Perinatal Development of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Rat Lung, Liver and Brain

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ABSTRACT

The developmental pattern of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34), which catalyzes the rate-limiting step of cholesterol biosynthesis, was studied in lung, liver and brain of Sprague-Dawley rats. Each tissue exhibited a distinct pattern. Reductase activity in the fetal lung reached a peak at 19 days of gestation, which corresponds to the onset of active surfactant production. This observation is consistent with the suggestion that the fetal lung synthesizes all surfactant components including cholesterol. In the liver, reductase activity varied in a reciprocal fashion with serum cholesterol levels. The peak of brain reductase activity occurred at 3 days after birth at the onset of rapid brain growth despite rapidly rising serum cholesterol levels.

A deficiency in the production of lung surfactant is presently considered a principle cause of the Respiratory Distress Syndrome seen in the newborn (1). Since surfactant contains large amounts of fully saturated phosphatidylcholines, mainly dipalmitoyl phosphatidylcholine, the synthesis of this phospholipid by alveolar cells has been extensively investigated (1). The chemical composition of surfactant shows that this material is not solely dipalmitoyl phosphatidylcholine but rather a complex mixture of several species of phospholipids, neutral lipids and proteins (2). The neutral lipid is mostly cholesterol which on a molar basis comparises 10-25% of the total lipid of surfactant (2) and may serve an extremely important physiochemical function. Although dipalmitoyl phosphatidylcholine would by itself provide a sufficiently low alveolar surface tension, its phase transition temperature is 41 C which means that it would be in a "solid gel" state at body temperature (3). The addition of cholesterol to dipalmitoyl phosphatidylcholine lowers the transition between the gel and liquid crystalline phase to below 37 C (3). Monoenoic phospholipids exhibit a similar effect. The presence of these lipids in surfactant would be expected to lower the phase transition temperature of the complex and thus insure proper adsorption of the surface active material at the alveolar interface (2).

Surfactant is produced and secreted by alveolar type II epithelial cells (4,5). These cells have the capability to synthesize monoenoic and fully saturated phospholipids (1,6). Developmental studies indicate that the enzyme activities responsible for the synthesis of these lipids reach maximal levels at 85 to 90% of term which corresponds to the onset of lung surfactant production (1). It is implied that all surfactant components are synthesized in the lung. However, no information is presently available concerning the levels of the enzyme activities involved in lung cholesterol biosynthesis during the fetal and neonatal periods.

In view of these observations, we have investigated the development of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) activity in lungs from fetal and neonatal rats. This enzyme catalyzes the rate-limiting reaction of cholesterol biosynthesis (7). For the purposes of controls and comparison, we also measured HMG-CoA reductase in livers and brains during development.

MATERIALS AND METHODS

Timed, pregnant Sprague-Dawley rats were purchased from ARS/Sprague Dawley, Madison, WI. The animals were housed in individual cages in a windowless room with lights on from 600 hr to 1800 hr and were fed Wayne Lab Blox and water ad libitum. Pregnant females at 16.5 to 21.5 days of gestation were killed by decapitation at 900 hr. The fetuses were removed by hysterotomy and immediately decapitated. A blood sample was obtained. The lungs, livers and brains from littermates were pooled and weighed. Suckling rats were killed at 900 hr also. For postweanling studies, the pups were removed from their mothers at 18 days of age and fed solid food in a windowless room with lights on from 2200 hr to 1000 hr the following day. All postweanling animals were killed at 1300 hr.

The tissues were minced and then homogenized with a motor-driven Teflon-glass Potter Elvehjem homogenizer using 10 ml of ice cold 0.3 M sucrose containing 1 mM dithiothreitol (SD) per gram of tissue. The broken cell preparation was centrifuged at 10,500 x g for 15 min. The resulting supernatant fraction was

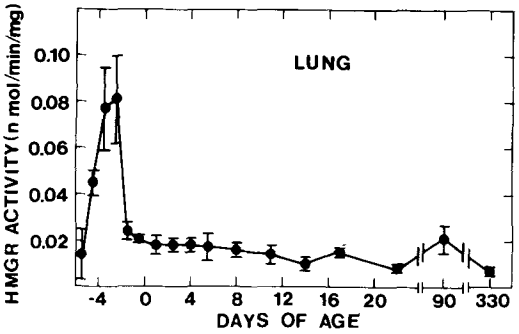

FIG. 1. Rat lung microsomal HMG-CoA reductase activity during development. The means and standard deviations are presented. Each point represents at least 3 litters or in the case of suckling pups at least 6 animals.

