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

By Mulchand S. PATEL*

Departments of Medicine and Biochemistry, General Clinical Research Center,
Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140, U.S.A.

(Received 4 July 1979)

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 μ Ci of carrier-free ^{131} I 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.

* Present address: Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, U.S.A.

The mitochondria derived from non-synaptic and synaptic fractions were isolated from 4-week-old normal and hypothyroid rats as described by Lai & Clark (1976). The procedure was adapted with minor changes (D. R. Deshmukh & M. S. Patel, unpublished work) to use only three brains in each experiment. In our laboratory, mitochondrial populations isolated from rat brain routinely show functional integrity (respiratory-control ratios of 5 or higher with 5 mM-pyruvate plus 2.5 mM-malate or 5 mM-glutamate plus 2.5 mM-malate) (D. R. Deshmukh & M. S. Patel, unpublished work).

Animals were decapitated and whole brain homogenates were prepared in buffered sucrose (0.25 M-sucrose/1 mM-2-mercaptoethanol/10 mM-Tris/HCl, pH 7.4). A portion of the homogenate was centrifuged at 100 000g for 45 min to remove the cytosolic acetoacetyl-CoA thiolase. The pellet was resuspended in the same volume with ice-cold sucrose/Tris buffer and sonicated for two 30 s periods at 60 W (model W185; Heat Systems Ultrasonics, Plainview, NY, U.S.A.). 3-Hydroxybutyrate dehydrogenase (EC 1.1.1.30) and acetoacetyl-CoA thiolase (EC 2.3.1.9) were measured in these preparations (Williamson *et al.*, 1971; Middleton, 1973). A portion of the initial homogenate was treated with Triton X-100 (final concn. 0.5%, w/v) before it was used to assay activities of other enzymes in whole homogenates and mitochondrial preparations. The activities of 3-oxo acid CoA-transferase (EC 2.8.3.5) (Williamson *et al.*, 1971), pyruvate carboxylase (EC 6.4.1.1) (Ballard & Hanson, 1967; Utter & Keech, 1963), citrate synthase (EC 4.1.3.7) (Ochoa, 1955), NAD⁺-glutamate dehydrogenase (EC 1.4.1.3) (Lai & Clark, 1976) and active and total pyruvate dehydrogenase complex (EC 1.2.4.1) (Land *et al.*, 1977; D. R. Deshmukh & M. S. Patel, unpublished work) were assayed spectrophotometrically. One unit of enzyme activity is defined as 1 μ mol of substrate utilized or product formed/min at 37°C. Protein was measured by the

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

Fig. 1. Effect of neonatal hypothyroidism on the postnatal development of ketone-body-metabolizing enzymes and pyruvate carboxylase in rat brain

Activities of these enzymes were measured as described in the Experimental section: ○, normal rats; ●, hypothyroid rats. The results are means $\pm$ S.E.M. for six experiments.

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 $\pm$ S.E.M. for six experiments. *P* values: *P < 0.05; **P < 0.025; ***P < 0.005; †*P* < 0.001.

Enzymes	Animals	Activity (munits/mg of mitochondrial protein)	
		Non-synaptic mitochondria	Synaptic mitochondria
3-Oxo acid CoA-transferase	Normal	1691 $\pm$ 65	1759 $\pm$ 153
	Hypothyroid	919 $\pm$ 62†	1293 $\pm$ 38**
Acetoacetyl-CoA thiolase	Normal	118 $\pm$ 10	86 $\pm$ 10
	Hypothyroid	53 $\pm$ 3†	48 $\pm$ 1***
Pyruvate dehydrogenase complex (active)	Normal	105 $\pm$ 10	32 $\pm$ 2
	Hypothyroid	68 $\pm$ 6***	27 $\pm$ 1
Pyruvate dehydrogenase complex (total)	Normal	136 $\pm$ 9	67 $\pm$ 8
	Hypothyroid	69 $\pm$ 14***	33 $\pm$ 9**
Citrate synthase	Normal	1968 $\pm$ 48	2020 $\pm$ 82
	Hypothyroid	1094 $\pm$ 23†	1237 $\pm$ 23†
NAD ⁺ -glutamate dehydrogenase	Normal	2148 $\pm$ 141	1129 $\pm$ 58
	Hypothyroid	2011 $\pm$ 148	1309 $\pm$ 95

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 $\pm$ 2 munits/mg of protein, *n* = 6) compared with age-matched normal animals (121 $\pm$ 4 munits/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 (Hamburgh & 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.

This work was supported in part by the National Institutes of Health grants NS-11088, AG-01345 and HD-12643, and General Clinical Research Center grant 5 MO1 RR349. I thank Dr. O. E. Owen for support, and Cindy Raefsky and Carolyn Brown for technical assistance.

References

- Balazs, R., Kovacs, S., Teichgraber, P., Cocks, W. A. & Eyrs, J. T. (1968) *J. Neurochem.* **15**, 1335-1349
- Ballard, F. J. & Hanson, R. W. (1967) *J. Lipid. Res.* **8**, 73-79
- Goldberg, R. C. & Chaikoff, I. L. (1959) *Endocrinology* **45**, 64-70
- Grave, G. D., Satterthwaite, S., Kennedy, C. & Sokoloff, L. (1973) *J. Neurochem.* **20**, 495-501
- Hamburgh, M. & Flexner, L. B. (1957) *J. Neurochem.* **1**, 279-288
- Klee, C. B. & Sokoloff, L. (1967) *J. Biol. Chem.* **242**, 3880-3883
- Krebs, H. A., Williamson, D. H., Bates, M. W., Page, M. A. & Hawkins, R. A. (1971) *Adv. Enzyme Regul.* **9**, 387-409
- Lai, J. C. K. & Clark, J. B. (1976) *Biochem. J.* **154**, 423-432
- Land, J. M., Booth, R. F. G., Berger, R. & Clark, J. B. (1977) *Biochem. J.* **164**, 339-348
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Middleton, B. (1973) *Biochem. J.* **132**, 731-737
- Ochoa, S. (1955) *Methods Enzymol.* **1**, 735-739
- Owen, O. E., Patel, M. S. & Boden, G. (1978) in *Biochemical and Clinical Aspects of Ketone Body Metabolism* (Soling, H.-D. & Seufert, C.-D., eds.), pp. 155-165, Georg Thieme Publishers, Stuttgart
- Page, M. A., Krebs, H. A. & Williamson, D. H. (1971) *Biochem. J.* **121**, 49-53
- Pasquini, J. M., Kaplun, B., Garcia Argiz, C. A. & Gomez, C. J. (1967) *Brain Res.* **6**, 621-634
- Pull, I. & McIlwain, H. (1971) *J. Neurochem.* **18**, 1163-1165
- Schwark, W. S., Singhal, R. L. & Ling, G. M. (1972) *J. Neurochem.* **19**, 1171-1182
- Sokoloff, L. (1971) in *Handbook of Neurochemistry* (Lajtha, A., ed.), vol. 5B, pp. 525-549, Plenum Press, New York
- Thaler, M. M. (1972) *Nature (London)* **236**, 140-141
- Tildon, J. T., Cone, A. L. & Cornblath, M. (1971) *Biochem. Biophys. Res. Commun.* **43**, 225-231
- Utter, M. F. & Keech, D. B. (1963) *J. Biol. Chem.* **238**, 2603-2608
- Weinberg, M. B. & Utter, M. F. (1979) *J. Biol. Chem.* in the press
- Wilbur, D. O. & Patel, M. S. (1974) *J. Neurochem.* **22**, 709-715
- Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) *Biochem. J.* **121**, 41-47