Effect of Environmental Temperature Changes on Rat Liver Fatty Acid Desaturases

SUSANA GONZÁLEZ, ANÍBAL M. NERVI[1], RAÚL O. PELUFFO[1] and RODOLFO R. BRENNER*,[1], *Instituto de Investigaciones Bioquímicas de la Plata (INIBIOLP), CONICET-UNLP, Facultad de Ciencias Médicas, calles 60 y 120, (1900) La Plata, Argentina*

ABSTRACT

Female rats warm-adapted at 30-32 C for 20-25 days and then shifted to 13-15 C for 12, 24, 48, 72 and 120 hr showed that Δ9 desaturase and fatty acid synthetase activity decay after 24 hr of cold exposure, while Δ6 and Δ5 desaturases were increased after this period of time. These results were confirmed by an increase of arachidonic acid of heart and liver microsomes phosphatidylcholine and a decrease of oleic acid. Neither NADH-cyt b_5 reductase nor NADH-cyt c reductase activity of liver microsomes were significantly affected. Male rats warm-adapted under the same conditions and then shifted to 13-15 C for 120 hr did not show significant changes in fatty acid synthetase, Δ9 and Δ6 desaturases and enzymes of the microsomal electron transport chain. Therefore, the desaturase response to environmental temperature changes could be plausibly linked to female hormones.
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INTRODUCTION

Good evidence has been gathered showing the effects of environmental temperature on unsaturated fatty acid biosynthesis in microorganisms (1,2). Martin et al. (3) and Skrivers and Thompson (4) have made contributions concerning temperature changes in *Tetrahymena piriformis*, which alter the fatty acid desaturation activity of endoplasmic reticulum membrane. In this organism, the endoplasmic reticulum would constitute a kind of self-regulated system for maintaining an optimal physical state by means of activation or deactivation of desaturation reactions. In more evolved poikilothermic organisms as fish, Torrengo and Brenner (5) have shown that the temperature acclimation of *Pimelodus maculatus* from 38 C to 18 C increases the specific activity of Δ6 desaturase.

Although the effect of temperature on membrane fluidity is not obvious in homeothermic animals as it is in poikilotherms, other reactions may be triggered by a change of environmental temperature altering the unsaturated fatty acid biosynthesis. In 1974, Peluffo and Brenner (6) showed in the rat that Δ6 and Δ9 desaturases are not only diet-dependent enzymes but also change their activity according to seasons. Other authors have demonstrated that cold-exposed homeotherms show an increase on catecholamine levels (7,8), oxygen consumption (9,10), oxidations (11,12), thermal generation (13,14), glucagon and free fatty acids (15, 16). Cold exposure also affects prostaglandins (17), corticosteroids (18), cyclic AMP (19) and enzyme levels in mitochondria, peroxisomes and lysosomes (20).

Taking into account the above information, we are interested to study the effect of environmental temperature on the saturated and polyunsaturated fatty acid biosynthesis in the female rat.

MATERIALS AND METHODS

[1-^{14}C]Palmitic acid (56 mCi/mmol), [1-^{14}C]linoleic acid (55 mCi/mmol), [1-^{14}C]-eicosa-8,11,14-trienoic acid (61 mCi/mmol) were provided by New England Nuclear, Boston, MA. Cytochrome c was provided by Sigma Chemical Company Inc., St. Louis, MO. Cofactors for enzyme reactions were purchased from Boehringer Argentina, Buenos Aires, Argentina.

Animal Treatment

Thirty female Wistar rats, 130-150 g weight, were divided into groups of 5 animals each, and were placed in a warm room at 30-32 C for 20-25 days under purina chow diet and water, ad libitum. After that period of time, 5 groups were placed in a temperature-controlled chamber at 13-15 C for 12, 24, 48, 72 and 120 hr, respectively, under the same diet conditions.

Ten male Wistar rats were divided in 2 groups of 5 animals each and were warm-adapted at 30-32 C for the same period of time and then one of them was placed in the cold chamber for

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

*Author to whom correspondence should be addressed.

