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Effects of Octanoic Acidemia on Palmitic Acid Metabolism in Rat Liver (41121)

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Abstract. This study examined the effects of octanoic acidemia on the metabolism of radioactive palmitic acid in rat liver. Octanoic acidemia, produced either by an intraperitoneal bolus injection or sustained intravenous infusion of sodium octanoate, markedly influenced biochemical pathways which are concerned with the hepatic disposition of palmitic acid into the different classes of rat liver lipids. During acute octanoic acidemia, the incorporation of [16-^{14}C]palmitic acid into hepatic triacylglycerols was greatly increased, while uptake of this fatty acid into phospholipids, especially phosphatidylcholine, was sharply reduced. When the octanoic acidemia resulted from intravenous infusion of sodium octanoate for 4 hr prior to the injection of [16-^{14}C]palmitic acid, the pattern of isotope incorporation was one which would support hepatic steatosis.

Intravenous or intraperitoneal injections of neutralized short-chain fatty acids, such as sodium octanoate, have been shown to produce narcotic actions in a number of animal species including the rat (1). The specific mechanism of short-chain fatty acid-induced coma has not been delineated, although it was demonstrated (2) that the injection of ammonium salts and short-chain fatty acids at the same time acted synergistically to produce coma and it was suggested that these agents played a significant role as toxic factors in experimental hepatic coma. Other workers (3–5) have postulated that accumulation of medium-chain fatty acids, including octanoic acid, may contribute to the pathophysiology of fatty infiltration of the liver and the acute encephalopathy seen in certain clinical diseases. The present work describes the effects of octanoic acidemia on aspects of neutral lipid and phospholipid metabolism in rat liver.

Materials and Methods. *Surgical procedures.* More than 20 different surgical approaches have been tried to develop the most efficient procedure to accomplish simultaneous infusion of fatty acids and serial blood sampling in the rat. We have found that an animal preparation, utilizing a silastic cannula (0.04-in. i.d. × 0.085-in. o.d.) inserted by way of the right jugular vein into the right superior vena cava to the level of the right atrium and a smaller cannula passed via the left jugular vein and left superior vena cava into the level of the inferior vena cava, can be maintained for about a month before sclerotic reaction in the veins prevents efficient blood withdrawal from the left side. A rat, so prepared, is placed in a metabolism cage and connected to a free-moving swivel apparatus attached to a pump, which allows freedom of movement for the animal and permits fatty acid infusion at controlled rates, the intravenous injection of radioactive fatty acids at controlled times, and serial blood withdrawals.

Production of fatty acidemia. Two methods, either intravenous infusion of sodium octanoate using the above-described animal preparation or a bolus intraperitoneal injection of sodium octanoate, were employed to obtain a fatty acidemia.

In order to study the immediate acute effects of short-chain fatty acids on hepatic fatty acid metabolism the following procedure was employed. Sodium octanoate, pH 7.4, was injected intraperitoneally into male Wistar rats (175–225 g) at a dose rate of 5.0 mmole/kg body wt. The administration of such a bolus injection invariably led to an unconscious state in 3–6 min. One minute after the rat became comatose, the abdomen was opened and 0.2 ml of a 0.001 *M* sodium palmitate–10% beef serum albumin complex (containing 1 μCi of [16-^{14}C]-palmitic acid) was injected into the portal vein. Sixty seconds after the isotope injection, the liver was rapidly excised and

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immediately homogenized in chloroform/methanol (2/1, v/v) and the lipids were extracted according to the procedure of Folch *et al.* (6). Separation of the total lipid extract into individual phosphatides (7) and neutral lipids (8) was accomplished by thin-layer chromatography. Specific radioactivities (cpm/μM lipid phosphorus) were determined by liquid scintillation in Aquasol and phosphorous analysis by the procedure of Bartlett (9). When radioactivity was expressed per lipid fraction, the determination represented the total radioactivity in all lipids in the fraction, prior to separation of lipids into individual components (Table I and Fig. 1). Control animals were treated in an identical manner except that they were injected with an equivalent volume of physiological saline and the unconscious state was ether induced.

