Activity of 3-Hydroxy-3-methylglutaryl-Coenzyme A Reductase in Brains of Adult and 7-Day-Old Rats

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Rat brain contains 3-hydroxy-3-methylglutaryl-CoA reductase activity, but this enzyme is far more active in 7-day-old brain than in adult brain. This difference may partly explain why cholesterol biosynthesis is more rapid in growing than in adult rat brain.

A feature of brain development is the so-called growth spurt, during which the brain grows rapidly and, together with other processes, myelination takes place; in the rat, this growth spurt begins at about the time of birth and continues for about 10 days (Dobbing, 1972). Cholesterol biosynthesis is known to be more rapid in young than in adult rat brain (Srere *et al.*, 1950), although evidently adult rat brain can synthesize cholesterol at a measurable rate (McMillan *et al.*, 1957; Nicholas, 1957; Nicholas & Thomas, 1958). Little is known of the enzymes of cholesterol biosynthesis in the brain, so that the biochemical basis for this difference in biosynthetic ability is poorly understood. In this present paper, the activity of 3-hydroxy-3-methylglutaryl-CoA reductase in adult rat brain is compared with that in 7-day-old rat brain. This enzyme was chosen for study because in the liver its activity varies enormously in response to various stimuli, and it is considered to be a regulatory enzyme of cholesterol biosynthesis.

Experimental

Animals

Wistar rats were obtained from Bantin and Kingman, Grimston, Aldbrough, Yorkshire, U.K. Adult rats weighed 200–250 g and females only were used; male and female 7-day-old rats were used. Animals were killed by decapitation, and the brains were immediately removed and placed in ice-cold medium containing 0.35 M-sucrose, 25 mM-KCl and 50 mM-Tris/HCl buffer, pH 7.6. The brains were then homogenized in this buffer (2.5 ml of buffer to 1 g of brain) in a motor-driven Teflon-glass homogenizer. The homogenate was then centrifuged, at 4°C, at 500g for 10 min and the supernatant was used as the source of 3-hydroxy-3-methylglutaryl-CoA reductase.

Enzyme assay

Brain 3-hydroxy-3-methylglutaryl-CoA reductase activity was assayed essentially as previously des-

cribed for the liver microsomal enzyme (Gregory *et al.*, 1972), except that the characteristics of mevalonate production as a function of incubation time and of the quantity of protein incubated were determined with the brain preparation described above. Mevalonate formation, by adult or 7-day-old brain, was observed to be linear for up to 40 min at a concentration of brain protein not exceeding 4 mg/ml. These conditions were observed in all subsequent assays. One unit of 3-hydroxy-3-methylglutaryl-CoA reductase activity forms 1 pmol of mevalonate in 1 min. Enzyme specific activity is expressed as units/mg of protein.

Chemicals

3-Hydroxy-3-methyl[3-¹⁴C]glutaryl-CoA was prepared as previously described (Gregory *et al.*, 1972). CoA (grade I) and NADPH (tetrasodium salt, grade II) were purchased from Boehringer Corp., London W.5, U.K.

Table 1. Sedimentation of 7-day-old rat brain 3-hydroxy-3-methylglutaryl-CoA reductase by centrifugation

Brains were homogenized as described in the Experimental section and centrifuged as indicated in the Table. The assay for 3-hydroxy-3-methylglutaryl-CoA reductase activity is described in the Experimental section, where units of enzyme activity are defined.

