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Correlation Between Decreased Expression of Mitochondrial F_0F_1-ATP Synthase and Low Regenerating Capability of the Liver after Partial Hepatectomy in Hypothyroid Rats

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In hypothyroid rats, partial hepatectomy does not induce liver regeneration until 120 h after surgical operation, when, instead, in normal rats a complete recovery of the liver mass, in this interval, is observed. In normal rats, a good efficiency of mitochondrial oxidative phosphorylation is needed as an energy source for liver regeneration (Guerrieri, F. *et al.*, 1995); in hypothyroid rats the efficiency of mitochondrial oxidative phosphorylation is low in the 0–120 h interval after partial hepatectomy. This low efficiency of oxidative phosphorylation appears to be related to a low mitochondrial content of F_0F_1-ATP synthase, in liver of hypothyroid rats, which does not recover after partial hepatectomy. In the liver of hypothyroid rats, low levels of the nuclear-encoded mitochondrial catalytic βF_1 subunit and of its transcript are observed and they do not increase, as occurs in normal rats, after partial hepatectomy.

KEY WORDS: Liver regeneration; hypothyroidism; mitochondria; oxidative phosphorylation; F_0F_1-ATP synthase.

INTRODUCTION

Surgical removal of 70% of the liver induces the transition of hepatocytes from G_0 state in the prereplicative G_1 phase, which lasts for 12–14 h and is followed by DNA synthesis, which peaks at 22–24 h after partial hepatectomy (PH) and by mitosis 6–8 h later (Bucher, 1991). It is commonly accepted that cellular energy metabolism is one of the rate-limiting steps of the regenerative process (La Brecque, 1994). Recently our group showed that, during the prereplicative phase of liver regeneration after PH, the efficiency of mitochondrial oxidative phosphorylation decreases (Guerrieri *et al.*, 1995) and this appears to be related to oxidative damage of the F_0F_1-ATP synthase complex (Guerrieri *et al.*, 1999). During the replicative phase of liver regeneration the progressive recovery of liver mass is associated with the recovery of the efficiency of oxidative phosphorylation (Guerrieri *et al.* 1995, 1999).

Several hormones (Michalopulos, 1990; Bucher, 1991) appear to be essential to liver regeneration. In particular, hepatic regeneration is significantly reduced in thyroidectomized rats and restored after thyroid hormone administration (Canzanelli *et al.*, 1949; Fran-

[1] Key to abbreviations: F_1, catalytic sector of mitochondrial F_0F_1-ATP synthase: β-F_1, subunit of the catalytic sector of F_0F_1-ATP synthase; A_p5A, p1,p5-Di[adenosin-5′-] pentaphosphate; RCI, respiratory control index; T_3, 3,5,3′-triiodo-L-thyronine; T_4 3,5,3′,5′-tetraiodo-L-thyronine (thyroxine), PTU, 6-*n*-propyl-2-thiouracil; PAGE polyacrylamide gel electrophoresis; PH, partial hepatectomy; ATP synthase (E.C.3.6.1.34).

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cavilla *et al.*, 1994). Thyroid hormones influence the mitochondrial function in several tissues, such as liver, kidney, and skeletal muscle (Soboll, 1993; Pillar and Seitz, 1997). Moreover, they play an important role in hepatic mitochondrial maturation (Izquierdo *et al.*, 1990; Almeida *et al.*, 1995) by regulating the expression of both mitochondrial and nuclear genes. In particular, it was observed that thyroid hormones regulate the expression of the catalytic β-F_1 subunit of the mitochondrial F_0F_1-ATP synthase in the liver of neonatal rats (Izquierdo *et al.*, 1990; Izquierdo and Cuezva, 1993) and that, in the liver of hypothyroid rats, both the expression of the β-F_1 subunit and the efficiency of oxidative phosphorylation are decreased (Guerrieri *et al.*, 1998).

As liver regeneration is an *in vivo* system in which active cell growth takes places, it can be used as model system to investigate the role of mitochondrial energy supply for cell proliferation. In this paper, we compared the time course of the changes of various parameters of mitochondrial oxidative phosphorylation during liver regeneration in normal and hypothyroid rats. The results obtained showed that partial hepatectomy in hypothyroid rats does not induce liver regeneration and this appears to be associated with a low efficiency of mitochondrial oxidative phosphorylation. The molecular aspects have been investigated by measuring, at different times after PH, the changes in levels of ATP synthase complex, β-F_1 mRNA, and β-F_1 subunit in normal and hypothyroid rats.

MATERIALS AND METHODS

Materials

6-*n*-Propyl-2-thiouracil (PTU) was purchased from Sigma Chemical Co. Hexokinase and p1,p5-di[adenosine-5′] pentaphosphate (Ap5A) were obtained from Boehringer. SDS, goat anti-rabbit IgG labeled with horseradish peroxidase color development reagent, molecular mass standards, and agarose were from Bio-Rad and nitrocellulose membrane (0.45 μm pore size) were from Schleicher and Schüll. [α-^{32}]dATP (specific activity 3000 Ci/mmol) was from Amersham-Pharmacia. The rat-cDNA probe for β-F_1 subunit was a generous gift from Prof. P.L.Pedersen (Johns Hopkins University, Baltimore, Maryland). All other chemicals were of high purity grade.