120 hr. After these periods of time, all the animals were killed at 7 A.M. to avoid circadian effects. Livers were homogenized in a solution containing 0.25 M sucrose, 0.15 M KCl, 62 mM phosphate buffer (pH 7) and 1.5 mM glutathione. The homogenate was centrifuged at 10,000 x g for 20 min, the pellet was discarded and the supernatant was centrifuged again at 110,000 x g during 60 min to obtain the microsomes and cytosol.

Enzyme Assays

Fatty acid desaturase assay was performed using 50 nmol of labeled palmitic, linoleic or eicosa-8,11,14-trienoic acids. Each acid was incubated with 3 mg of microsomal protein at 35 C for 10 min. In these conditions, the enzymes were saturated by the substrate. The incubation solution contained: 0.25 M sucrose, 0.15 M KCl, 0.04 M phosphate buffer (pH 7.0), 1.5 mM glutathione, 0.04 M KF, 1.3 mM ATP, 0.06 mM CoA, 0.87 mM NADH, 5 mM $MgCl_2$ and 0.33 mM nicotinamide in final volume of 1.6 ml. Fatty acids were saponified, esterified and the conversion was measured by gas liquid radiochromatography in a Packard apparatus with a proportional counter (21). NADH-cyt b_5 reductase activity was measured by NADH oxidation at 340 nm, using potassium ferricyanide as terminal electron acceptor. The reaction mixture contained ferricyanide (70 nmol), microsomal protein (2-10 μg) and NADH (30 nmol) in a final volume of 0.27 ml of 0.05 M Tris acetate (pH 8.1), 1 mM EDTA. An extinction coefficient of 6.22 $mM^{-1} \times cm^{-1}$ was used. NADH cyt c reductase activity was measured at 550 nm using cytochrome c as a terminal electron acceptor. The reaction mixture contained 20 nmol of cyt c, 30 nmol of NADH and 2-10 μg of microsomal protein in a final volume of 0.27 ml of 0.05 M Tris acetate (pH 8.1), 1 mM EDTA. The absorption increase at 550 nm was followed as a function of time. An extinction coefficient of 18.5 $mM^{-1} \times cm^{-1}$ was used.

The fatty acid synthetase activity was assayed by the method of Bruckdorfer et al. (22) measuring the NADPH oxidation at 340 nm.

Phosphatidylcholine Fatty Acid Analyses

Liver, heart and liver microsomal total lipids were extracted, phosphatidylcholine isolated and methyl esters were prepared and analyzed by gas liquid chromatography (GLC) in a Hewlett-Packard 5840-A gas chromatograph equipped with the 5840-A GC terminal and using a 6-ft column filled with 10% Sp 2330 on 100-200 Chromosorb WAW (23).

RESULTS AND DISCUSSION

Liver fatty acid desaturase activity is modified by several factors such as diets, hormones (24), cytosolic soluble proteins (25), ethanol (26) and clofibrate (27). In this experiment, the effect of environmental temperature variations is described. Figure 1 shows that Δ9 desaturase conversion increases in a nonstatistically significant way during the first 12 hr of temperature, shifting from 30-32 C to 13-15 C, being followed by a marked decrease. At 48 hr, the Δ9 desaturase reaches the lowest value that remains constant after this time. Similar behavior was shown by the fatty acid synthetase that also decreases but reaching the lowest value with a slight delay of 24 hr. The similarity of Δ9 desaturase and fatty acid synthetase behavior promoted by diet modification has already been pointed out by Jeffcoat and James (28) and other authors (26,27), and the present results are new evidence that the activity of both enzymes may be intimately related. This decrease of Δ9 desaturation is confirmed by the decrease of oleic acid shown on the fatty acid composition of both heart and liver microsomes phosphatidylcholine (Table 1).

