COENZYME A TRANSFERASE ACTIVITY IN RAT BRAIN

J. Tyson Tildon, A. Lynne Cone and Marvin Cornblath

Department of Pediatrics, School of Medicine, University of Maryland, Baltimore, Maryland 21201 and Research Department of Rosewood State Hospital, Owings Mills, Maryland 21208.

Received March 5, 1971

Summary

Succinyl-CoA:3-ketoacid CoA transferase (CoA transferase) activity in the rat brain has been demonstrated to be 3-4 fold higher at 12 to 20 days of life than at birth or throughout adult life. No increase in the transferase activity was observed as the result of starvation. These data indicate that the metabolism of ketone bodies by the brain during early development proceeds through the activation of acetoacetate as catalyzed by CoA transferase.

Although it is generally accepted that the brain utilizes glucose as its primary source of energy, recent reports indicate that ketone body oxidation can be an additional energy source for this tissue in the newborn animal (1,2,3). Owen et al (4) have also demonstrated that prolonged starvation in humans results in the replacement of glucose by ketone body oxidation as the predominant fuel for brain metabolism, and Smith et al (5) have proposed that starvation of the adult rat will also result in an increase in ketone utilization by the brain.

Three enzymatic steps have been postulated for the conversion of ketone bodies to acetyl CoA which then permit the continued oxidation of these substrates via the tricarboxylic acid cycle.

1. D β-hydroxybutyrate ↔ Acetoacetate

2. Acetoacetate + Succinyl CoA ↔ Acetoacetyl CoA + Succinate

3. Acetoacetyl CoA + CoA ↔ 2 Acetyl CoA

These reactions are catalyzed by the enzymes (1) β-hydroxybutyrate dehydrogenase (EC 1.1.1.30), (2) 3-keto acid CoA transferase (EC 2.8.3.5) and (3) keto thiolase (EC 2.3.1.16).

Klee and Sokoloff (6) have shown that β-hydroxybutyrate dehydrogenase in the immature (15 day old) rat brain had a specific activity which was 3 times the activity found in the adult rat, and studies have shown that in the adult brain this enzyme increased at least 3 fold after 24 to 48 hours of starvation (5). Thiolase activity has also been demonstrated in both the infant and adult rat brain (7,8). This paper reports changes in rat brain CoA transferase that occur during early maturation, and it demonstrates that there was no increase in enzyme activity after 72 hours of starvation.

MATERIALS AND METHODS

Albino Wistar rats were used in all experiments and they were sacrificed by decapitation. Each homogenate was prepared from the brain of a single adult animal or from the brains of 3 to 5 pups of either sex. Fetal brain homogenates were prepared by pooling the brains from an entire litter (10-12 animals). Ten percent homogenates were made with 0.3% sodium deoxycholate in 0.25 M sucrose, then centrifuged 15 minutes at 20,000 X g at 4°C; the supernatant was used for enzyme measurements. Samples not assayed immediately were kept frozen at -20°C. Experiments showed that no change in activity occurred after 5 weeks of storage at -20°C (9).

CoA transferase activity was assayed by a modification of the method described by Hersh and Jencks (10) which measured the decrease in absorbance at 313 mμ of the enolate form of acetoacetyl CoA. The reaction mixture consisted of 0.003 M magnesium chloride, 0.067 M tris HCl, pH 8.40, 0.067 mM acetoacetyl CoA, and 0.0067 M succinate. All assays represent initial rates at 25°C. One unit of enzyme activity is expressed as the decrease of 1 mμmole of acetoacetyl CoA per minute in the presence of added succinate. Since no previous reports have definitively demonstrated CoA transferase in the brain, several criteria were used to substantiate that the measured activity was that of the enzyme. These included the partial purification of the enzyme, studies of substrate specificity in both the forward and reverse direction, acrylamide gel electrophoresis, and heat denaturation. Under the conditions of our assay, the A_{313} for the acetoacetyl CoA was 11.9 X 10^3 which is in good agreement with reported values (10). Protein was measured by the method of Lowry et al (11) and ketone bodies were determined by the method of Britton (12).

RESULTS AND DISCUSSION

The total amount of CoA transferase activity in the 0.3% sodium deoxycholate homogenate was consistently more than 5 times the activity obtained in the absence of the detergent. This increase has been shown to result from the solubilization of mitochondrial bound enzyme (9).

Using the 0.3% deoxycholate homogenate preparation, CoA transferase activity was measured in brain homogenates prepared