Animals

Male Wistar rats (180–200 gm) were housed at a temperature of 22C with food and water *ad libitum*. Hypothyroidism was induced by administration of 0.1% w/v PTU in drinking water for 21 days.

Partial Hepatectomy

Normal and hypothyroid rats were anesthetized with ether/oxygen and the median and left lateral lobes of the liver (corresponding to 65–70% net weight of the whole liver) were excised (Guerrieri *et al.*, 1995). After surgery, the rats were kept on a standard diet until they were sacrificed by decapitation. The trunk blood was collected and the liver was removed, weighed (wet weight), and the regenerated liver used to prepare mitochondria (Bustamante *et al.*, 1977). All operations were carried out under sterile conditions. The study was approved by the State Commission on animal experimentation.

Determination of Thyroid Hormones

Blood, collected from animals, was quickly mixed with equal volume of ice-cold 0.9% NaCl containing 0.24 mg EDTA/100 ml. Plasma was separated by centrifugation in the cold and the samples stored at −70°C until assayed. Plasma triiodothyronine (T_3) was determined using commercial T_3 LIA kits (Diagnostic Products BYK-GULDEN, Italy). After incubation, the tubes were thoroughly decanted and the luminescence was determined. Standard curves were constructed by plotting the amount of total luminescence against hormone concentration.

Electrophoresis and Immunoblotting Procedures

Blue-native PAGE (Schägger and von Jagow, 1991) was performed on a nonlinear (5–10%) polyacrylamide gradient (0.75 mm thickness). Mitochondrial proteins (2.5 mg protein/ml) were incubated at 0°C for 45 min in 750 mM 6-amino-*n*-caproic acid, 50 mM bis-Tris-HCl pH 7.0 and 1% laurylmaltoside. Subsequently, Coomassie Brillant Blue G-250 was added (final concentration, 0.25%). The slab gels were loaded with 0.035 mg of mitochondrial proteins (Guerrieri *et al.*, 1999).

For immunoblotting procedures, mitochondrial proteins (0.05 mg) were separated by SDS-PAGE with a linear gradient of polyacrylamide (15–20%) (Guerrieri *et al.*, 1989). Gels were subjected to immunoblot using polyclonal antibodies against bovine F_1 (Guerrieri *et al.*, 1998).

Nitrocellulose sheets were scanned at 590 nm with a CAMAG TLC Scanner. The quantity of antigen detected was evaluated from computed peak area and expressed in arbitrary units (Guerrieri *et al.*, 1998).

Northern Blot Hybridization

Total liver RNA was extracted from about 250 mg of liver tissue (Chirgwin *et al.*, 1979). For Northern blot hybridization, 20 μg of RNA were loaded on a 1.1% formaldehyde agarose gel (Sambrook *et al.*, 1989). The gel was first photographed under UV light and then blotted onto a Hybond filter (Hybond-Amersham). The membrane was incubated with β-F_1-cDNA labeled by random priming. Labeling, hybridization, and washing conditions were as described elsewhere (Cantatore *et al.*, 1987). After autoradiography, the relative amounts of β-F_1 mRNA was determined by densitometry, comparing the intensity of the hybrid band with that of 28 and 18S rRNA present in the same gel and used as an internal standard.

Assays

Respiratory rate was measured by a Clark oxygen electrode. Mitochondrial proteins (750 μg) were suspended in 1.5 ml of a solution containing 200 mM sucrose, 3 mM $MgCl_2$, 1 mM EDTA, 10 mM K-P_i (pH 7.4), 20 mM glucose, 0.25% bovine serum albumin, 5 units of hexokinase, 300 μM Ap5A, and 2 μg/ml rotenone. After 2 min of equilibration, K-succinate (20 mM, pH 7.4) was added and the respiratory rate in State 4 was measured. After 4 min, Mg-ADP 300 μM was added and the respiratory rate in State 3 was followed. The ADP-stimulated respiratory rate was measured in the presence of the regenerative system for ADP (glucose plus hexokinase) and was linear until oxygen was exhausted (Guerrieri *et al.*, 1998). After 5 min of respiration in State 3, 500 μL of the suspension were taken, treated with 500 μL of 28% $HClO_4$ and the rate of synthesis of ATP determined spectrophotometrically, as described by Papa *et al.* (1969).

