Free Rad. Res. Comms., Vol. 7, No. 2, pp. 67–72
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CARDIAC PEROXISOMAL ENZYMES AND STARVATION

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(Received May 4, 1989)

In mice subjected to 3-day periods of food deprivation an increase in plasma free fatty acids occurred together with a rise in the cardiac content of fatty acyl CoA-oxidase (+ 15.2%) and catalase (+ 136.2%)activities. Stimulation of hydrogen peroxide production by the heart was found after 30 hours of fasting and this phenomenon was almost completely eliminated by 6 hours of refeeding. These data suggest that high myocardial loads of free fatty acids involve the peroxisomal enzymes in the beta-oxidation process. The resulting increase in hydrogen peroxide production could be partly responsible for the myocardial injury caused by starvation.

KEY WORDS: Starvation, lipolysis, peroxisomal enzymes, H_2O_2 production, cardiac injury.

INTRODUCTION

The presence of peroxisomes (microbodies) has been reported in the mouse myocardium.[1] Among other enzymes, these intracellular organelles contain fatty acyl CoA-oxidase.[2] They also contain catalase[1] which decomposes the hydrogen peroxide generated by the former enzyme or by other oxidases. In addition, there is a general consensus that fatty acids are an important metabolic fuel for the myocardium. It is also known that fasting activates the oxidation of fatty acids which are preferentially utilized by the myocardium in this condition (for a review see reference[3]). Thus, one could assume that in the fasting state an increased peroxisomal oxidation of fatty acids occurs in the myocardium together with a greater production of hydrogen peroxide. However, notwithstanding these statements, there is still some controversy as to whether the myocardial peroxisomal enzymes play any role in the handling of fatty acids *in vivo*. On the basis of the close morphological association of cardiac peroxisomes with mitochondria and sarcoplasmic reticulum, Herzog and Fahimi[1] suggested that they are involved in lipid cardiac metabolism. Accordingly, Crane and Master[4] reported that the interruption of the peroxisomal beta-oxidation can cause perturbation of lipid metabolism in the heart of living animals. However, Kerckaert and Roels[3] studied *in vivo* hydrogen peroxide production by the hearts of rats

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subjected to increased myocardial load or fasting, and concluded that the beta oxidation of fatty acids by peroxisomes does not contribute in a substantial way to energy production in the heart.

In this paper we present evidence that in the mouse heart fasting induces an activation of acyl CoA-oxidase and of catalase together with an increase in hydrogen peroxide production. These findings give rise to the possibility that the elevation of this oxidant may contribute to the onset of the cardiac damage induced by starvation.

MATERIALS AND METHODS

Animals and starvation

Female CD 1 mice of an average weight of 20 g were used in the experiments. They were supplied by Charles River Italia SpA, Calco, Italy and normally fed a standard laboratory diet with free access to water. Groups of mice were subjected to periods of complete food deprivation with free access to water and then sacrificed. The weight of each animal was recorded before the start of starvation and before sacrifice.

Triglycerides and free fatty acids in plasma

The mice were sacrificed by decapitation. Blood was collected in tubes containing sodium edetate 0.5 mol/l (25 μmoles/ml of blood) and centrifuged at 500 × g for 10 min at 4°C. For each observation the pooled plasma of 3 mice was used. Plasma triglycerides and free fatty acids were determined according to standard spectrophotometric techniques and using commercial kits (supplied by Boehringer Biochemia Srl, Divisione Diagnostici, Milan, Italy).

Acyl CoA-oxidase activity

The animals were sacrificed by cervical fracture. The thorax of the animals was opened and their hearts were perfused for 4 min. with cold saline, in order to avoid blood contamination. After that, the hearts were rapidly removed, blotted dry and weighed. They were frozen in liquid nitrogen and pulverized using an iron mortar. After resuspension in volumes (10 ml per g) of 0.03 M sucrose 5 mM phosphate buffer (pH 7.4) the homogenates were centrifuged at 12000 × g for 30 min. at 4°C. On the supernatants acyl CoA-oxidase activity was determined and expressed according to[5] using leuco-2,7-dichlor-fluorescein (E. Merck, Darmstadt, F.R. Germany) as detector and palmitoyl-CoA (Sigma Chemical Co., St. Louis, USA) as substrate. Proteins were measured by the commercially available Bio-Rad protein assay (Bio-Rad, ECS Division, Anaheim, USA).