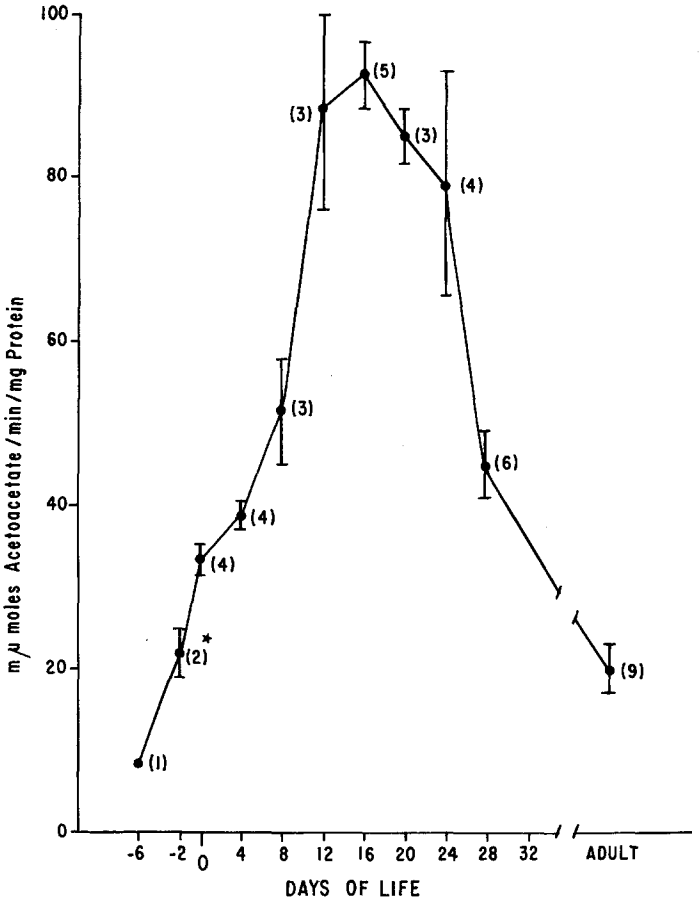

Figure 1 - CoA transferase activity in rat brain as a function of age. Enzyme activity was measured as described in the text. All results are expressed as mμmoles of acetoacetate formed per minute per mg protein. Each point represents the mean ± standard deviation. (* = mean and the range of two brain homogenates), ( ) indicates the number of separate homogenates used to determine each point.

---

from rats of various ages. Although the activity was low, measurable amounts of enzyme could be detected in the earliest (15 day old) fetal brain sample examined. At birth, the activity was 33.5 mμmoles/min/mg protein; this activity progressively increased during the next 14-18 days until it reached a maximum of 92.5 mμmoles/min/mg protein (Figure 1). The specific

TABLE I

COENZYME A TRANSFERASE ACTIVITY IN FED AND FASTED RATS

Hours of Fasting	Blood Ketone* Levels µmole/ml	Enzyme Activity Mµmoles Acetoacetate/min/mg Protein		
		Adults	Weanlings (26 Day Old)	15 Day Old Pups
0	0.24 ± .06	21.9 ± 3.1 (4)	38.1, 37.7, 48.9	71.3, 75.1
24	0.83 ± .22	19.5 ± 2.5 (4)	--	--
48	0.69 ± .25	19.9 ± 5.5 (4)	29.8, 38.4, 40.6	83.8, 77.1
72	0.83 ± .42	15.2 ± 1.5 (4)	--	--

* Ketone Determinations were Made on Adult Blood Samples Only. ( ) Indicates the Number of Animals.

activity then fell very abruptly until it reached a value of 20 mμmoles/min/mg protein which was maintained throughout adult life.

In contrast to the differences observed between the neonatal and the adult brain enzyme activity, essentially no difference was observed between fasted and fed rats (Table I).

The results reported indicate that CoA transferase is present in the rat brain. The observed changes in the enzyme activity that occurred during the early maturation of the rat brain, closely resembled the comparative rates of ATP generation from ketone bodies by mature and immature rat brain mitochrondria as reported by Klee and Sokoloff (6). In addition, this maturation pattern for CoA transferase also resembled the activity changes for β-hydroxybutyrate dehydrogenase that occurred during maturation as demonstrated by these same authors.

These data suggest that the increased ketone utilization by the rat brain during the neonatal period results at least in part, from the increase in CoA transferase, but the results do not support the conclusion that a starvation induced ketone utilization by the brain would be a consequence of an increase in this enzyme.

ACKNOWLEDGEMENTS

This investigation has been supported in part by Public Health Service Grant HD-03959-02 and a grant from the Bressler Reserve Fund.

REFERENCES

1. Drahota, Z., Hahn, P., Mourek, J., and Trojanova, M., Physiologia, Bohemoslovenica (Praha) 14, 134 (1965).
2. Itoh, T., and Quastel, J.H., Biochemical Journal, 116, 641 (1970).
3. Itoh, T., and Quastel, J.H., Science, 164, 79 (1969).
4. Owen, O.E., Morgan, A.P., Kemp, H.G., Sullivan, J.M., Herrera, M.G., and Cahill, G.F., Journal of Clinical Investigation, 46, 1958 (1967).
5. Smith, A.L., Satterthwaite, H.S., and Sokoloff, L., Science, 163, 79 (1969).
6. Klee, C.B., and Sokoloff, L., Journal of Biological Chem., 242, 3880 (1967).
7. McGarry, J.D., and Foster, D.W., Journal of Biological Chem., 244, 4251 (1969).
8. Dierks-Ventling, C., Tildon, J.T., and Cone, L., Journal of Cell Biology, 47, 50A (1970).
9. Tildon, J.T., Dierks-Ventling, C., Cone, L., and Sevdalian, D., Journal of Cell Biology, 47, 212A (1970).
10. Hersh, L.B., and Jencks, W.P., Journal of Biological Chem., 242, 3468 (1967).
11. Lowry, O.H., Rosebrough, N.J., Farr, H.L., and Randall, R.J., Journal of Biological Chem., 193, 265 (1951).
12. Britton, H.G., Analytical Biological Chem., 15, 261 (1966).