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SHORT COMMUNICATION

The Effect of Triiodothyronine on Cell Oxidative Capacity in Regenerating Rat Liver

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Summary

The recovery of total DNA content and recovery of total cytochrome c oxidase activity in the rat liver after partial hepatectomy is accelerated by triiodothyronine applied in three doses, two before and one immediately after liver resection. Triiodothyronine-treated animals already have higher cytochrome c oxidase activity before resection. The recovery of the tissue oxidative capacity after partial hepatectomy is more rapid in triiodothyronine-treated animals. These data indicate that hormonal activation of the liver regeneration process is involved.

Key words

Liver regeneration – Triiodothyronine – Mitochondria – Cytochrome c oxidase – Glycerol-3-phosphate cytochrome c reductase

Liver regeneration after partial hepatectomy is a useful model for studying controlled growth *in vivo* (Bresnick 1971). After partial hepatectomy (70 % of liver tissue), the original mass is doubled in size in 48 hours and completely restored in 7–10 days (Bucher and Swaffield 1964). This rapid biogenetic process is highly energy-dependent and requires activation of cell energy metabolism. It is now commonly accepted that the energy metabolism of hepatocytes is one of the rate-limiting steps of the regenerative process (LaBrecque 1994).

The available data indicate that structural changes of mitochondria occur in residual hepatocytes immediately after partial hepatectomy (Murray *et al.* 1981). In the period of 12–24 h, both the capacity and the efficiency of the ATP-producing system are depressed (Buckle *et al.* 1986, Guerrieri *et al.* 1994, Svátková *et al.* 1996). Activities of mitochondrial oxidative enzymes are not changed during this period. A temporary increase of various oxidative enzymes was found to have a peak 7–10 days after partial hepatectomy (Nagino *et al.* 1989, Inomoto *et al.* 1994) at which time the depressed activity of the ATP generating system is also normalized (Guerrieri *et al.* 1994).

It is known that hypothyroidism affects both the structure and the function of mitochondria and that thyroid hormones activate liver oxidative capacity (Lanni *et al.* 1992). Hepatocyte proliferation is also activated (Francavilla *et al.* 1994). In the regenerating liver, we found that DNA synthesis is activated by triiodothyronine (Červinková *et al.* 1984) and decreased in experimentally induced hypothyroidism (Červinková and Šimek 1992). It has been suggested that triiodothyronine is a physiological effector of mammalian mitochondrial biogenesis

(Mutvei *et al.* 1989). We therefore used cytochrome c oxidase activity as an indicator of tissue oxidative capacity and we tried to establish to what extent the administration of triiodothyronine might affect the recovery of original enzyme capacity during the regeneration process.

The experimental animals were two-month-old male Wistar rats fed a standard laboratory diet *ad libitum* and maintained under standard light and temperature conditions. Partial hepatectomy (67.5 % of liver mass) was performed according to the method of Higgins and Anderson (1931). Triiodothyronine (Lyothyronine Gedeon Richter) (200 μg/kg body weight), was administered by stomach tube 48 and 24 hours before liver resection and immediately after the operation. Control animals received an equivalent amount of physiological solution. The DNA content was determined according to Burton (1956). Liver mitochondria were isolated as described earlier (Svátková *et al.* 1996). Cytochrome c oxidase and glycerol-3-phosphate cytochrome c reductase activities as well as the protein concentration were determined as described before (Kalous *et al.* 1989, Rauchová *et al.* 1993). The statistical differences between individual groups were calculated by Student's t-test.

In agreement with our previous data (Červinková *et al.* 1984), we found that the recovery of total DNA during the regeneration process is accelerated (Table 1). The total DNA content in the liver was not changed by two doses of triiodothyronine applied 48 and 24 hours before resection. However, the increase of total liver DNA three days after liver resection, was about 25 % higher in animals that had received triiodothyronine.

Table 1. The effect of triiodothyronine on the increase of liver DNA during the regeneration process after partial hepatectomy

	Residual tissue after resection (A)	3-days of regeneration (B)	Increase (B/A)
Total weight (g)			
$-T_3$	2.89±0.30	5.54±0.40	1.92$^+$
$+T_3$	2.46±0.30	6.31±0.65	2.57$^+$
mg DNA/g			
$-T_3$	1.70±0.15	2.13±0.13	1.25
$+T_3$	1.97±0.17	2.33±0.14	1.18
Total DNA (mg)			
$-T_3$	4.90±0.45	11.80±0.35	2.40$^+$
$+T_3$	4.84±0.52	14.70±0.42	3.03$^+$

Triiodothyronine was applied ($+T_3$) in three doses (200 μg/kg), 48 and 24 hours before liver resection and immediately after resection. Control groups ($-T_3$) received equivalent amount of physiological solution. Data indicate averages from six animals ± S.E.M., $^+$ *$p<0.01$.*

The recovery of total oxidative capacity after partial hepatectomy was more rapid in triiodothyronine-treated animals. We found an increase in liver cytochrome c oxidase activity already after the administration of two doses of the hormone before resection and the activity further increased during the regeneration process. In animals without triiodothyronine treatment the enzyme activity remained unchanged (Table 2).