Preparation	Total 3-hydroxy-3-methylglutaryl-CoA reductase activity (units)
(a) Homogenate	15.0
500g × 10 min supernatant	12.0
500g × 10 min sediment	1.5
(b) Homogenate	22.0
10000g × 10 min supernatant	6.5
10000g × 10 min sediment	10.0

Table 2. 3-Hydroxy-3-methylglutaryl-CoA reductase activity in 500g supernatant of 7-day-old and adult rat brain homogenates

All values (except for brain wet weight) refer to the 500g supernatant of brain prepared as described in the Experimental section. 3-Hydroxy-3-methylglutaryl-CoA reductase activity was assayed as described in the Experimental section, where units of enzyme activity are also defined. Protein was estimated by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Each value in the Table is the mean $\pm$ s.e.m. of separate measurements on the number of rats shown in parentheses. Differences between means were evaluated by using Student's *t* test; values of *P* are given, where relevant, in the Results and Discussion section.

	7-day-old rats	Adult rats
Brain wet weight (g)	0.53 $\pm$ 0.02 (5)	1.8 $\pm$ 0.1 (5)
Protein (mg/brain)	25.5 $\pm$ 3.3 (5)	126 $\pm$ 13 (5)
Protein (mg/g wet wt. of brain)	48.0 $\pm$ 4.9 (5)	67.0 $\pm$ 8.3 (5)
3-Hydroxy-3-methylglutaryl-CoA reductase (units/mg of protein)	126 $\pm$ 8 (5)	3.6 $\pm$ 0.5 (5)
3-Hydroxy-3-methylglutaryl-CoA reductase (units/brain)	3260 $\pm$ 560 (5)	409 $\pm$ 57 (5)
3-Hydroxy-3-methylglutaryl-CoA reductase (units/g wet wt. of brain)	6100 $\pm$ 800 (5)	218 $\pm$ 26 (5)

Results and Discussion

Table 1(a) shows that when brain homogenate was centrifuged at 500g for 10min about 80% of the 3-hydroxy-3-methylglutaryl-CoA reductase remained in the supernatant. This 500g supernatant was subsequently used in preference to whole homogenate, which was observed to contain large particles, presumably clumps of unbroken cells, likely to introduce errors into the assay by preventing the removal of representative samples. Centrifugation of brain homogenate at 10000g for 10min resulted in the sedimentation of about 50% of the reductase activity [Table 1(b)]. In their study of mouse brain, Kandutsch & Saucier (1969) assayed a fraction that they termed 'microsomes', prepared by applying to brain a procedure designed to isolate liver microsomal fraction. It seems possible, from the results shown in Table 1(b), that much of the mouse brain reductase would have been lost in the sediment formed on centrifuging at 8000g for 10min, which they apparently discarded without assaying it for 3-hydroxy-3-methylglutaryl-CoA reductase activity. In the liver, 3-hydroxy-3-methylglutaryl-CoA reductase is exclusively microsomal, but in rat brain much of the enzyme seems to be associated with more rapidly sedimentable structures [Table 1(b)].

3-Hydroxy-3-methylglutaryl-CoA reductase activities in 7-day-old and adult rat brain are compared in Table 2. Adult brains were about 3½ times heavier than 7-day-old brains and contained about 5 times the amount of protein. However, the protein content per unit weight of brain was not significantly higher in adult than in 7-day-old brain ($P > 0.1$). 3-Hydroxy-3-methylglutaryl-CoA reductase specific activity was 35 times higher in 7-day-old brain than in adult brain ($P < 0.001$), and the reductase activity per unit weight of brain was about 30 times higher in the younger rat

brain than in the adult ($P < 0.01$). In terms of total activity per brain, 7-day-old brain contained about 8 times the 3-hydroxy-3-methylglutaryl-CoA reductase of the adult brain ($P < 0.01$). Since a comparison is being made between organs of different size, probably the most instructive way of expressing the enzyme activity is as units/g of brain, especially since the protein content per unit weight of brain is not significantly different between the two groups of rats.

The results reported here demonstrate that rat brain contains 3-hydroxy-3-methylglutaryl-CoA reductase activity, and that this enzyme is significantly more active in 7-day-old brain than in adult brain. Loss of certain enzymes may therefore partly explain the low rate of cholesterol biosynthesis in adult brain compared with that in growing rat brain.

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