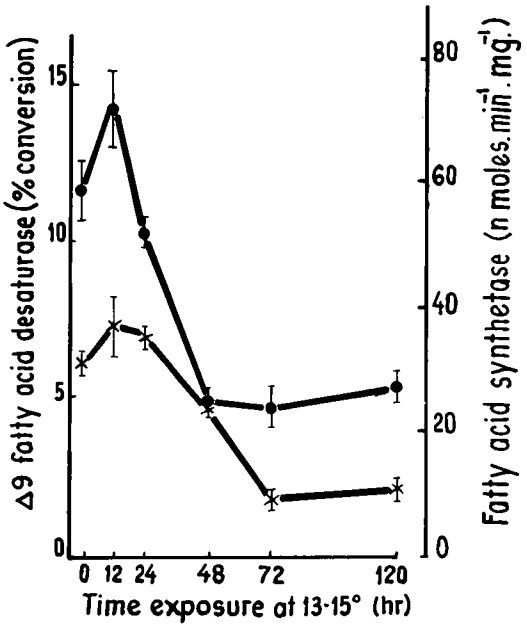

FIG. 1. Fatty acid synthetase (X—X) and Δ9 desaturase (•—•) variations in female rat liver previously warm-adapted (20-25 days at 30-32 C) and then shifted to 13-15 C for 12, 24, 48, 72 and 120 hr. Results are the mean of 5 samples ± SE.

TABLE 1

Liver Microsomes and Heart Phosphatidylcholine Fatty Acid Composition of Female Rats Exposed at 30-32 C for 20 Days and Then Shifted to 13-15 C for 120 hr

Fatty acids	Liver microsomes		Heart	
	30-32 C	13-15 C	30-32 C	13-15 C
16:0	25.6 ± 0.5	19.0 ± 0.3[b]	19.3 ± 0.4	18.0 ± 0.8
16:1	2.6 ± 0.1	2.3 ± 0.5	1.5 ± 0.2	1.2 ± 0.1
18:0	28.8 ± 0.6	31.4 ± 0.2[a]	26.0 ± 0.6	25.5 ± 1.0
18:1	12.9 ± 0.4	10.1 ± 0.1[b]	13.0 ± 0.3	10.4 ± 0.3[b]
18:2(ω6)	7.9 ± 0.2	8.5 ± 0.3	14.1 ± 0.8	9.1 ± 0.7[a]
20:3(ω6)	1.9 ± 0.1	1.2 ± 0.1[a]	–	–
20:4(ω6)	12.6 ± 0.9	20.7 ± 0.7[b]	20.9 ± 0.7	27.6 ± 0.9[b]
22:5(ω3)	1.9 ± 0.5	0.9 ± 0.2	1.6 ± 0.1	2.8 ± 0.4
22:6(ω3)	5.8 ± 0.6	5.9 ± 0.2	3.6 ± 0.3	5.4 ± 1.0

Minor components were not considered. Results are the mean of 5 samples ± 1 SE. 30-32 C vs 13-15 C: [a]$p < 0.01$, [b]$p < 0.001$.

The decreased environmental temperature effect on the conversion of 18:2 to 18:3 by Δ6 desaturase was completely different since the conversion increased progressively according to the time of cold exposure after a lag period of 24 hr. A similar effect was shown on Δ5 desaturase, since the conversion of 20:3 to 20:4 was also increased after a lag period of 24 hr. However, a plateau of maximal conversion was reached at 48 hr (Fig. 2).

The activity of enzymes of the microsomal electron transport chain such as NADH-cyt b_5 reductase and NADH-cyt c reductase, which are involved in the fatty acid desaturation reaction, were not modified significantly (Fig. 3) by the environmental temperature shift. Therefore, it may be admitted that the effect would be exerted on the terminal fatty acid desaturase and, whatever the mechanism of desaturase temperature-dependence might be, the results mentioned above show that saturated and monounsaturated fatty acid synthesis is diminished, while that of polyunsaturated fatty acids is increased, when warm-adapted female rats are shifted from 30-32 C to 13-15 C.

Since the analysis of the fatty acid composition of phospholipids by GLC may be used to show the status of a desaturase more precisely than does its enzymatic assay (29), the fatty acid composition of the phosphatidylcholine of liver microsomes and heart was also investigated. In our experiments, the increase of Δ6 and Δ5 fatty acid desaturation suggested an increase of arachidonic acid. The total lipid fatty acid composition of microsomal membrane (not reported here) showed only a small increase of arachidonic acid that was not statistically significant. However, the increase of

