DIRECT PROOF OF PROPIONATE INVOLVEMENT IN CHOLINE SYNTHESIS IN RATS

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Choline, which belongs to the vitamins group, is a biologically important substance found in animals [5, 6]. Endogenous choline is formed in animals by the transfer of methyl groups from three S-adenosylmethionine molecules to the amino group of the ethanolamine residue in the structure of phosphatidylethanolamine. As a result of this reaction phosphatidylcholine is formed. However, the endogenous pathway of choline synthesis is insufficient to supply the physiological needs of the body, and for that reason choline intake in the diet is essential.

Choline synthesis *de novo* from [1-^{14}C]- and [2-^{14}C]acetate has been demonstrated only in the tissues of ruminants [1]. No direct evidence has been obtained of choline biosynthesis from free precursors in monogastric animals. Incorporation of labeled carbon from [1-^{14}C]acetate and [1-^{14}C]glycine into the nonfatty-acid moiety of cerebral cortical phospholipids has been demonstrated in Wistar rats [4]. There is also evidence that radioactivity from the C3-precursors [1-^{14}C]propionate and [2-^{14}C]malonate in rat liver and brain is incorporated into the nonfatty-acid moiety of glycerophospholipids, namely phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine; compared with malonate, propionate is the preferred precursor [3].

Since propionyl-CoA, like acetyl-CoA, is a key metabolite in animals, the study of its role in choline synthesis is most interesting. We know that the sources of formation of propionyl-CoA in the tissues are amino acids (valine, isoleucine, methionine, threonine), cholesterol, unsaturated fatty acids with an odd number of carbon atoms, etc. Oxidation of propionyl-CoA in mitochondria leads to the formation of acetyl-CoA, whereas carboxylation followed by isomerization leads to the formation of succinyl-CoA, a substrate for the tricarboxylic acid cycle. Propionyl-CoA can thus be incorporated both into the pathway of complete oxidation and into the pathway of neogenesis of many different substances: glucose, fatty acids, cholesterol, certain amino acids, etc.

The aim of this investigation was to obtain proof of propionate involvement in choline synthesis in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male Wistar rats weighing 180-240 g 2 h after feeding. Throughout the period of the experiment the animals received water *ad lib*. The animals were given an intraperitoneal injection of [2-^{14}C]propionate in a dose of 30 μCi/100 g body weight and they were decapitated 2 h after injection of the isotope. Specific activity of samples obtained from liver and brain tissue was measured on the Mark 2 instrument (from Nuclear Chicago, USA). Liver lipids were fractionated by chromatography in a thin layer of silica-gel (L 5/40μ, Czechoslovakia).

Preparative separation of the phospholipids was carried out in a chloroform—methanol—water system (65:25:4) [2]. Phosphatidylcholine was subjected to acid hydrolysis in 6N HCl for 24 h at 110°C in sealed ampuls. After hydrolysis, higher fatty acids were extracted from the digest with hexane, the water-soluble fraction was concentrated on a vacuum rotor evaporator, and glycerol and choline were isolated preparatively from the residue by ascending chromatog-

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TABLE 1. Specific Radioactivity (in cpm/g tissue) of Brain Lipids and also of Liver Lipids, Phosphatidylcholine, and Its Structural Components (M ± m)

Component	Brain	Liver
Total lipids	190±22	2595±152
Phosphatidylcholine:		
Total fraction	—	490±30
Fatty acids	—	295±23
Glycerol	—	572±144
Choline	—	94±10

raphy on paper (FN-12, East Germany) in a system of isopropanol—water—7 N NH_4OH. To purify the choline and glycerol they were chromatographed three times in the same system of solvents. In this system glycerol migrates with the solvent front whereas choline remains at the start.

EXPERIMENTAL RESULTS

The results showed that after injection of [2-^{14}C]proprionate into rats at the height of digestion the radioactivity of the liver lipids was more than 13 times higher than that in the brain lipids (Table 1).

The largest quantity of labeled carbon in the phosphatidylcholine fraction of the liver was found in glycerol, rather less in the total fatty acids fraction, and least of all in choline.

Considering that propionate is predominantly a substrate for biosynthesis of fatty acids with an odd number of carbon atoms in the animal body, the relative content of which in liver phosphatidylcholine usually does not exceed 3-4%, it can be postulated that the main part of the specific radioactivity of the total fatty acid fraction is concentrated in the structure of fatty acids with an odd number of carbon atoms.

The fairly high level of incorporation of the ^{14}C-atom of propionate into glycerol is a somewhat unexpected fact, for the view was held that the pathway of its neogenesis, linked with the partial section of the glyconeogenesis pathway, does not operate at the height of active digestion. As regards incorporation of label from [2-^{14}]propionate into choline, it may be pointed out that this was observed for the first time.

It is thus evident that propionate is incorporated into hydrophobic and hydrophilic components of phosphatidylcholine in satiated rats at the height of active digestion.

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