RESULTS

PTU is frequently used to induce chemical hypothyroidism in laboratory animals (Blake and Henning, 1985). It acts on the thyroid inhibiting the thyrosine iodination and the coupling reaction (Gilman and Murad, 1975), causing a decrease of both thyroxine (T_4) and T_3. In the periphery, PTU inhibits the conversion of T_4 to T_3 (Gilman and Murad, 1975), thus it would have a relatively greater effect on the concentrations of serum T_3 than on the concentrations of T_4. The present study shows that, in the PTU-treated rats, the serum level of T_3 was significantly reduced as compared to normal animals ($p < 0.01$) which confirms the hypothyroid state (Fig. 1A). In hypothyroid rats, the serum level of T_3 did not change during liver regeneration and always remained significantly lower than in the serum of normal rats (Fig. 1A). In these animals, the T_3 serum level slightly decreased (~26%) 24 h after PH, but returned to about normal values after 96 h (Fig. 1A). In hypothyroid rats, no significant increase of the liver mass is observed until 120 h after PH (Fig. 1B). In normal rats, the time course of recovery of liver total mass showed a lag phase of about 24 h. Liver mass then progressively increased until the total mass was recovered, between 96–120 h after PH (Fig. 1B).

Figure 2A compares the time course of the changes in the mitochondrial respiratory control index (RCI) after PH in normal and hypothyroid rats using succinate as substrate. While in normal rats the RCI first decreased and then progressively recovered in the second phase of liver regeneration (Guerrieri *et al.*, 1995), in hypothyroid rats the RCI was lower than in normal rats and did not change until 120 h after PH (Fig. 2A). The decrease of RCI, observed during the prereplicative phase of liver regeneration in normal rats, is not due to an increase of respiratory rate in State 4, but is related to a decrease of the respiratory rate in State 3 (Fig. 2B). During the replicative phase of liver regeneration, a progressive recovery of respiratory rate in State 3 is observed, so that, 96 h after PH, the respiratory rate in State 3 was almost equal to that observed in liver mitochondria isolated before PH (Fig. 2B). In hypothyroid rats, consistent with previous data (Verhoeven *et al.*, 1985; Horrum *et al.*, 1990; Paradies *et al.*, 1991; Guerrieri *et al.* 1998), the respiratory rates, both in State 3 and State 4, using succinate as substrate, were lower than in liver mitochondria isolated from normal rats, and did not change substantially until 96 h after PH (Fig. 2B). The changes in respiratory rate

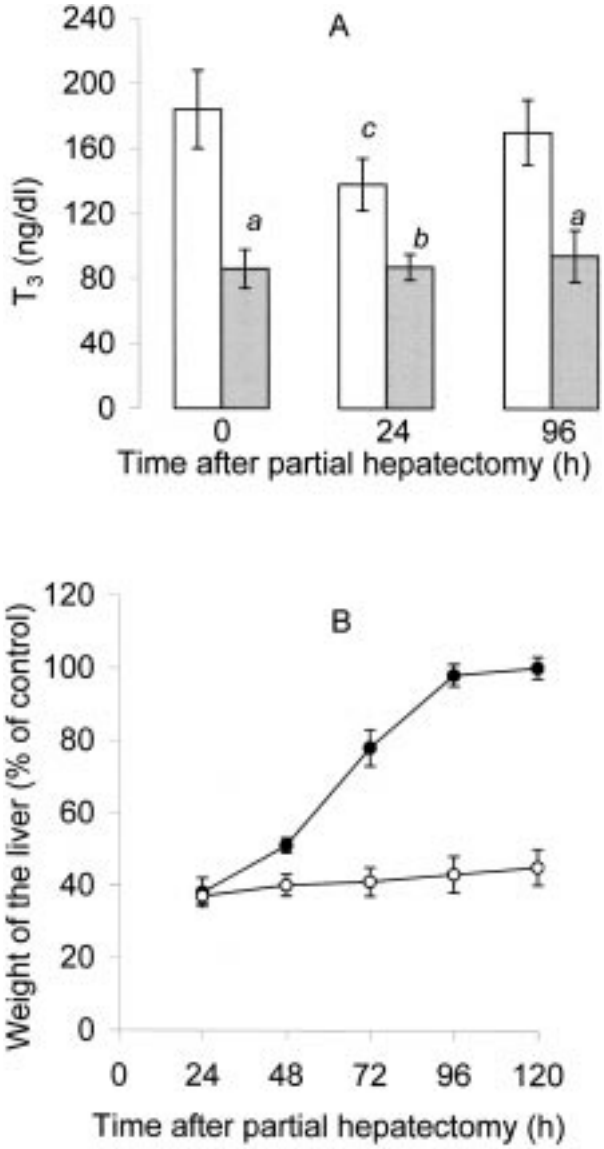

Fig. 1. T_3 serum levels (panel A) and time course of changes in liver mass (panel B) in normal and hypothyroid rats after PH. For treatment of rats with PTU, partial hepatectomy and determination of the T_3 serum levels see section on Materials and Methods. (A) Empty columns: T_3 serum levels in normal rats; filled columns: T_3 serum levels in hypothyroid rats. Data reported are the means ± S.E.M. of 10 different rats. *a*, $p < 0.01$ vs. normal rats at 0 and 96 h after PH, respectively; *b*, $p < 0.02$ vs. normal rats at 24 h after PH; *c*, $p < 0.02$ vs. normal rats at 0 h after PH. (B) The mass of the liver is expressed as a percentage of the weight of the liver of sham-operated normal rats (12.3 ± 1 gm) or hypothyroid rats (7.5 ± 0.5 gm) respectively. Symbols: (●) normal rats; (○) hypothyroid rats.