When the fatty acidemia was achieved by the whole animal perfusion technique, the animals were surgically cannulated and allowed a week to recover before being connected to the pump and infused with various fatty acids. In the infusion experiments, 1.0 *M* sodium octanoate at pH 7.4 was administered at a rate of 1.0 ml per hour for 4 hr, at which time 0.5 ml of 0.001 *M* sodium palmitate–10% beef serum albumin complex (containing 3 μCi of [16-^{14}C]palmitic acid was injected intravenously. Rats were killed at 10-, 20- and 30-min intervals, the livers were rapidly removed, and the total lipids were isolated and fractionated by the same techniques used in the acute experiments. Control animals were treated in a similar manner except that they were infused with physiological saline. In all experiments, animals were fasted overnight but had access to water.

Chemicals. Octanoic acid, Sequanal grade, was obtained from Pierce Chemical Company. [16-^{14}C]Palmitic acid was purchased from New England Nuclear Corporation and made up in 0.001 *M* with respect to palmitic acid (Hormel Institute) and 10% with respect to beef serum albumin (Sigma).

Results. *Degree of fatty acidemia.* Plasma free fatty acids were determined as described by Zieve *et al.* (2) which recovered 80–85% of octanoic acid when added to plasma. For the measurement of plasma free fatty acids, blood was collected by cardiac puncture 3–5 min after ip injection of sodium octanoate in the acute experiments, and at the time of sacrifice of animals during sodium octanoate infusion experiments. In the acute experiments the average plasma fatty acid level of rats (N = 10) receiving sodium octanoate at a dose rate of 5.0 mmole/kg body wt was 2250 nmole/ml (range 2080–2510) as compared to plasma fatty acid levels in control rats (N = 10) of less than 450 nmole/ml. In the *in vivo* animal experiments (N = 10) where the fatty acidemia was induced by intravenous infusion of sodium octanoate, the level of plasma free fatty acids was predictably lower, an average of 1505 nmole/ml (range 1120–1860).

Hepatic metabolism of [16-^{14}C]palmitic acid during acute octanoic acidemia. The incorporation of [16-^{14}C]palmitic acid, administered via portal vein injection, into the neutral lipids and phospholipids of rat liver in control animals and rats rendered comatose by a bolus injection of sodium ocatanoate is shown in Table I. In such acute experiments, the control animals consistently demonstrated that approximately 50% of the radioactive fatty acid entered the neutral lipid pool and the remaining isotope was distributed among the phospholipids, with phosphatidylcholine exhibiting the highest specific radioactivity. In the experimental octanoate-injected animals, there was a significant increase in the incorporation of radioactive palmitate into the hepatic neutral lipids with a concomitant decrease in isotope incorporation into the phosphatides. Upon separation of the neutral lipids into individual fractions, it was found that the increase in palmitate incorporation was distributed equally between the triacylglycerols and the free fatty acids. Analysis of the specific radioactivities of individual phosphatides demonstrated that the labeling of phosphatidylcholine was only 33% of that observed in control animals.

*Hepatic metabolism of [16-^{14}C]palmitic

TABLE I. EFFECT OF SODIUM OCTANOATE ON THE INCORPORATION OF INTRAPORTALLY INJECTED [16-^{14}C]PALMITIC ACID INTO RAT LIVER NEUTRAL LIPIDS AND PHOSPHOLIPIDS

Animal group (No.)	cpm/neutral lipids	SEM	cpm/phospholipids	SEM
Experimentals (6)	583,026*	±31,271	91,878	±9,045
Controls (6)	369,046	±39,313	405,320	±12,416
Significance**		$P < 0.001$		$P < 0.001$

Note. Experimental animals were injected with sodium octanoate, pH 7.4, at a rate of 5 mmole/kg body wt; 1 min after they became unconscious, 1 μCi of [16-^{14}C]palmitic acid (0.001 *M* bound to 10% BSA) was injected via the portal vein and the livers were rapidly removed 1 min later and treated as described under Materials and Methods. Control animals treated identically except unconscious state was induced with ether after saline injection.

* Numerical values are means obtained with six male rats (175–225 g), Wistar.

