Hepatic $\Delta^9$ and $\Delta^6$ Desaturase Activities during the Recovery Period Following Carbon Tetrachloride Poisoning

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ABSTRACT

The liver microsomal $\Delta^9$ and $\Delta^6$ desaturase activities have been studied in rats with carbon tetrachloride-induced hepatitis. Immediately after poisoning, significant decreases were observed for both types of desaturase activity. However, recovery kinetics were slower for the $\Delta^6$ desaturase than for the $\Delta^9$ desaturase. The activities of NADH-ferricyanide and NADH-cytochrome C reductases, proteins involved in the electron transfers associated with microsomal desaturation, were also measured. There was a fall in both activities after poisoning, but this decrease was less than that of the desaturase activities.

INTRODUCTION

Some insight into the process of liver regeneration may be gained from a sequential study of the activities of certain enzymes after injury by hepatocellular poisoning (1-3). During tissue regeneration, the unsaturated fatty acids produced by acyl-CoA desaturases are among the essential components of cell membranes to be rebuilt. Therefore, an investigation of the liver microsomal desaturation system after poison ingestion could contribute to the analysis of the recovery process.

The microsomal acyl-CoA desaturase systems involve several proteins. For instance, the system which desaturates stearyl-CoA into oleyl-CoA is composed of 3 different proteins (4,5): NADH-cytochrome b5 reductase (6-8), cytochrome b5 (9,10) and the desaturase itself (11,12). Cytochrome b5 also is involved in the desaturation of linoleate into γ-linolenate (13,14). As is the case with all membrane enzymes, the activities of the enzymes in the acyl-CoA desaturase systems could be controlled by the lipid environment of the active sites of the proteins (4,15,16).

In this report, we describe the variations of the activities of the $\Delta^9$ and $\Delta^6$ desaturases from rat liver microsomes during the regeneration period following CCl₄ poisoning. The levels of microsomal NADH-ferricyanide and NADH-cytochrome C reductase have also been measured; their activities are coupled to NADH-cytochrome b5 reductase and to the reduction of cytochrome b5, respectively. The measurement of these enzymes would enable us to follow the different components of several microsomal desaturase systems during the regeneration process.

MATERIAL AND METHODS

Treatment of Animals

Five- to 6-wk-old male rats (Wistar CF), weighing 110-120 g, were maintained on a commercial lab chow and ad libitum water. Carbon tetrachloride (Merck Chem., Darmstadt, Germany) diluted in liquid paraffin (1:1, v/v) was administered intragastrically. The dose of the carbon tetrachloride given was 150 µl of CCl₄/100 g body weight diluted in 350 µl of liquid paraffin. The activity of the desaturases was measured at 3, 6 and 12 hr as well as 1, 2, 4, 7 and 14 days postingestion of poison. In each experiment, control animals were given 500 µl of liquid paraffin.

Chemicals

$[1^{14-\text{C}}]$Stearic acid (50 mCi/mmol) and $[1^{14-\text{C}}]$linoleic acid (50 mCi/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Corresponding acyl-coenzyme A esters were prepared according to the Ailhaud and Vagelos procedure (17) as modified by Bourre and Daudu (18). NADH was purchased from Sigma (St. Louis, MO).

Preparation of Microsomes

The liver was rapidly removed from rats killed by decapitation, weighed and homogenized in 3 vol (v/w) of ice-cold solution of 250 mM sucrose. A postmitochondrial supernatant fraction, obtained by centrifugation at 15,000 x g for 30 min, was further centrifuged at 100,000 x g for 60 min to give the microsomal pellet. This pellet was suspended in 0.5 vol (v/w) 250 mM sucrose solution. Protein concentration was determined according to Lowry et al. (19).
Enzyme Assays

Acyl-CoA desaturases. The final incubation mixture (1 ml) contained 0.1 M sodium phosphate (pH 7.4), 1 mM NADH, 50 nmol [114-C]-acyl-CoA (120,000 cpm) and 1.0 mg microsomal protein (20). Prior to the addition of microsomes, the assay medium was aerated by bubbling with oxygen for 10 sec. The incubations were performed in a thermoregulated shaking water bath at 37 C for 20 min.

The reaction was terminated by the addition of 1.0 ml of 10% methanolic KOH and heating at 80 C for 30 min. The fatty acids were liberated by the addition of 1.0 ml 7 N HCl and heating at 80 C for 10 min. Fatty acids were extracted twice by 2 ml n-hexane/extraction. Fatty acid methyl esters were prepared with N methanolic anhydrous HCl, as described by Carreau and Dubacq (21). Radiolabeled methyl esters were then analyzed either by radio gas chromatography (radio-GC) (Packard Instrument, series 894) or measured with a liquid scintillation spectrometer (Intertechnique, series SL 30) after thin layer chromatography (TLC) on silver nitrate-impregnated Silica Gel G plates. Commercial Silica Gel G-(Merck)impregnated plates were immersed for 10 sac in a 80% ethanol solution containing 10% silver nitrate. Benzene/hexane (7:3, v/v) was used as the developing solvent to separate monounsaturated and saturated fatty acid methyl esters (22). To separate linoleic and γ-linolenic acid methyl esters, a mixture of benzene/hexane/diethyl ether (7:3:0.75, v/v/v) was used. The spots were revealed by rapid immersion of the plates in 0.1% 1.7-dichlorofluorescein solution and examination under ultraviolet (UV) light. The marked spots were scraped off and coated silica gel was placed in 15 ml of a toluene solution containing PPO and POPOP.

RESULTS

Δ9 Desaturase Activity

Six hr after CCl4 ingestion stearyl-CoA desaturase activity in liver microsomes from poisoned rats was decreased (Table I). The lowest values were observed between 12 and 24 hr: 25-30% of control values. After 48 hr, Δ9 desaturase activity increased again but a return to normal values still was not complete after 14 days.

Δ6 Desaturase Activity

The activity of linoleyl-CoA desaturase was also affected by CCl4 poisoning and a decrease was observed 3 hr after ingestion with maximal effect at 12 hr (Table I). A progressive restoration process began at 24 hr but was slower than that of Δ9 desaturase activity since at 14 days the Δ6 desaturase activity remained at 70-75% of controls.

NADH-ferricyanide Reductase and NADH-cytochrome C reductase Activities

The NADH-ferricyanide reductase activity of liver microsomes from rats having ingested 150 μl of CCl4/100 g body weight decreased rather slowly after poisoning (Table II). By 12 hr after CCl4 ingestion, there was a drop of 15% in the NADH-ferricyanide reductase activity. This decrease reached 40% by 24 hr, which was then followed by a gradual increase to normal values by the 7th day.

Three to 6 hr after CCl4 ingestion, the NADH-cytochrome C reductase activity in liver microsomes from poisoned rats was higher than that of control animals (Table II). Thereafter a decrease occurred but, in comparison to that of NADH-ferricyanide reductase, was less immediate and became obvious only 48 hr postingestion.

Fatty Acid Composition of the Total Lipid of Liver Microsomes

As a consequent of CCl4 poisoning, the content of palmitic acid in liver microsomes increased from the 12th hr through the 4th day (Table III). During this same period, the content of arachidonic and stearic acids decreased but that of linoleic acid remained slightly higher than that of control rats.

Hydrolysis of Acyl-CoA and Incorporation of Precursors into Complex Lipids

The influence of CCl4 on the hydrolytic

LIPIDS, VOL. 15, NO. 9