When the total cytochrome c oxidase activity was calculated, it was quite evident that the increase of total oxidative capacity of liver after partial hepatectomy is more rapid in triiodothyronine-treated animals (Table 2). Before the resection, cytochrome c oxidase activity in the liver was higher by 50 %. Within three days after partial hepatectomy, the total activity increased to 300 %, but only to 200 % in animals without triiodothyronine treatment (Table 2).

The specific activity of cytochrome c oxidase of isolated mitochondria was about 20–30 % higher in triiodothyronine-treated animals. However, in neither group did we find any changes in mitochondrial cytochrome c oxidase activity during the regeneration period (Table 2).

Table 2. Changes of liver cytochrome c oxidase during the regeneration process after partial hepatectomy

Time after resection	$-T_3$ (A)	$+T_3$ (B)	B/A
Cytochrome c oxidase activity of liver homogenate (μmole cyto c reduced per min per mg protein)			
Control	0.655 ± 0.030	1.032 ± 0.110	1.55*
24 hours	0.607 ± 0.062	1.045 ± 0.116	1.72*
48 hours	0.688 ± 0034	1.207 ± 0.039	2.05+
72 hours	0.650 ± 0.025	1.252 ± 0.081	1.93+
Total liver cytochrome c oxidase (μmole cyto c reduced per min per organ)			
Control	376 ± 27	483 ± 22	1.28+
24 hours	453 ± 50	726 ± 110	1.60*
48 hours	587 ± 69	1112 ± 90	1.89+
72 hours	819 ± 79	1560 ± 19	1.90+
Cytochrome c oxidase activity of isolated liver mitochondria (μmole cytochrome c reduced per min per mg mitochondrial protein)			
Control	1.57 ± 0.16	2.10 ± 0.10	1.34
24 hours	1.33 ± 0.17	1.92 ± 0.11	1.44+
48 hours	1.64 ± 0.08	2.28 ± 0.14	1.42*
72 hours	1.50 ± 0.11	2.12 ± 0.31	1.41*

Triiodothyronine was administered, where indicated ($+T_3$), in three doses (200 μg/kg 0, 48 and 24 hours before liver resection and immediately after resection. Control groups ($-T_3$) received the equivalent amount of physiological solution. The data indicate averages from six animals ± S.E.M., $^+ p<0.01$, $^* p<0.05$.

The fact that liver mitochondria are affected under our experimental conditions by triiodothyronine treatment may be demonstrated by measurements of mitochondrial FAD-linked glycerol-3-phosphate dehydrogenase activity. We found, in agreement with Lee and Lardy (1965), that the activity of this enzyme in mitochondria isolated from triiodothyronine-treated animals is twice as high as in control animals.

We may thus conclude from the data on cytochrome c oxidase activity in liver homogenate and isolated mitochondria that the recovery of liver oxidative capacity in the early period of the regeneration process (first three days after partial hepatectomy) is due to activation of mitochondrial biogenesis, because the specific activity of cytochrome c oxidase of isolated mitochondria was not changed. Data in the literature indicate that a temporary increase of various oxidative enzymes occurs 4–10 days after resection (Nagino *et al.* 1989) which further enhances the liver oxidative capacity during the regeneration process in this period.

In triiodothyronine-treated animals, specific cytochrome c oxidase activity is increased by almost 50 % before liver resection and, is further stimulated during the first three days of the regeneration process. Because the level of thyroid hormones is already normalized by the third day after the last dose of triiodothyronine, it is evident from our data that triiodothyronine potentiates the increase of liver oxidative capacity during the regeneration process. Triiodothyronine in the regenerating liver may thus help to overcome the problems of energy deficiency for biogenetic processes (LaBrecque 1994).

Our recent observations have shown that triiodothyronine also activates the biogenesis of ATP synthase in the liver (Guerrieri *et al.* unpublished data). Therefore we may expect that in the presence of triiodothyronine both parts of the oxidative phosphorylation system, namely the

respiratory chain and ATP synthase, are able to better cover the high energetic demands of the regenerating process in the liver after partial hepatectomy.

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