birth. In contrast with normal animals, the brains of hypothyroid rats did not show decreases in the activities of these enzymes during the immediate postweaning period. The activities of ketone-body-oxidizing enzymes were markedly diminished in both non-synaptic and synaptic mitochondria isolated from 4-week-old hypothyroid rats compared with age-matched normal animals.

The importance of the ketone bodies, 3-hydroxybutyrate and acetoacetate, as metabolic fuels for developing and mature brain is well recognized (for reviews see Krebs et al., 1971; Owen et al., 1978). The ketone-body-metabolizing enzymes, namely 3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase, develop rapidly in immature rat brains during the first 2-3 postnatal weeks and attain activities 2-3-fold higher than in adult rat brain (Klee & Sokoloff, 1967; Page et al., 1971; Tildon et al., 1971; Thaler, 1972; Middleton, 1973). The activities of these enzymes in brains of suckling rats decline soon after weaning to adult values within a few weeks. Maternal starvation, deprivation of food immediately after birth (Thaler, 1972) or after weaning (Pull & McIlwain, 1971), and hyperthyroidism (Grave et al., 1973) have been shown to influence the postnatal development of 3-hydroxybutyrate dehydrogenase in suckling rats. Since neonatal hypothyroidism markedly influences the development of rat brain (Sokoloff, 1971), its effect on ketone-body-metabolizing enzymes and their distribution in non-synaptic and synaptic mitochondria was investigated.

Experimental

Pregnant (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.), lactating and weaned rats were maintained on Wayne Lab-Blok (Allied Mills, Chicago, IL, U.S.A.) and water ad libitum. Radiothyroidectomy was produced by administering intraperitoneally 150 μCi of carrier-free 131I to each rat at 1 day of age (Goldberg & Chaikoff, 1949). The control pups received the vehicle (0.1 ml of 0.9% NaCl) only. Litters were decreased to eight to diminish competition for the mother's milk.

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method of Lowry et al. (1951), with bovine serum albumin as standard.

Results and Discussion

Thyroidectomy induced soon after birth caused marked decreases in body and brain weights, which were evident only after the second postnatal week. During the third and fourth postnatal weeks hypothyroid rats grew poorly compared with normal animals (body weights of normal and hypothyroid rats were 41.5 ± 0.3 and 34.6 ± 0.6 g respectively at 3 weeks). A significant decrease in brain weights of hypothyroid rats was first observed in the third postnatal week (1448 ± 8 and 1316 ± 29 mg for normal and hypothyroid rats respectively; n = 8 each; P < 0.05). At 10 weeks the brain weight of hypothyroid animals was approx. 65% of that of normal controls. These findings are consistent with previous reports (Pasquini et al., 1967; Schwark et al., 1972).

The postnatal developmental patterns of d-3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase in brains of normal rats were similar to that reported previously (Klee & Sokoloff, 1967; Page et al., 1971; Tildon et al., 1971; Middleton, 1973). The activity of 3-oxo acid CoA-transferase in developing normal brain observed in our study is higher than that reported previously (Page et al., 1971; Tildon et al., 1971). This may be due to the measurement of this activity at 37°C in whole homogenates treated with Triton X-100. The activities of three ketone-body-metabolizing enzymes in brains of hypothyroid pups accumulated slowly as compared with those in normal rats during the first four postnatal weeks (Fig. 1). Very little postweaning decline was observed in activities of these enzymes in brains of 6-10-week-old hypothyroid rats (Fig. 1). In fact, at 6-10 weeks the activities of these enzymes were significantly higher in brains of hypothyroid rats compared with normal animals (Fig. 1). Similar alterations in the developmental pattern of 3-oxo acid CoA-transferase in brains of suckling rats treated with propylthiouracil have also been observed by F. Valdivieso of Madrid, Spain (personal communication). The patterns of pre- and post-weaning developmental changes in ketone-body-metabolizing enzymes between hypothyroid and normal rats remained unchanged whether the activities were expressed per g wet wt. (Fig. 1) or per mg of protein (results not shown). The development of pyruvate carboxylase in brains of normal rats has been reported previously (Wilbur & Patel, 1974). Fig. 1 shows that neonatal hypothyroidism also markedly retarded the developmental pattern of this enzyme (Fig. 1).