in State 3, observed in normal rats, reflect changes in the activity of oxidative phosphorylation. This was shown by measuring, during liver regeneration, the rate of ATP synthesis in isolated mitochondria, whose pattern, was similar to that of RCI (Fig. 3A). It is interesting to observe that the recovery of the oxidative phosphorylation, in the 24–96 h interval after PH, is linearly related to the recovery of the liver mass (inset B, Fig. 3A). In hypothyroid rats, as already reported (Guerrieri *et al.*, 1998), the rate of ATP synthesis was much lower as compared to the normal rats. Then, following partial hepatectomy, a further decrease of the rate of ATP synthesis took place during the prereplicative phase (Fig. 3A), but contrary to that which occurred in normal rats, no significant recovery of the efficiency of oxidative phosphorylation was observed in mitochondria from hypothyroid rats. Thus, the rate of ATP synthesis remained at 10–15% of the rate of the control until 120 h after PH (Fig. 3A).

Blue native PAGE of liver mitochondria allows the separation of the complexes of respiratory chain and of the F_0F_1-ATP synthase (Schägger and von Jagow, 1991). Figure 3C shows a representative blue native PAGE of liver mitochondria isolated at 0, 24, and 96 h after PH in normal (N) and hypothyroid rats (H).The changes in the content of F_0F_1-ATP synthase (Guerrieri *et al.*, 1999) were analyzed by semiquantitative densitometric analysis at 590 nm. The results obtained show, in liver mitochondria from normal rats, a pattern similar to that of the rate of ATP synthesis, with a decrease (to about 40% of the control) in the prereplicative phase of liver regeneration (24 h after PH), followed by a recovery, so that at 96 h after PH, the mitochondrial content of F_0F_1-ATP synthase amounted to about 90% of the content of the enzyme in liver mitochondria before PH (Fig. 3D). In liver mitochondria from hypothyroid rats, the amount of F_0F_1-ATP synthase was, before hepatectomy, about 46% with respect to the liver mitochondria from normal rats and, at 24 h after PH, a further decrease to about 25% was observed (Fig. 3D). Afterward, a slight tendency to recover took place, but at 96 h the mitochondrial level of the enzyme was about as low as that observed 24 h after PH in normal rats (Fig. 3D).

The catalytic β-F_1 subunit of the mitochondrial F_0F_1-ATP synthase is encoded by a single copy gene which is ubiquitously expressed in mammalian cells (Neckelmann *et al.*, 1989). In order to study the molecular events responsible for the changes in content and function of F_0F_1-ATP synthase, we compared the changes in mitochondrial β-F_1 content with the changes in the transcription of its gene during liver

