Oxidative Desaturation of α-Linolenic, Linoleic, and Stearic Acids by Human Liver Microsomes

IRMA N.T. DE GÓMEZ DUMM[1] and **RODOLFO R. BRENNER**[1], Instituto de Fisiología, Facultad de Ciencias de la Salud, Universidad Nacional de La Plata, La Plata, Argentina

ABSTRACT

The desaturation of stearic, linoleic, and α-linolenic acids by human liver microsomes were studied. The microsomes were isolated from liver biopsies obtained during operations. It was shown that human liver microsomes are able to desaturate 1-^{14}C-α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid; 1-^{14}C-linoleic acid to γ-linolenic acid; and 1-^{14}C-stearic acid to oleic acid in the same system described in the rat. However, the desaturation activity obtained was low compared to other mammals. This effect was attributed to fasting, premedication, or the anaesthesia.

INTRODUCTION

It is well established that stearate, linoleate, and α-linolenate are converted to oleate, γ-linolenate, and octadeca-6,9,12,15-tetraenoate, respectively, by animal liver microsomes in a reaction requiring oxygen and nicotinamide adenine dinucleotide, reduced form (NADH) or nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) (1-3).

The existence of similar enzymatic systems in human liver is assumed. However, as far as we know, the microsomal desaturation activity of human liver has not been measured directly until now. Therefore, it was considered important to determine these enzymatic activities in fresh human liver.

The present paper reports that human liver biopsies provide enough material to measure microsomal fatty acid desaturation activities. From these biopsies, stearic, linoleic, and α-linolenic acid microsomal desaturation activities were determined.

MATERIALS AND METHODS

Chemicals

1-^{14}C-stearic acid (56 mCi/mmole, 99% radiochemically pure) and 1-^{14}C-linoleic acid (56.2 mCi/mmole, 99% radiochemically pure) were purchased from New England Nuclear, Boston, Mass. 1-^{14}C-α-linolenic acid (41.5 mCi/mmole, 99% radiochemically pure) was obtained from The Radiochemical Centre, Amersham, England.

Subjects

The patients in this investigation were between the age of 27-52, males and females. There was no clinical or laboratory evidence of liver diseases. Patients designated RN, YA, AS, and MR suffered from cholelithiasis and the others from tumors of the digestive system. Before their operations, the patients were premedicated with atropine and meperidine hydrochloride. The general anaesthesia consisted of thiopental sodium, halothane and methoxyfluorane or thipental sodium, halothane, and succinylcholine and was initated a few min before the operation.

Liver biopsies (3-5 g) were taken within 15 min after the abdomen had been opened and were immersed immediately in cold homogenizing medium. The liver samples were homogenized in a cold solution (3:1, v/w), consisting of 0.15 M KCl, 0.005 M $MgCl_2$, 0.004 M ethylenediaminetetraacetic acid (EDTA), 0.004 M N-acethyl-cysteine, 0.05 M phosphate buffer (pH 7), and 0.25 M sucrose. The crude homogenate was centrifuged at 20,000 x g for 10 min. The microsomal fraction was obtained by centrifugation of the 20,000 x g supernatant at 140,000 x g for 60 min in a Spinco model L2 centrifuge. The pellets were resuspended in 0.5 ml homogenizing medium. Microsomal protein (10-50 mg) was obtained from the liver biopsies.

Assay for Oxidative Desaturation of Fatty Acids

The desaturation of the fatty acids by human liver microsomes was measured by estimation of the percentage conversion of 1-^{14}C-stearic to oleic acid; 1-^{14}C-linoleic to γ-linolenic acid; and 1-^{14}C-α-linolenic to octadeca-6,9,12,15-tetraenoic acid.

Labeled acids (10-50 nmoles) were incubated with 1-5 mg microsomal protein in a Dubnoff shaker at 35 C in a total volume of 1.5 ml 0.15 M KCl, 0.25 M sucrose solution. The time period tested was 20 min. The solution contained 4 μmoles adenosine 5'-triphosphate (ATP), 0.1 μmole CoA, 1.25 μmoles NADH, 5 μmoles $MgCl_2$, 2.25 μmoles glutathione, 62.5

[1]Member of the Carrera del Investigador Científico of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

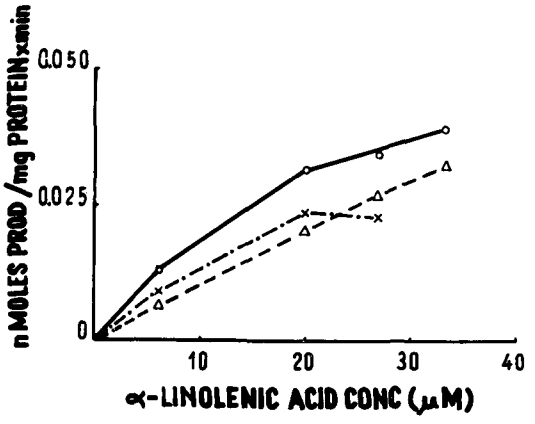

FIG. 1. Effect of the α-linolenic acid concentration upon the specific desaturation activity of human liver microsomes. Patients designated: RN (•————•), AP (x———•———x), and YA (Δ----Δ). 1, 2.5, or 5 mg microsomal protein was incubated at 35 C for 20 min under the conditions described in the text.