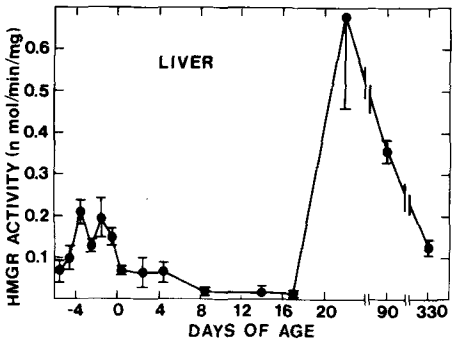

FIG. 2. Rat liver microsomal HMG-CoA reductase activity during development. The means and standard deviations are presented. Each point represents at least 3 litters or in the case of suckling pups at least 6 animals. Rat pups were weaned at 18 days of age.

centrifuged for 45 min at 145,000 x g. The pellet which contained the microsomes was resuspended in cold SD media using 1.5 ml per gram of tissue.

HMG-CoA reductase activity of these microsomal preparations was determined as described by Shapiro et al. (8). This method measures the formation of radioactive mevalonate from [^{14}C]HMG-CoA. The labeled mevalonate was converted to mevalonolactone and separated from the substrate by thin layer chromatography using acetone/benzene (1:1). Separation of labeled mevalonolactone from HMG-CoA by the frequently used anion exchange method (9) leads to erroneous values for HMG-CoA reductase activity caused by interfering enzyme activities present in isolated microsomes (10). Reductase activity was expressed in terms of nmol of mevalonate formed per min per mg of microsomal protein.

Microsomal protein was determined by a biuret method (11). Serum cholesterol levels were determined by the method described by Abell et al. (12).

RESULTS

If the cholesterol needed for the production of surfactant is derived from synthesis by the fetal lung, one would expect the development of cholesterol biosynthesis to parallel that of the other surfactant components. It has been shown that lung phospholipid synthesis and surfactant production increase sharply at ca. 90% of term (13). The developmental pattern of HMG-CoA reductase activity in rat lung microsomes is shown in Figure 1. A pronounced peak of reductase activity occurred at ca. 19 days of gestation or 86% of term (full term is 22 days). The activity then fell sharply before birth and remained at low levels. This pattern closely parallels that of fetal lung lecithin biosynthesis by the CDP-choline pathway (1). The peaks of lung cholesterol and phospholipid biosynthesis both occur at the onset of lung surfactant production.

Since the liver is considered to synthesize most body cholesterol, the development of hepatic HMG-CoA reductase was examined. As shown in Figure 2, hepatic microsomal HMG-CoA reductase activity is elevated during the last 4 days of gestation, when surfactant is being actively produced. Liver reductase activity declines after birth and reaches very low levels at 8 to 17 days of age. The activity then rises sharply as the rat pups are weaned onto solid food. These levels are considerably higher than those observed in adult rats. This general pattern for hepatic reductase activity agrees with that reported by McNamara et al. (14).

Since rat milk contains cholesterol and since it has been established that hepatic reductase activity decreases markedly in adult rats fed cholesterol (15), serum cholesterol levels were determined as a function of development (Fig. 3). Serum cholesterol levels increased 2- to 3-fold during the suckling period, when liver reductase levels reached their lowest values. Upon weaning, serum cholesterol levels fell concomitant with the sharp rise in hepatic reductase activity.

In contrast to the pattern in lung and liver, brain microsomal HMG-CoA reductase activity increases after birth reaching a peak at 3 days of age (Fig. 4). The time at which peak activity occurs corresponds to the onset of rapid brain growth. After 3 days of age, the specific activity of brain reductase steadily declines and does not increase when the rat pups are weaned

onto solid food as does the liver enzyme. Thus, brain microsomal reductase appears to be regulated independent of changes in serum cholesterol levels.

DISCUSSION

The essentiality of cholesterol biosynthesis to normal cell growth and function has been emphasized by the experiments of Kandutsch and Chen (16). These investigators have shown that when cholesterol biosynthesis in cultured cells is inhibited by the addition of oxygenated cholesterol derivatives, a number of consequence follow. These include: a decrease in intracellular sterol concentrations; followed by a decline in DNA synthesis; decreased rate of cell growth and ultimately cell death (16). These consequences can be prevented by adding mevalonate or cholesterol to the culture media. In experiments with phytohemagglutinin-stimulated lymphocytes, it was shown that a period of increased sterol synthesis must precede the synthesis of DNA and blastogenic transformation (17). Thus, it is of particular interest that HMG-CoA reductase, which catalyzes the rate-limiting reaction in cholesterol biosynthesis, reaches its peak activity in brain at 3 days after birth which corresponds to the onset of rapid brain growth. The high levels of brain HMG-CoA reductase observed in suckling rats (Fig. 4) are consistent with the previous suggestion (18) that the cholesterol required for myelin formation is derived from synthesis within the neural tissue.