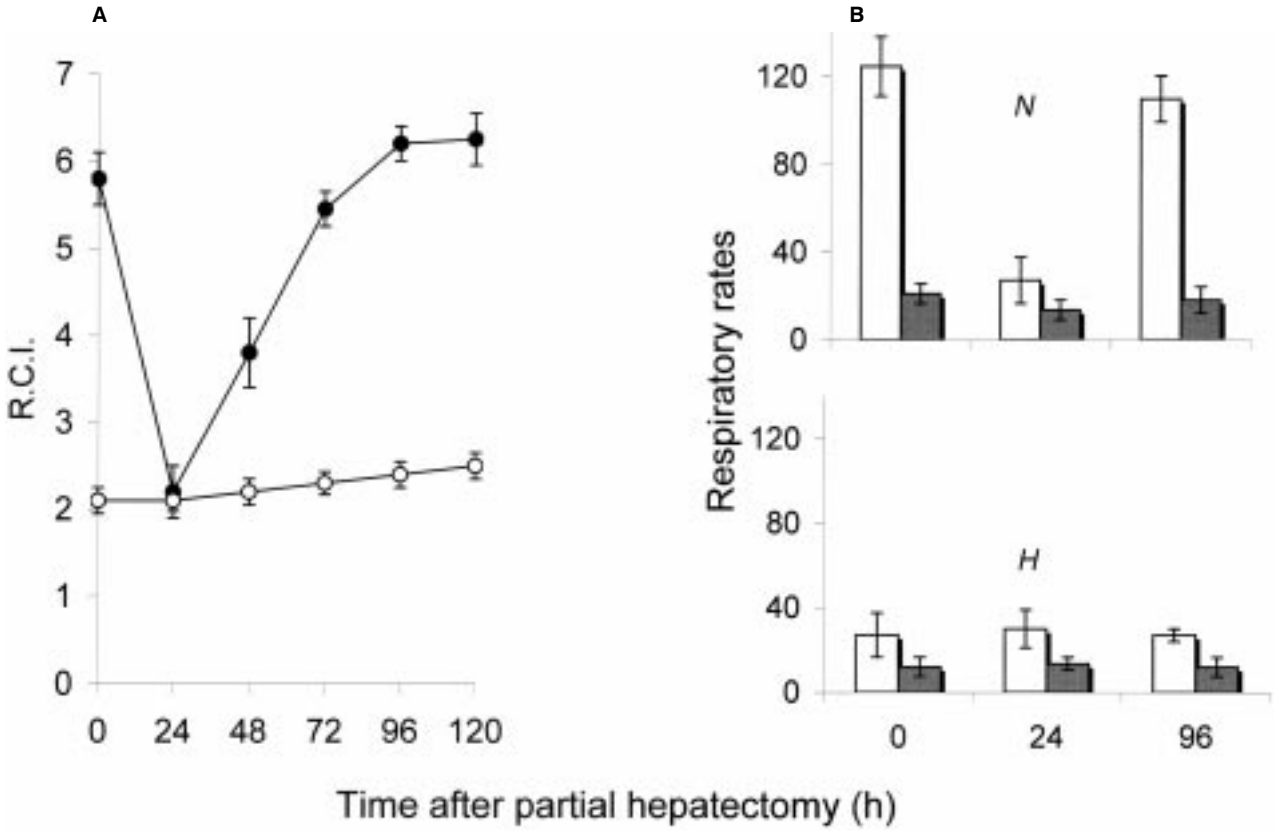

Fig. 2. Respiratory control index (panel A) and respiratory rates (panel B) in liver mitochondria isolated after PH. (A) (●) Normal rats; (○) hypothyroid rats. The respiratory control index (R.C.I.) is obtained as a ratio between the rate of oxygen consumption in State 3 and the rate of oxygen consumption in State 4. The data reported are the means ± SEM of 6 different experiments. (B) Respiratory rates in liver mitochondria from normal (N) and hypothyroid (H) rats at 0, 24, and 96 h after PH. Empty columns refer to respiratory rates in State 3; filled columns refer to respiratory rates in State 4. The respiratory rates are expressed as nanogram atom O/min/milligram mitochondrial protein and determined as described in the section on Materials and Methods. The data are the means ± SEM of 6 different experiments.

regeneration in normal and hypothyroid rats. Figure 4A shows representative Northern blot, for hybridization between RNA extracted from livers of normal (N) or hypothyroid (H) rats at various time after PH and a radioactive β-F_1 probe. Figure 4C shows a representative Western blot for immunodetection of β-F_1 subunit in mitochondria,isolated from livers of normal (N) or hypothyroid (H) rats at various time after PH. Semiquantitative densitometric analysis of immunoblots and Northern blots, showed a decrease of the content of the β-F_1 subunit during the prereplicative phase of liver regeneration in normal rats, while the level of β-F_1 mRNA increased (Fig. 4). In hypothyroid rats, the level of the β-F_1 subunit was about 50% of that observed in liver mitochondria from normal rats (Fig. 4D) and no significative change was observed in the prereplicative phase of liver regeneration, so that 24 h after PH, the level of immunodetected β-F_1 in liver mitochondria from normal and hypothyroid rats was about the same (Fig. 4D). In normal rats, during the proliferative phase, the increase of the transcript for β-F_1 subunit continued until 48–72 h after PH and the mitochondrial level of protein progressively recovered (Fig. 4). At 96–120 h, the mitochondrial level of the β-F_1 was completely recovered, while the liver level of its transcript tended to the normal value (Fig. 4). In hypothyroid rats, although a small increase of the β-F_1 mRNA was observed, the level of the transcript did not exceed that observed in nonproliferating liver of normal rats before PH and no increase of the low level of the mitochondrial β-F_1 was observed for 120 h after PH (Fig. 4).

DISCUSSION

Liver regeneration after partial hepatectomy is characterized by a prereplicative phase in which a low