Catalase activity

After sacrifice the hearts of the animals were perfused for 4 min. with a cold solution containing NaCl (8.1 g/l) and 0.01 M sodium phosphate buffered at pH 7.4. After that, each heart was removed and homogenized for 30 sec. in volumes (10 ml per g) of the solution described above containing also ethanol (22 mM). A Kinematica model PT 10S Polytron working at medium speed was used in order to obtain the

homogenates. On the homogenates catalase activity was determined according to[6] and expressed as a calculated rate constant (k).

In vivo hydrogen peroxide production

In order to study hydrogen peroxide production *in vivo* we employed, with slight modifications, the method of residual catalase activity as described by.[3] It is based on the *in vivo* competition between 3-amino-1,2,4-triazole (subsequently abbreviated as aminotriazole, obtained from Sigma Chemical Co., St. Louis, USA), which irreversibly blocks the catalase-hydrogen peroxide complex, and methanol which prevents such interaction. The extent of inhibition of catalase activity caused by aminotriazole allows the determination of the *in vivo* hydrogen peroxide production by the heart or, at least, the amount of hydrogen peroxide which reaches catalase. For example, if more hydrogen peroxide is present in the heart, as it is following the administration of glycolic acid (a substrate for a peroxisomal oxidase), catalase is more inhibited by aminotriazole. The reverse is also true. The administration of diethyldithiocarbamate, which is an inhibitor of superoxide dismutase and decreases hydrogen peroxide production, diminishes the inhibition of cardiac catalase activity caused by aminotriazole (see references[3,7].

Briefly, the animals received aminotriazole (1 g/Kg) and methanol (5 mmol/Kg) by intraperitoneal injection. One hour later they were sacrificed. The catalase activity present in their hearts was determined following a procedure identical to that described in the "Catalase activity" section.

Statistical analysis

This was performed by the two-tailed t test for independent means and, in the case of the weight determinations, by the t test for paired data.

RESULTS

After a 3-day period of food deprivation the mice had 74.1 $\pm$ 4.7% (S.D.) of their initial weight (12 observations, $P < 0.01$). Control mice had 101.6 $\pm$ 3.1% of their initial weight (not significant).

In starved mice plasma triglycerides were decreased by 43.8% if compared to controls and free fatty acids were increased by 29.3% (Table 1).

Cardiac acyl CoA-oxidase increased by 31.5% when the activity was expressed as U/mg protein and by 15.2% when the activity was expressed as U/heart (Table 1). Cardiac catalase activity (which was expressed as k/heart) increased by 136.2% in starved mice (Table 1).

In contrast with the other parameters, hydrogen peroxide production was studied after only 30 hours of fasting. This was to prevent the poor general condition of the animals after 3 days of fasting from altering the cardiac distribution of aminotriazole and methanol and creating artifacts.

In vivo hydrogen production by the heart was increased in the fasted animals (Table 2). In fact, the residual catalase activity in fasted mice treated with aminotriazole and methanol was 26.1% of that of the fasted animals. Instead, the residual catalase activity of control mice treated with aminotriazole and methanol was 40.6% of the

TABLE 1
Biochemical parameters in control or starved mice (3 days of food deprivation). Data are expressed as means ± S.D. (for details see "Materials and Methods").

	Plasma triglycerides (mg/100 ml)	Plasma free fatty acids (mEq/l)	Heart acyl CoA-oxidase		Heart catalase (k/heart)
			U/heart	U/mg protein	
Controls (4)	105 ± 15	862 ± 73	4.46 ± 0.20	1.11 ± 0.05	1.74 ± 0.07
Starved (4)	59 ± 9*	1115 ± 81*	5.14 ± 0.31*	1.46 ± 0.08*	4.11 ± 0.39*

Number of observations in parenthesis. In the case of acyl CoA-oxidase the number of observations was 6 in both the groups. * $P < 0.01$ with respect to controls.