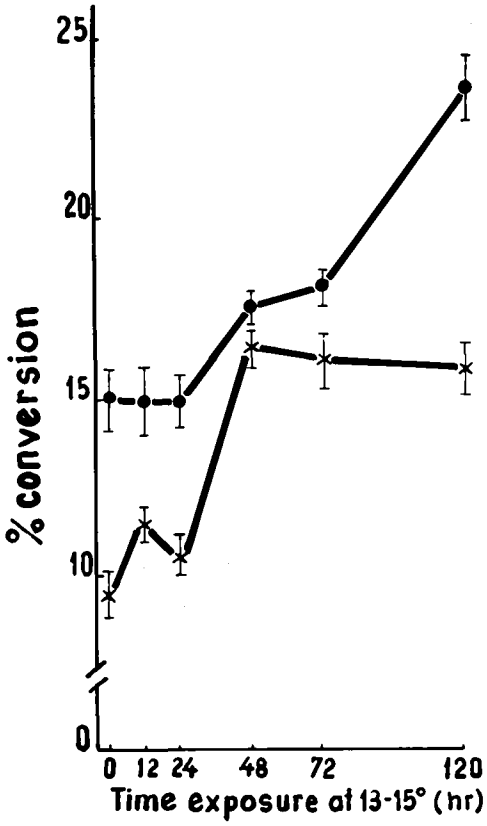

FIG. 2. Δ6 (• –•) and Δ5 (X --X) desaturase variations in female rat liver microsomes previously warm-adapted (20-25 days at 30-32 C) and then shifted to 13-15 C for 12, 24, 48, 72 and 120 hr. Results are the mean of 5 samples ± SE.

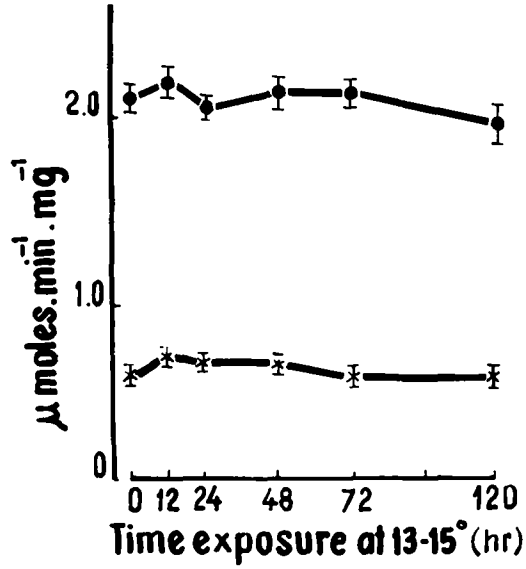

FIG. 3. NADH-cyt b_5 reductase (•—•) and NADH-cyt c reductase (×—×) activity variations in female rat liver microsomes warm-adapted (20-25 days at 30-32 C) and then shifted to 13-15 C; for 12, 24, 48, 72 and 120 hr. Results are the mean of 5 samples ± SE.

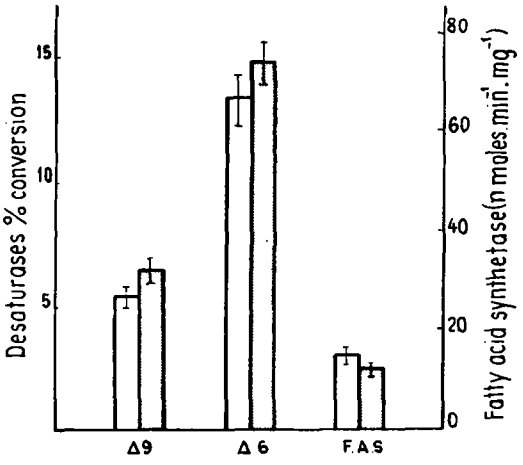

FIG. 4. Fatty acid synthetase, Δ9 and Δ6 desaturase variations in male rat liver warm-adapted (20-25 days at 30-32 C) (light bars) and then shifted to 13-15 C for 120 hr (heavy-strippled bars). Results are the mean of 5 samples ± SE.

arachidonic acid was statistically significant when the fatty acid composition of phosphatidylcholine was investigated in liver microsomes and heart (Table 1).

It is interesting that the changes of Δ9 and Δ6 fatty acid desaturase activity found in female rats were not present in males (Fig. 4). This result would suggest that female sex hormones could be involved in the temperature effect, and although it is too early to predict a mechanism for the process, it is important to consider Holloway's contribution showing that estradiol may alter microsomal Δ9 fatty acid desaturase in the rooster (30). This research is in progress.

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