Essential Fatty Acid Deficiency: Metabolism of 20:3(n-9) and 22:3(n-9) of Major Phosphoglycerides in Subcellular Fractions of Developing and Mature Mouse Brain

GRACE Y. SUN, The Department of Chemistry, University of Missouri, Kansas City, Missouri 64110, and H. WINNICZEK, J. GO, and S.L. SHENG, The Ohio Mental Health and Mental Retardation Research Center, Cleveland, Ohio 44109

ABSTRACT

Essential fatty acid deficiency was initiated in young and mature mice. The metabolism of 20:3(n-9) and 22:3(n-9) in brain subcellular fractions was followed after the mice were switched from the deficient diet to a corn oil supplemented diet. After switching to the supplemented diet, the proportions of (n-9) polyunsaturated fatty acids in brain in both groups of mice decreased with time. The rate of disappearance of (n-9) polyunsaturated fatty acids was faster in the young groups than in the mature group. In the developing mice, the half-lives of the (n-9) polyunsaturated fatty acids in the total ethanolamine phosphoglycerides of brain microsomal, synaptosomal, and myelin fractions were 3, 10, and 15 days, respectively. In the mature group, the half-lives for 20:3(n-9) in diacyl-glycerophosphorylethanolamine of microsome, synaptosome, and myelin fractions were 8-10, 10, and 22 days, respectively; and the half-lives for 22:3(n-9) in alkenylacyl-glycerophosphorylethanolamine of the same subcellular fractions were 8-12, 28, and 35-40 days, respectively. In general, the rate of disappearance of 20:3(n-9) in brain was faster in the diacyl-glycerophosphorylethanolamine than in the alkenylacyl-glycerophosphorylethanolamine. These results demonstrate that the metabolism of (n-9) polyunsaturated fatty acid in brain phosphoglycerides during recovery from essential fatty acid deficiency not only varies with age, but also depends upon individual phosphoglycerides present in each subcellular fraction.

INTRODUCTION

It has been well recognized that during essential fatty acid (EFA) deficiency, there is a decrease in the proportion of 20:4(n-6) in the acyl groups of phosphoglycerides among various body tissues (1). The decrease in proportion of 20:4(n-6) usually is marked by an increase of 20:3(n-9), a new fatty acid presumably derived from 18:1(n-9) during the deficient state. Past studies on lipid changes among various body tissues during EFA deficiency have been rather extensive (2). However, in the more recent investigations, changes in brain lipids during the deficiency have been studied (3-7). Unlike other body tissues, the brain is especially rich in long chain polyunsaturated fatty acids (PUFA). Not only are specific acyl group profiles attributed to individual phosphoglycerides, they are also different in the subcellular fractions (8).

In a previous study, we have demonstrated that the increase in proportions of (n-9) PUFA in brain was greater when the deficient diet was initiated during the early developmental period (9). The ability of brain tissue to “recover” from EFA deficiency has been examined by White, et al., (10) with rats by measuring the decrease of 20:3(n-9) in brain after switching the animals to a corn oil supplemented diet. In the present investigations, we have further examined the metabolism of (n-9) PUFA in brain phosphoglycerides at a subcellular level during brain recovery from EFA deficiency. We intend to use this information to evaluate the metabolism of these acyl groups in brain phosphoglycerides during the early developmental period and after maturation.

MATERIALS AND METHODS

Animal Subjects and Induction of EFA Deficiency

Two experiments were performed with C57BL/10J mice which were purchased from Jackson Laboratory (Bar Harbor, Maine). In the first experiment, pregnant mice were obtained ca. 10 days prior to delivery. After being transferred to the laboratory, they were individually placed into plastic cages with stainless steel covers. All of the animals received water and diet ad libitum. One group of the pregnant mice was given a fat free diet and the other group was given a control diet supplemented with corn oil (General Biochemicals, Chagrin Falls, Ohio). The fatty acid-deficient diet contained the following ingredients (g/kg): casein, 240; sucrose, 710; salt mix no. 2, U.S.P. XIII (catalog no. 170870), 39; vitamin supplement
Body weight

Brain weight

FIG. 1. (A) A comparison of the brain and body wt of control and essential fatty acid (EFA) deficient mice at 21 days of age and at 10 and 40 days after supplementing with a corn oil diet. (B) A comparison of the brain and body wt of control and EFA deficient mice at 4.5 months of age. □ = control and ■ = deficient.

(GBI Technical Bulletin V-17), 10; and non-nutritive fiber, 9. The fatty acid-supplemented diet had the same composition as the deficient diet, except that corn oil (2%, w/w) was added. Chromatographic analysis of the fatty acid composition showed that the corn oil supplemented diet contained 16:0, 13%; 18:0, 2%; 18:1, 25%; and 18:2, 60%. After delivery, the litters were kept with the same mothers until 21 days. After this period, the litters were weaned. At 21 days, 3 young mice from each of the dietary groups were sacrificed. The brains were dissected and individually homogenized in 0.32 M sucrose. The remaining young mice in the deficient group then were switched to a control diet supplemented with corn oil. The weanlings in the control group also were given the corn oil supplemented diets at this time. At 10 and 40 days after switching to the supplemented diet, 3 mice from each dietary group were sacrificed. The brains were homogenized individually and homogenized in 0.32 M sucrose. The remaining young mice in the deficient group then were switched to a control diet supplemented with corn oil. The weanlings in the control group also were given the corn oil supplemented diets at this time. At 10 and 40 days after switching to the supplemented diet, 3 mice from each dietary group were sacrificed. The brains were homogenized individually in 0.32 M sucrose, and the brain homogenates were further subjected to subcellular fractionation and lipid analysis.

In the second experiment, mice were treated similarly, except that the pregnant mice were given Purina Lab Chow instead of the synthetic diets. After weaning, the young mice also were divided into two groups, one of which was given the fatty acid deficient diet and the other the corn oil supplemented diet. These young mice were reared on this dietary scheme for a period of 3 months, after which 3 mice from each dietary group were sacrificed. The brains were dissected individually and homogenized in 0.32 M sucrose as described above. The remaining group of mice with the deficient diet then was switched to the corn oil supplemented diet. After the dietary reversal, groups of three mice were sacrificed at 1, 3, and 5 weeks, and the brain homogenates were prepared similarly.

Subcellular Fractionation of Brain Homogenates

At the end of each period, the brains were dissected and individually homogenized in 20 volumes of 0.32 M sucrose solution containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM MgCl₂, and 15 mM Tris buffered at pH 7.4. The brain homogenates were further subjected to differential and sucrose gradient centrifugation to obtain the myelin, synaptosome-rich, and microsomal fractions. The procedure for subcellular fractionation was essentially the same as described previously (11), except that discontinuous sucrose gradient centrifugation to obtain the myelin, synaptosome-rich, and microsomal fractions. The procedure for subcellular fractionation was essentially the same as described previously (11), except that discontinuous sucrose gradient centrifugation to obtain the myelin, synaptosome-rich, and microsomal fractions. The synaptosome-rich fraction was obtained by isolating the material at the 0.8 M and 1.2 M sucrose interphase after gradient centrifugation. Purity of the synaptosome-rich fractions.