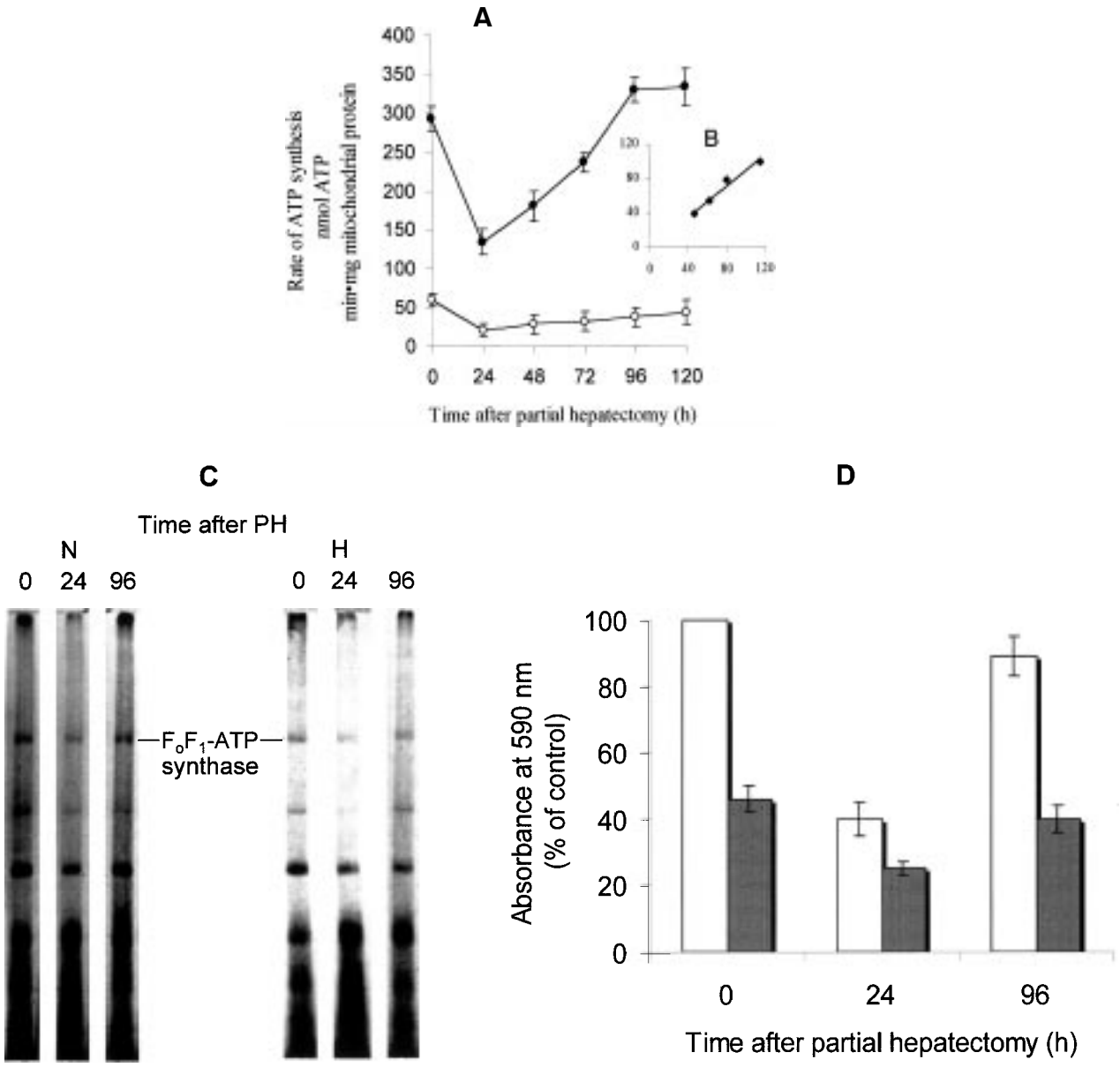

Fig. 3. Changes in the rates of ATP synthesis and in the content of F_0F_1-ATP synthase complex in mitochondria isolated from livers of normal and hypothyroid rats after PH. (A) For experimental procedures, see section on Materials and Methods. Symbols: (●) normal rats; (○) hypothyroid rats. Data reported are the means ± SEM of 6 different experiments. The inset of A (B) shows the linear relationship between recovery of the liver mass (expressed in the ordinate axis as percentage of the liver mass of sham-operated rats, which was 12.3 ± 1 gm, see Fig.1B) in function of the rate of ATP synthesis (expressed in the abscissa axis as percentage of the rate of ATP synthesis in mitochondria isolated at 0 h for normal rats, which was 290 ± 15 nmol ATP/min/mg mitochondrial protein) in the 24–120 h interval after PH in normal rats. (C) Coomassie blue stained analytical native gels of mitochondria isolated from livers at 0, 24, and 96 h after PH of normal (N) and hypothyroid (H) rats The F_0F_1-ATP synthase has been identified by resolution in the second dimension gel and immunoblot (see Guerrieri *et al.*, 1999). (D) Semiquantitative analysis of mitochondrial F_0F_1-ATP synthase content by densitometry at 590 nm of Blue native PAGE of liver mitochondria isolated at 0, 24, and 96 h after PH from normal (empty columns) or hypothyroid rats (filled columns), taking the area peak of the band of the F_0F_1-ATP synthase of the gels of liver mitochondria from control rats as 100% (Guerrieri *et al.*, 1999). For experimental conditions see the section on Materials and Methods and Guerrieri *et al.*, 1999. The data reported are the means ± SEM of 6 different experiments.