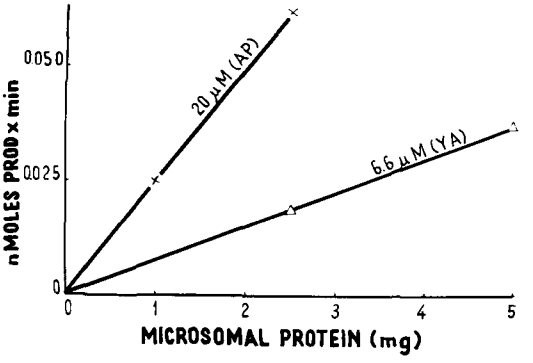

FIG. 2. Effect of the amount of microsomal protein upon the oxidative desaturation of 1-^{14}C-α-linolenic acid. Patients designated: AP (x————x), α-linolenic acid concentration, 20 μM; and patient YA (Δ——Δ), α-linolenic acid concentration, 6.6 μM. Incubation time 20 min at 35 C. Technical details described in the text.

μmoles KF, 0.5 μmole nicotinamide, and 62.5 μmoles phosphate buffer (pH 7). The reaction was stopped by addition of 2 ml 10% KOH in methanol. The fatty acids were recovered by saponification of the incubation mixture (40 min at 85 C) and extraction with petroleum ether (bp 30 C). The acids were esterified with methanolic 3 M HCl (3 hr at 68 C), and the distribution of radioactivity between substrate and product was determined by gas liquid radiochromatography in an apparatus equipped with a Packard proportional counter (4). The samples were analyzed using a column packed with 10% diethylene glycol succinate on Chromosorb W (80-100 mesh) at 180 C (3). The specific enzymatic activity expressed as μmoles product/mg microsomal protein/min was calculated from these data. The methyl esters of fatty acids were identified by equivalent chain length determination and by comparison with authentic standards. The protein content of the microsomal fraction was determined by the biuret method of Gornall, et al., (5) using crystalline bovine serum albumin as a standard.

TABLE I

Linoleic Acid Desaturation Activity in Human Liver

Subjects[a]	Substrate (μM)	Desaturation activity[b] (nmoles product/mg protein x min)
JR	6.7	0.007
	33.3	0.060
MR	20.0	0.037
	33.3	0.052

[a]Initials designate different patients.

[b]Microsomal protein (5 mg) incubated at 35 C for 20 min under the conditions described in the text.

TABLE II

Stearic Acid Desaturation Activity in Human Liver

Subjects[a]	Substrate (μM)	Desaturation activity[b] (nmoles product/mg protein x min)
AP	6.7	0.008
	20.0	0.025
AS	20.0	0.015
	33.3	0.018

[a]Initials designate different patients.

[b]Microsomal protein (5 mg) incubated at 35 C for 20 min under the conditions described in the text.

RESULTS AND DISCUSSION

The determination of the fatty acid desaturation activity in human liver microsomes is difficult because of different factors. The main problem is to obtain enough liver tissue to isolate ca. 1 mg microsomal protein. The second problem, probably as important as the first one, is to get this sample of the tissue with absolutely unaltered enzymes. Since the enzymatic desaturation system is rather unstable, this means that the sample must be obtained directly from living tissue and immediately cooled to 0-4 C. Under this condition, the desaturase activity is stable for at least 1 hr. For this reason, two procedures were tested. The first one was to obtain the sample by direct puncture of the liver. However, the amount obtained by this procedure was so small that it was impossible to isolate the microsomes with

our technique. The second procedure tested was the use of biopsies. This technique requires an operation. Therefore, it only could be used in operations where the liver was exposed and where biopsies were obtained by the surgeon. In these cases, enough tissue was obtained to separate microsomes and measure the fatty acid desaturation activity.

Figure 1 shows that the microsomes of human liver biopsies desaturate α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid. Substrate saturation curves were obtained. Desaturation enzymatic activities were calculated, since a linear relationship was found between the amount of product formed and the mg microsomes incubated (Fig. 2). The Δ6-desaturation activity for α-linolenic acid was rather low compared to other mammals (6,7).

Tables I and II show, respectively, that human liver microsomes also desaturate linoleic acid to γ-linolenic acid and stearic acid to oleic acid. However, in some patients, the linoleic acid desaturation activity was not measurable under our experimental conditions. The number of analyses was not enough to build a saturation substrate curve. The Δ6-desaturation activity for linoleic acid and Δ9-desaturation activity for stearic acid are low compared to other mammals (6,7).

Since adult subjects were analyzed, the low fatty acid desaturations may be a consequence of the age. An age effect has been found in rats (6). However, the low desaturation values also may be due to the 12 hr fasting prior to the operation, since fasting has been shown to decrease the Δ6 and Δ9-desaturation activity of rat liver microsomes (8,9). Besides, interpretation of data from most human studies is complicated by the additional influences of drugs used for premedication and induction and maintenance of anaesthesia. Ether, halothane, and barbiturates also affect enzymes involved in glucose homeostasis, and they also may alter the enzymes of lipid metabolism.

Nevertheless, the data obtained show that it is possible to measure fatty acid desaturation activity in human biopsies and that stearic, linoleic, and α-linolenic acids are desaturated by the same system described in the rat.

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