The developmental pattern of brain cholesterol biosynthesis has been examined in mice by Kandutsch and Saucier (19). These investigators found a peak of synthesis at ca. 11 days after birth followed by a steady decline. In the only previous study of the developmental pattern of HMG-CoA reductase activity in rat brain, Aragon et al. (20) reported two peaks of enzymic activity; one at 10 days of age and the other at 20 dys of age immediately following weaning. These invetigators (20) also report only a 2- to 3-fold decrease in HMG-CoA reductase specific activity in the adult as compared to the newborn rat. These results differ considerably from those reported in Figure 4. The very low levels of HMG-CoA reductase activity found in adult brain in the present study are in good agreement with previous reports (21,22) of the rate of acetate incorporation into cholesterol. In these earlier studies, cholesterol synthesis was found to be markedly reduced with age and in some cases was not detectable.

In comparing the developmental patterns of

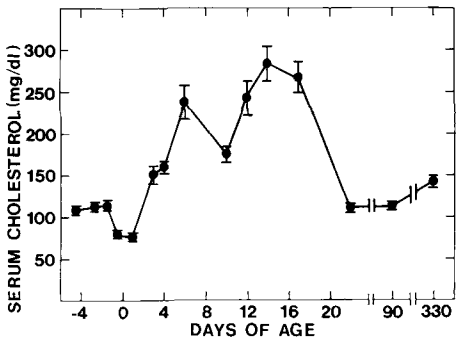

FIG. 3. Changes in serum cholesterol levels during development. The means and standard deviations are presented. Each point represents at least 3 liters or in the case of suckling pups at least 6 animals.

microsomal HMG-CoA reductase from lung, liver and brain, it is evident that the enzyme is not coordinately regulated. Rather, the enzyme activity appears to be responsive to the physiological demands of each tissue for cholesterol synthesis. For example, hepatic HMG-CoA reductase activity appears to vary in response to dietary intake of cholesterol. In the adult rat, these adaptive changes in hepatic HMG-CoA reductase activity appear to be mediated by changes in the intracellular concentrations of cholesterol and cholesterol esters (23,24). In the suckling rat, McNamara et al. (14) showed that liver cholesterol levels did not differ from those of the adult, but yet hepatic reductase activity was markedly lower. From the data presented in Figures 2 and 3, it is apparent that in the suckling rat hepatic reductase activity is inversely related to serum cholesterol levels.

The observation that in fetal lung the

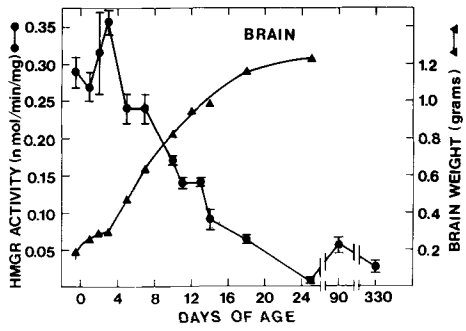

FIG. 4. Rat brain microsomal HMG-CoA reductase activity during development. The means and standard deviations are presented. Each point represents at least 3 litters or in the case of suckling pups at least 6 animals. Brain weight is presented in terms of wet weight. The values represent the means of at least 4 individual brains.

enzyme, which catalyzes the rate-limiting reaction in cholesterol biosynthesis, undergoes a sharp increase in activity at the time when surfactant production begins provides support for the view that the fetal lung has the capability of synthesizing each of the major components of surfactant including cholesterol. Studies on the development of surfactant production by the fetal lung have centered on the enzymes involved in phospholipid biosynthesis, particularly those responsible for the synthesis of dipalmitoyl phosphatidylcholine. As pointed out by Farrell and Avery (1), relevant data on the synthesis of apoproteins and other lipid constituents by the fetal lung is presently unavailable. A defect in the biosynthesis of any of the constituents of surfactant could result in a deficiency of surfactant and in impaired respiratory function.

ACKNOWLEDGMENTS

This work was supported in part by USPHS research grants HL 18094 and 5 S07 RR 05749.

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[Received August 25, 1978]