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Impaired calcium sequestration activity in liver microsomes from fasted rats

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Calcium uptake activity was assayed in liver microsomal vesicles from fed and fasted rats. This activity required ATP and was stimulated by the calcium trapping agent oxalate. The most striking feature was the low rate of calcium accumulation in liver microsomes from fasted rats. Maximal rate was inhibited up to 66 and 82% after 1 and 3 days starvation, respectively. This defective microsomal calcium handling suggests its possible involvement in the massive glycogen breakdown during starvation.

Microsome; Calcium uptake; Starvation; (Liver)

1. INTRODUCTION

Intracellular free calcium seems to be involved in the hormone-evoked signal transduction system in hepatic tissue. In fact, glycogen breakdown in liver in response to α-receptor activations results from a single signal, namely cytosolic calcium which stimulates glycogenolysis through the activation of phosphorylase kinase [1–3]. Moreover, glucagon, at physiological concentrations, stimulates inositol phospholipid breakdown [4], which in turn may account for the increase in intracellular free calcium [3] since inositol 1,4,5-triphosphate releases calcium from endoplasmic reticulum in permeabilized hepatocytes [5].

The aim of the present work was to study the calcium uptake capacity of the endoplasmic reticulum from liver tissue in fed and fasted rats, considering its possible involvement in the massive glycogen breakdown during starvation. The results show an impaired calcium sequestration activity in liver microsomes from fasted rats.

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2. MATERIALS AND METHODS

Microsomal vesicles were prepared from livers of 200–250 g fed or fasted male Wistar rats [6]. Calcium uptake was measured by membrane filtration through type HA Millipore filters of 0.45 μm pore size previously soaked in 100 mM KCl, 30 mM Hepes (pH 6.8). Liver microsomes were incubated at 37°C in a medium consisting of 100 mM KCl, 5 mM $MgCl_2$, 5 mM ATP, 5 mM azide, 5 mM oxalate, 30 mM Hepes, 40 μM $CaCl_2$ and 0.1 μCi/ml $^{45}CaCl_2$, pH 6.8. Unless indicated otherwise microsomal protein concentration ranged from 0.15 to 0.3 mg/ml. Aliquots of the microsomal suspension were removed and filtered under vacuum conditions. The filters were washed once with 10 ml of the soaking buffer, dried and then dissolved in the scintillation cocktail.

Protein was determined as in [7] with bovine serum albumin as standard. Cyanide-insensitive NADPH–cytochrome-*c* reductase activity was assayed as described in [8]. ATP, Hepes, NADPH and cytochrome *c* were from Boehringer Mannheim and $^{45}CaCl_2$ from Amersham.

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3. RESULTS AND DISCUSSION

Liver microsomes from fed and fasted rats were prepared by differential centrifugation and final sedimentation at 100000 × *g* according to Moore et al. [6]. Microsomal vesicles accumulated calcium provided that MgATP was present in the incubation medium. The rate of calcium uptake was strongly stimulated by the calcium trapping agent oxalate and remained linear for at least 60 min. Maximal rate of calcium accumulation approached 7.2 ± 0.068 nmol·mg protein^{-1}·min^{-1}. The most striking feature was the low rate of calcium accumulation in liver microsomes from fasted rats (table 1). In fact, calcium uptake by liver microsomes was inhibited up to 66 and 82% after 1 and 3 days starvation, respectively (table 2).

The mitochondrial inhibitor sodium azide slightly inhibited calcium uptake by liver microsomes, indicating a minor contamination by mitochondria of microsomal vesicles from either fed or fasted rats (table 1). On the other hand, calcium uptake by liver mitochondria from fed and fasted rats did not differ significantly (not shown, but see [9]).

Table 1

Effect of azide and oxalate on ATP-dependent microsomal calcium uptake

Additions	Fed	Fasted
A None	1.2 ± 0.11	0.9 ± 0.08
B 5 mM oxalate	2.5 ± 0.23	1.5 ± 0.13
C 5 mM ATP	12.9 ± 0.97	9.1 ± 0.75
D ATP + 5 mM NaN_3	11.2 ± 1.1	7.1 ± 0.72
E ATP + oxalate	56.5 ± 5.2	21.2 ± 1.9
F ATP + oxalate + NaN_3	42.5 ± 4.1	19.2 ± 1.8