** *P* values calculated by unpaired Student's *t* test.

acid subsequent to sodium octanoate infusion. The intravenous injection of 0.001 *M* sodium palmitate, containing 3 μCi of [16-^{14}C]palmitic acid, into rats that had been infused with sodium octanoate for 4 hr allowed for the comparison of the hepatic metabolism of this fatty acid in such animals with rats which were infused with physiological saline. Animals were killed at 10-, 20-, and 30-min intervals following administration of isotope, after which hepatic neutral lipids and phospholipids were isolated from each group of animals. The total incorporation of [16-^{14}C]palmitic acid into the neutral lipids and phospholipids over the time span studied is shown in Fig. 1. At all three time periods, the octanoate infused animals demonstrated increased incorporation of palmitic acid into the hepatic neutral lipids, with 93–96% of the radioactivity located in the triacylglycerol fraction. The peak incorporation of radioactivity into neutral lipids was delayed in the octanoate-infused animals as compared to controls and the amount of radioactivity in the livers of the experimental animals was still approximately fourfold that in control animals at the 30-min interval. Additional experiments, not shown in Fig. 1, had demonstrated that [16-^{14}C]palmitic acid uptake into neutral lipids and phosphatides at a time earlier than 10 min was on the ascending parts of the curves. The incorporation of [16-^{14}C]palmitic acid into the phosphatides of the octanoate-infused and control rats was similar.

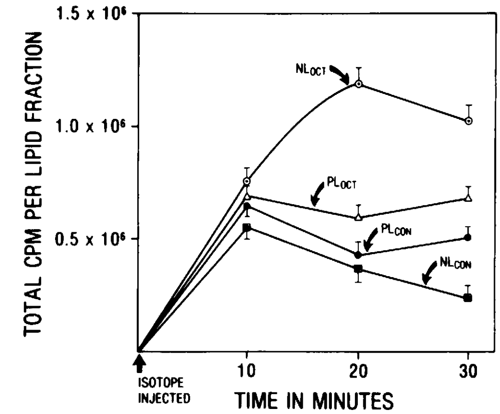

FIG. 1. Incorporation of [16-^{14}C]palmitic acid into hepatic neutral lipids (NL) and phospholipids (PL) of control (CON) and octanoate (OCT)-infused rats. Controls were infused with physiological saline and experimentals with 1.0 *M* sodium octanoate for 4 hr at a rate of 1.0 ml per hour prior to isotope injection. Each point represents the average value for three animals and the vertical bar represents SEM.

A major problem was encountered during infusion of other long-chain fatty acids, such as oleic acid, since such infusion resulted in a dose-related hemolysis leading to the demise of the rat due to severe anemia accompanied by fatal pulmonary and renal effects. This complication prevented us from studying [16-^{14}C]palmitic acid incorporation into hepatic lipids under a condition of long-chain fatty acidemia.

Discussion. The data presented demonstrate that a short-chain fatty acid, e.g., octanoic acid, either administered by intravenous infusion or intraperitoneal bolus injection, affected important metabolic pathways associated with rat liver lipid metabolism. An intraperitoneal injection of sodium octanoate, at a level resulting in unconsciousness, resulted in increased incorporation of [16-^{14}C]palmitic acid into rat liver neutral lipids while decreasing entry of this fatty acid into phospholipids, especially phosphatidylcholine. Since the total amount of isotopic palmitate incorporated into the lipids of the rats with octanoic acidemia was not decreased when compared to control rats, it is unlikely that the octanoate interfered with fatty acid activation. The inhibition of [16-^{14}C]palmitate incorporation into lecithin, accompanied by accelerated incorporation of the fatty acid into the neutral lipid fraction, would best be explained by an inhibitory action of octanoate at some point in phospholipid synthesis.

When rats were infused with sodium octanoate for 4 hr prior to intravenous injection of [16-^{14}C]palmitic acid, the metabolism of this fatty acid was also found to be predisposed toward hepatic steatosis, again suggesting that octanoate has a direct adverse effect on mechanisms involved in liver clearance of long-chain fatty acids.

The hepatic fat accumulation observed in certain clinical situations, e.g., Reye's syndrome, is probably initiated from an increased uptake of fatty acids as a consequence of fatty acidemia. However, a direct action of short- or long-chain fatty acids on liver enzymes involved in triacylglycerol or phospholipid synthesis could contribute to the fatty congestion characteristically seen in such patients.

The degree of fatty acidemia observed with the acute bolus injections of sodium octanoate is higher than one would anticipate to be the case in clinical situations, but the levels of total plasma free fatty acids measured in the sodium octanoate infusion experiments are comparable to those reported in Reye's syndrome (4). Studies have been initiated using lower doses (ip and iv) of sodium octanoate in order to observe the effects on enzymatic reactions that determine the hepatic content of neutral lipid.

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