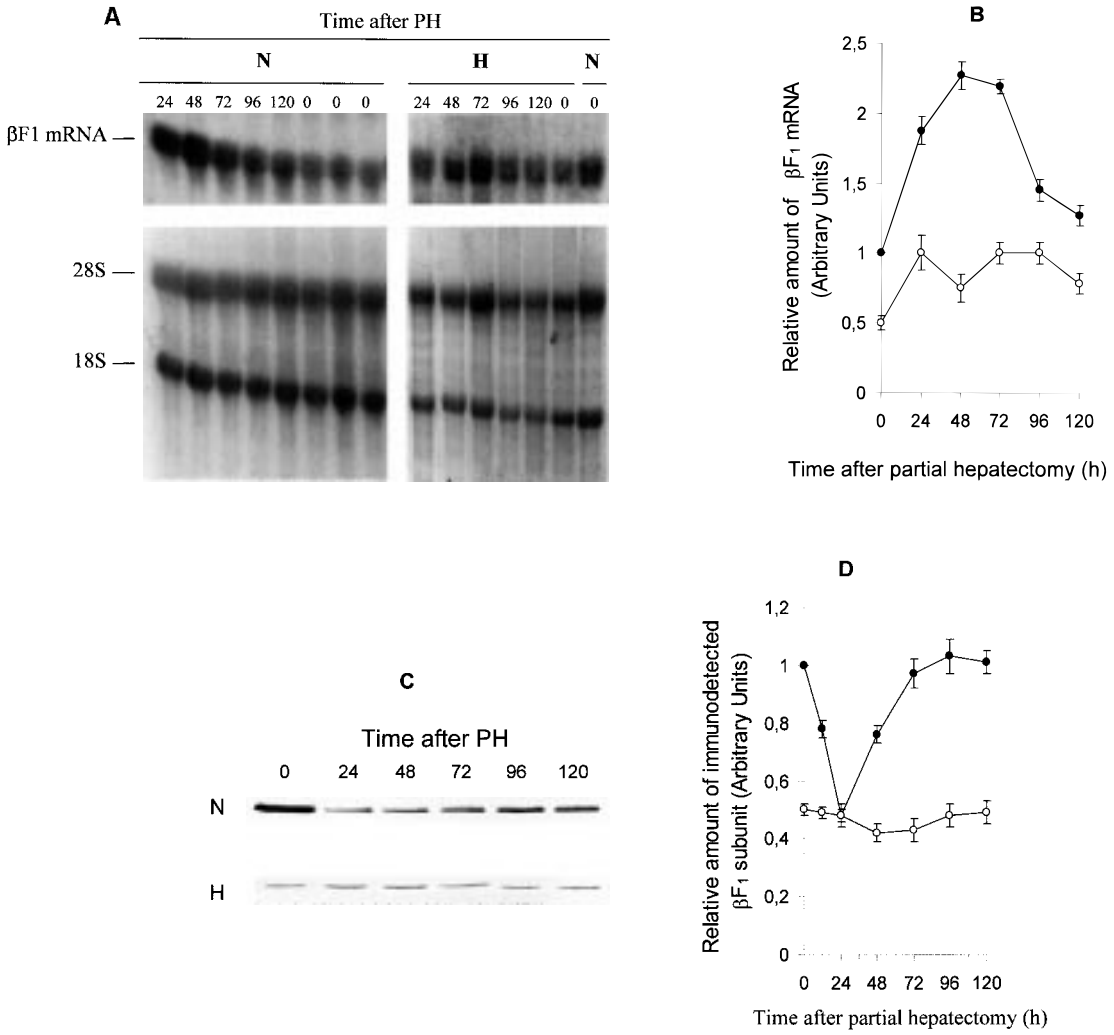

Fig. 4. Changes of β-F_1 mRNA by Northern blot in the liver and of β-F_1 subunit by immunoblots in the mitochondria during liver regeneration in normal and hypothyroid rats (A) Representative Northern blot hybridization between 20 μg of RNA, extracted from the liver of normal (N) or hypothyroid (H) rats at various time after PH, and a radioactive β-F_1 probe. The 28 and 18 S rRNA signal on the same ethidium bromide stained gel is also shown. (B) Semiquantitative analysis by densitometry of Northern blots. Symbols: (●) normal rats; (○) hypothyroid rats. Data represent the means + SEM of results using 6 different preparations. (C) Representative Western blots for immunodetection of β-F_1 subunit in mitochondria, isolated from livers of normal (N) or hypothyroid (H) rats at various times after PH. (D) Semiquantitative analysis by densitometry of Western blots. Symbols: (●) normal rats; (○) hypothyroid rats. Data represent the means ± SEM of results using 6 different preparations.

rate of DNA synthesis is observed (Steer, 1995). This lag in liver growth is accompanied by mitochondrial reactive oxygen species (ROS) production (Vendemiale *et al.* 1995; Lee *et al.*, 1999) with oxidative damage of mitochondrial proteins and, in particular, of the F_0F_1-ATP synthase (Guerrieri *et al.*, 1999), which is the key enzyme of mitochondrial oxidative phosphorylation. Evidence of some similarities between fetal liver and liver after partial hepatectomy, in the prereplicative phase, could be: (1) appearance of fetal isoenzymes (Michalopoulos and De Frances, 1997); (2) low efficiency of mitochondrial oxidative phosphorylation (Izquierdo and Cuezva 1993; Guerrieri *et al.*, 1995); (3) low content of ATP synthase complex (Izquierdo and Cuezva 1993; Guerrieri *et al.*, 1999). Moreover, as after birth, the increase of β-F_1 nuclear gene tran-

scription contributes to differentiation and maturation of liver mitochondria (Izquierdo and Cuezva 1993) during liver regeneration, increased expression of the nuclear encoded β-F_1 subunit is associated with the recovery of the mitochondrial oxidative phosphorylation (Guerrieri *et al.*, 1995).