Calcium uptake was assayed in liver microsomes from fed or 24 h fasted rats. Liver microsomes from fed and fasted rats were assayed in parallel within each experiment. The incubation medium consisted of 100 mM KCl, 5 mM $MgCl_2$, 30 mM Hepes, 40 μM $CaCl_2$ and 0.1 μCi/ml $^{45}CaCl_2$, pH 6.8 and was supplemented as indicated. Values, given as nmol Ca/mg protein per 10 min, are the means ± SE of 4 experiments. The differences in the mean values between fed and fasted conditions were statistically significant ($p < 0.02$) except in series A. The differences C − D and E − F were not statistically significant in either fed or fasted rats

Table 2

Effect of fasting on ATP-dependent microsomal calcium uptake

Alimentary conditions	Calcium uptake (nmol/mg protein per 10 min)
A Fed (controls)	71.3 ± 6.8 (n = 55)
B 24 h fasted	21.7 ± 4.3 (n = 31)[a]
C 48 h fasted	18.4 ± 3.6 (n = 14)[a]
D 72 h fasted	8.1 ± 3.4 (n = 5)[a,b]

[a] $p < 0.001$ vs controls
[b] $p < 0.05$ vs 24 h fasted

Liver microsomes were obtained from control fed rats and from rats fasted for the indicated periods. Calcium uptake was assayed for 10 min as indicated in section 2. Results are the means ± SE of the number of experiments indicated in parentheses

The results in fig.1 show the dependence of microsomal calcium uptake on protein concentration. Calcium accumulation and the concentration of microsomal vesicles were linearly correlated in

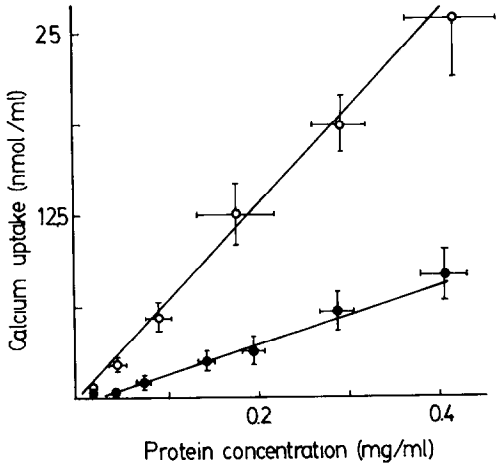

Fig.1. Dependence of calcium uptake on microsomal protein concentration. Liver microsomes were obtained from fed (○) and 24 h fasted (●) rats. In a set of 4 experiments, the uptake of calcium was assayed at increasing protein concentrations in the range 0.014–0.42 mg/ml. The values obtained were pooled with data of separate experiments performed at similar protein concentrations. Mean values of the pooled data were adjusted by linear regression. Each point represents the mean ± SE of 4–41 determinations.

the range 0.014–0.42 mg protein/ml. Starvation decreased the rate of calcium accumulation in liver microsomes by a factor of 0.33–0.14 as compared to fed rats. In five separate experiments, the specific activity of NADPH–cytochrome-*c* reductase in the microsomal pellets averaged 0.34 ± 0.09 and 0.51 ± 0.07 U/mg protein for fed and fasted rats, respectively. Although the difference is not statistically significant, the relatively higher specific activity in fasted rats could be a sufficient criterion to rule out a possible lower content of microsomal membranes in the pellets of fasted rats as a factor to explain the lower calcium accumulation.

The polarity of microsomes, i.e. the orientation of cytoplasmic and luminal surface in microsomal vesicles, does not differ significantly in liver microsomes from fed and fasted rats, as judged by the latency of glucose-6-phosphatase activity [10]. Nevertheless, in this study the polarity of microsomal vesicles was further sought by the accessibility of NADPH–cytochrome-*c* reductase to proteolysis. After a mild proteolysis nearly 80% of the reductase activity of liver microsomes was released into the incubation medium independently of the nutritional state of the rats. Moreover, 0.1% Triton X-100 produced a minor stimulation of the reductase activity. These results indicate the quite similar orientation of microsomal vesicles, with the cytoplasmic surface outside in liver microsomes from fed and fasted rats. Therefore a possible opposite orientation of membranes in microsomal vesicles from fed and fasted rats was ruled out to account for the impaired calcium uptake activity in microsomes from fasted rats.

Conceivably starvation could change membrane environment, i.e. alterations in lipid composition may account for differences in calcium uptake activity in liver microsomes [6]. The possibility that the glucose-6-phosphatase system [11] could be involved in the defective calcium handling described in the present work remains to be studied.

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