TABLE 2

In vivo hydrogen peroxide production in the hearts of mice after 30 hours of fasting or after 24 hours of fasting and 6 hours of refeeding. Data are expressed as catalase activity (k/heart) and are the mean of 5 observations ± S.D. (for details see "Materials and Methods").

	k/heart	% catalase activity
Controls	1.82 ± 0.11	
Controls + aminotriazole + methanol	0.74 ± 0.06*	40.6% of the controls
Fasted (30 hours)	3.21 ± 0.15*	176.3% of the controls
Fasted (30 hours) + aminotriazole + methanol	0.84 ± 0.08°	26.1% of the fasted
Fasted (24 hours) and refed (6 hours)	2.59 ± 0.13*°	142.3% of the controls
Fasted (24 hours) and refed (6 hours) + aminotriazole + methanol	0.96 ± 0.07^	37.0% of the fasted and refed

* $P < 0.01$ with respect to controls
° $P < 0.01$ with respect to fasted
^ $P < 0.01$ with respect to fasted and refed

control activity. This indicates that more catalase-hydrogen peroxide complex (which can be irreversibly blocked by aminotriazole) and hence more hydrogen peroxide was present in the hearts of the fasted animals.

Six hours of previous refeeding almost completely eliminated this phenomenon. In fact, in animals fasted for 24 hours and refed for 6 hours the residual catalase activity after aminotriazole and methanol was 37.0% of that of their own controls (fasted and refed but not treated with aminotriazole and methanol) (Table 2).

The increased hydrogen peroxide production in the heart of the fasted animals was also abolished by the administration of thioridazine (thioridazine hydrochloride, 50 mg/Kg orally, 3 hours before sacrifice), which has been reported to be an inhibitor of peroxisomal beta-oxidation *in vivo*[3] (data not shown).

DISCUSSION

In mice subjected to a 3-day period of food deprivation an increase in plasma free fatty acids occurred together with a rise in the cardiac content of fatty acyl CoA-oxidase and catalase activities. Stimulation of cardiac hydrogen peroxide production was found after 30 hours of fasting and this phenomenon was almost completely eliminated by refeeding which, according to[8] lowers plasma free fatty acid content. The increase in hydrogen peroxide production was also abolished by the administration of thioridazine which has been reported to be an inhibitor of peroxisomal beta-oxidation *in vivo*.[3] Overall, these findings strongly suggest that in the fasting animals cardiac acyl CoA-oxidase and catalase were induced by their respective substrates. Thus, unusually high myocardial loads of fatty acids seem to involve the peroxisomal enzymes in the beta-oxidative process. Indeed, induction of the same system has been shown in the hearts of rats fed on diets with a high content of long chain fatty acids (rapeseed oil or partially hydrogenated marine oil).[9] The myocardial toxicity of such diets has been described.[10] Other authors[11,12] have already shown that fasting leads to increased levels of catalase activity in the heart.

Our results differ from those of Kerckaert and Roels[3] who did not observe any stimulation of hydrogen peroxide production or increases in catalase activity in the heart of fasting rats. However, other than a different species, these authors used a shorter (20 hours) period of fasting than ours (30 hours), which was possibly inadequate to cause any activation of cardiac peroxisomal beta-oxidation.

It is well documented that starvation can cause myocardial injury both in animals[13–16] and in man.[17] The chief event is considered to be loss of cardiac protein probably due both to reduced synthesis,[15] particularly of actin,[14] and to increased degradation by the intervention of lysosomal activities.[13] On the other hand, lipolysis occurs and it is well documented that high circulating levels of free fatty acids can produce a number of noxious effects on the heart.[18] Our findings give evidence that free fatty acids may also activate hydrogen peroxide production by the heart and suggest that, owing to the scarce cardiac content of catalase,[19] this source of free radicals may participate in the cell injury caused by fasting at cell level. In this context an increasing amount of evidence indicates that oxidative stress occurs in the various tissues during nutritional deficiency or food deprivation (for review see reference).[20]

Acknowledgements

We thank Mr Giuseppe Saso for his technical assistance. This work was supported by Consiglio Nazionale delle Ricerche, Grant n. 85.00618.04

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Accepted by Prof. B. Halliwell