Although it has been postulated that thyroid hormones do not specifically regulate hepatic regeneration (Bucher, 1991), stimulation of both DNA synthesis (Francavilla *et al.*, 1994; Michalopoulos and De Frances, 1997) and translational activity of mRNA (Knopp *et al.*, 1992) has been observed following subcutaneous injection of T_3 to rats, indicating a role of the thyroid hormones for regeneration of this organ (Knopp *et al.*, 1992; Steer, 1995). On the other hand, hepatic regeneration following PH was significantly reduced in rats made hypothyroid either by thyroidectomy (Canzanelli *et al.*, 1949) or by oral administration of methimazole (Beyer, 1992). Our study shows that liver regeneration is accompanied, in the early phase (0–24 h after PH), by about 26% decrease of serum T_3 level. This could be related to surgical/anesthetic factors (Barsano *et al.*, 1987). In rats, made hypothyroid by PTU in drinking water, partial hepatectomy does not change the hypothyroid state and liver regeneration is significantly reduced. Changes in blood level of thyroid hormones alter the morphology of the mitochondria (Almeida *et al.*, 1997) and, in particular, hypothyroidism results in a decreased number, volume, and membrane area of mitochondria (Gustafsson *et al.*, 1965). Thyroid hormones regulate the expression of nucleus-encoded mitochondrial genes (Izquierdo and Cuezva, 1993; Nelson *et al.*, 1995; Pillar and Seitz, 1997) and of mitochondrial-encoded proteins (Gadaleta *et al.*, 1975, 1986; Enriquez *et al.*, 1999). In particular, it has been reported that thyroid hormones play an important role in postnatal differentiation and proliferation of rat liver mitochondria regulating the expression of the β catalytic subunit of mitochondrial F_0F_1-ATP synthase complex (Izquierdo *et al.*, 1990; Izquierdo and Cuezva, 1993). Chemically induced hypothyroidism decreases the expression and the amount of the β-F_1 in the liver of both neonatal (Izquierdo *et al.*, 1990; Izquierdo and Cuezva, 1993) and adult rats (Guerrieri *et al.*, 1998). Finally, it has been suggested that α- and β-F_1 subunits may have organizer functions for the correct assembly of F_1 sector into the mitochondrial inner membrane (Pillar and Seitz, 1997). The 26% T_3 decrease in the serum of normal rats, observed during the prereplicative phase of liver regeneration, does not seem to affect the expression of the nuclear gene for β-F_1 subunit, which increased during this phase. This implies that in regenerating liver, other factors than T_3 could be involved in the induction of the expression of the β-F_1 gene. In hypothyroid rats, following PH, the T_3 serum level remained low, but the level of the transcript for β-F_1 in the liver, which, before PH, was 50% of that found in normal rats, was doubled in 24 h, confirming that some other regulatory factors (Michaelopoulos, 1990) could contribute to the increase of the expression for the nuclear-encoded β-F_1 subunit following PH. Moreover, in hypothyroid rats, after PH, the increased level of β-F_1 transcript in the liver did not exceed that observed in nonproliferating liver of normal rats before PH and no increase of the low level of the β-F_1 subunit was observed until 120 h. These data agree with previous observations on mitochondrial biogenesis during postnatal differentiation of normal and hypothyroid rats (Luis *et al.*, 1993; Izquierdo *et al.*, 1995), where it was observed that changes in the level of β-F_1 mRNA do not correlate with its translational efficiency. The further decrease of both the amount and the activity of the F_0F_1-ATP synthase complex, observed in hypothyroid rats during the prereplicative phase of liver regeneration, might be related to oxidative damage of the residual mitochondrial F_0F_1-ATP synthase after surgical operation, as was observed in normal rats (Guerrieri *et al.* 1999).

The lack of increase of β-F_1 subunit, observed during the liver regeneration in hypothyroid rats, might depend on the low efficiency of oxidative phosphorylation which, in hypothyroid rats, was only 20% with respect of normal rats. This efficiency is lower than the decrease of the mitochondrial content of the F_0F_1-ATP synthase (46% of the normal rats) or of the β-F_1 subunit (50% of the normal rats) and could be related to alteration in hypothyroid rats of the adenine nucleotide and phosphate carriers (Chen and Hoch, 1977; Paradies *el al.*, 1991). In hypothyroid rats, the low efficiency in oxidative phosphorylation cannot support an active translation of the β-F_1 and no recovery of the mitochondrial activity and content of the F_0F_1-ATP synthase is observed.

In conclusion, our study on liver regeneration after partial hepatectomy in hypothyroid rats are an agreement with the view that the regenerating capability of the liver is correlated with the efficiency of oxidative phosphorylation. In fact, hypothyroidism in rats, causing a decrease of expression of β-F_1 ATP synthase in the liver, practically abolishes the capacity of the liver to regenerate after partial hepatectomy.

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