Since the activities of these enzymes in the brain are maximum in the fourth postnatal week, this age was chosen for mitochondrial studies. 3-Oxo acid CoA-transferase activity was similar in both non-synaptic and synaptic mitochondria isolated from 4-week-old normal rats (Table 1). In contrast with age-matched normal controls, the activity of 3-oxo acid CoA-transferase was markedly decreased (by 25-40%) in both mitochondrial populations from 4-week-old hypothyroid rats (Table 1). Table 1 also shows that the activity of acetoacetyl-CoA thiolase was significantly higher in non-synaptic mitochondria.
Table 1. Effect of neonatal hypothyroidism on several enzymes in non-synaptic and synaptic mitochondria from 4-week-old rats

Enzyme activities in the isolated mitochondria were measured at 37°C as described in the Experimental section. The results are means±S.E.M. for six experiments. P values: *P<0.05; **P<0.025; ***P<0.005; †P<0.001.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Animals</th>
<th>Activity (munits/mg of mitochondrial protein)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Non-synaptic mitochondria</td>
</tr>
<tr>
<td>3-Oxo acid CoA-transferase</td>
<td>Normal</td>
<td>1691±65†</td>
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<tr>
<td></td>
<td>Hypothyroid</td>
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<td>Acetoacetyl-CoA thiolase</td>
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<td>Pyruvate dehydrogenase complex (active)</td>
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<td></td>
<td>Hypothyroid</td>
<td>68±6***</td>
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<td>Pyruvate dehydrogenase complex (total)</td>
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<td></td>
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<td>Citrate synthase</td>
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<td>NAD⁺–glutamate dehydrogenase</td>
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<td>2148±141</td>
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<tr>
<td></td>
<td>Hypothyroid</td>
<td>2011±148</td>
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than in synaptic mitochondria from 4-week-old normal rats. Hypothyroidism markedly decreased (45–55%) the activity of this enzyme in both mitochondrial populations. 3-Hydroxybutyrate dehydrogenase was also significantly decreased (P<0.05) in non-synaptic mitochondria from brains of hypothyroid rats (111±2 mumits/mg of protein, n=6) compared with age-matched normal animals (121±4 mumits/mg of protein, n=6).

Although the activity of the active form of the pyruvate dehydrogenase complex was significantly altered only in non-synaptic mitochondria from 4-week-old hypothyroid rats compared with normal animals, the total pyruvate dehydrogenase complex was markedly decreased in both mitochondrial populations from hypothyroid rats (Table 1). A lack of increase in the total activity of the pyruvate dehydrogenase complex in hypothyroid rats suggests that in these animals this enzyme was present mostly in the active form. The activity of NAD⁺–glutamate dehydrogenase in the two mitochondrial populations was not altered by hypothyroidism. However, this treatment markedly decreased (40–45%) citrate synthase activity in both mitochondrial populations.

Neonatal thyroidectomy has been shown to cause a decrease in the postnatal increase in several glycolytic enzymes (Schwark et al., 1972) and mitochondrial enzymes, namely succinate dehydrogenase and aspartate aminotransferase (Hamburger & Flexner, 1957; Pasquini et al., 1967). Interestingly, the cytosolic aspartate aminotransferase was not altered in brains of hypothyroid rats (Pasquini et al., 1967). Administration of exogenous thyroxine to normal suckling rats was found to accelerate the postnatal development of 3-hydroxybutyrate dehydrogenase activity in brain by shifting the entire pattern of development of the enzyme to approx. 2 days earlier (Grave et al., 1973). It should be noted that the maximum activity of this enzyme per g of tissue in postweaning animals was, however, not altered in brains of hyperthyroid animals compared with normal rats. The data presented in Fig. 1 show that the developmental patterns of three ketone-body-metabolizing enzymes during the first 4 weeks of life were markedly diminished in brains of hypothyroid rats compared with age-matched normal animals. This cannot, however, be attributed to the diminished body and brain growth in hypothyroid rats, because decreased growth caused by dietary restriction (by increasing the size of the litter to 16 pups per nursing mother) had no adverse effect on the developmental pattern of these enzymes in malnourished pups (M. S. Patel, unpublished work). Further, the activities of the ketone-body-metabolizing enzymes in hypothyroid rats did not decline after maximal postnatal values had been reached (Fig. 1). This lack of decline during the postweaning period resulted in higher activities in brains of hypothyroid animals than in age-matched normal rats.

Weinberg & Utter (1979) observed a decrease in activities of pyruvate dehydrogenase, pyruvate carboxylase and citrate synthase in hepatic mitochondria from hypothyroid adult rats. Although protein synthesis is decreased in brains of suckling hypothyroid rats (Sokoloff, 1971), apparently only certain enzymes in the mitochondria are affected in brain (Table 1; Balazs et al., 1968) and liver (Weinberg & Utter, 1979) of hypothyroid rats. Since the developing brain derives energy from the oxidation of glucose and ketone bodies, the decrease in activity of several key enzymes involved in the oxidation of pyruvate and ketone bodies (Fig. 1; Table 1) may
contribute, in part, to the pathophysiology of growth and mental retardation associated with hypothyroidism.

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