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SHORT COMMUNICATION

Activation of Mitochondrial Glycerophosphate Cytochrome *c* Reductase in Regenerating Rat Liver by Triiodothyronine

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Summary

Triiodothyronine administration before partial hepatectomy increased the activity of mitochondrial glycerophosphate cytochrome *c* reductase. The enzyme activity was further activated after partial hepatectomy during the regenerative process. Our findings showed that: a) the increase of glycerophosphate cytochrome *c* reductase induced by triiodothyronine was further potentiated by the regeneration process, b) the high activity of the glycerophosphate shuttle was maintained after partial hepatectomy during the period, when most of the liver tissue had again been recovered.

Key words

Liver regeneration • Triiodothyronine • Mitochondria • Glycerophosphate cytochrome *c* reductase • Cytochrome *c* oxidase

Triiodothyronine (T_3) administration before partial hepatectomy (PH) stimulates rat liver regeneration as manifested by increased DNA synthesis and an increase of mitotic activity in regenerating tissue (Červinková *et al.* 1984). On the other hand, thyroidectomy or propylthiouracyl administration inhibited growth both in the intact and the regenerating liver (Červinková and Šimek 1992). T_3 administration before PH improved liver steatosis, increased oxidative capacity of hepatocytes in regenerating tissue (Červinková *et al.* 1998) and decreased changes of membrane fluidity induced by PH (Drahota *et al.* 1999). Thyroid hormones may thus be considered as a part of hormonal milieu that supports the regenerative process after PH (Leffert *et al.* 1979, LaBrecque 1994).

It is well known that T_3 application activates the expression of several nuclear encoded genes of mitochondrial energy coupling system in the rat liver. Besides cytochrome *c*, adenine nucleotide translocator, and some of cytochrome *c* oxidase subunits a very high increase of mRNA was also found for mitochondrial glycerophosphate dehydrogenase (mGPDH) (Müller and Seitz 1994). Flavoprotein-dependent mGPDH together with the NADH-dependent cytosolic glycerophosphate dehydrogenase (cGPDH) form a glycerophosphate shuttle, which has many important regulatory functions in cell metabolism. The maintenance of a high rate of glycolytic ATP production could be especially important for regenerative processes. The reoxidation of cytosolic NADH by the glycerophosphate shuttle also avoids

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lactate formation (Bücher and Klingenberg 1958). Furthermore, the fact that the cytosolic NADH formed by glycolysis through this pathway can be used as an additional energetic substrate for mitochondrial ATP production (Bücher and Klingenberg 1958) may be important during liver regeneration. In the liver, cGPDH activity exceeds the activity of mGPDH, which is thus a limiting factor for the glycerophosphate shuttle function. We used the enzyme activity as an indicator of hormonally activated biogenesis because it was demonstrated that the activity well correlates with expression of mGPDH mRNA (Müller and Seitz 1994). We therefore studied the question whether mitochondrial glycerophosphate cytochrome *c* reductase hormonaly activated before PH, was maintained during the regenerative process.

We used two-month-old male albino Wistar rats (Velaz Prague, Czech Republic) fed by a standard laboratory diet *ad libitum* and maintained under standard light and temperature conditions (Červinková *et al.* 1998). PH (removal of 67.5 % of liver tissue) was performed according to the method of Higgins and Anderson (1931). Triiodothyronine (Liothyronine, Gedeon Richter, Hungary) dissolved in saline (20 μg/ml) was applied by stomach tube in three portions 48 and 24 h before PH and immediately after the surgery in a dose of 200 μg/kg body weight. Control animals received an equivalent amount of physiological saline. Liver mitochondria were isolated as described earlier (Svátková *et al.* 1997). Glycerophosphate cytochrome *c* reductase activity was determined as described previously (Rauchová *et al.* 1993) in the presence of 0.05 % fatty acid-free bovine serum albumin (BSA). The content of cytochrome aa_3 in isolated mitochondria was determined as described by Kalous *et al.* (1989). The statistical significance was calculated using Student's t-test.

Table 1. Mitochondrial glycerophosphate cytochrome *c* reductase activity (nmol of cytochrome *c* oxidized per min per mg of mitochondrial protein) in the liver of control and T_3-treated rats

Time after PH (hours)	Control animals	T_3-treated animals
0	11.7 ± 0.25 (100 %)	21.4 ± 0.33 ** (182 %)
24	11.0 ± 0.49 (94 %)	38.4 ± 1.99 ** (342 %)
48	14.7 ± 0.38 (126 %)	40.8 ± 2.70 ** (349 %)
72	12.4 ± 0.52 (103 %)	39.4 ± 2.92 ** (337 %)

*All values represent means from 6 animals ± S.E.M. ** Significantly different ($p<0.01$) from control rats.*

It is evident from Table 1 that, in agreement with data in literature (Müller and Seitz 1994), the glycerophosphate cytochrome *c* reductase activity was increased after T_3 administration. This increase was further potentiated during the regenerative process, when the T_3 level had already been normalized (Francavilla *et al.* 1994).

We measured activity of glycerophosphate cytochrome *c* reductase in the presence of BSA to eliminate interference of free fatty acid inhibition (Houštěk and Drahota 1975), because we found a temporary increase of liver triacylglycerols after PH (Červinková *et al.* 1998). In the absence of BSA, the values of the enzyme activities in controls and T_3-treated rats were about 30 % of those in the presence of BSA (not shown), but the differences between particular groups were identical (Table 1).

Determination of glycerophosphate cytochrome *c* reductase activity, using cytochrome *c* as electron acceptor, also includes the activity of the bc_1 complex. Nevertheless, succinate and NADH cytochrome *c* reductases are not affected by T_3 (Lee and Lardy 1965). Therefore modified activity of the bc_1 complex cannot be responsible for the observed changes.

When we measured the cytochrome *c* oxidase activity in the liver mitochondria after PH we also found

Table 2. Content of cytochrome aa_3 (nmol/mg protein) in liver mitochondria of control and T_3-treated rats

	Control animals (A)	**T_3–treated animals** (B)	**B/A**
Before PH	0.184 ± 0.012	0.213 ± 0.029	1.15
72 h after PH	0.184 ± 0.029	0.297 ± 0.017 *	1.61

All values represent means from 6 animals ± S.E.M. Where indicated the difference between. control and T_3-treated group is significant ($p<0.02$). T_3 - 3 x 200 μg/kg b.w.*

a stimulatory effect of T_3 that was further increased during liver regeneration (Červinková *et al.* 1998). To extend these findings we also determined the content of cytochrome aa_3 in parallel to glycerophosphate cytochrome *c* reductase (Table 2). We found neither significant increase of cytochrome aa_3 after three doses of T_3, nor changes in control animals during the regenerative period. However, a combination of hormonal action and the regenerative process increased the content of cytochrome aa_3 by 60 % (Table 2). This further confirmed our previous findings that T_3 application before PH increases the oxidative capacity of mitochondria in the regenerating tissue. Our results clearly document that the metabolic changes induced by T_3 administration before PH are further activated during following regenerative period when the plasma hormone level is already normalized (Francavilla *et al.* 1994). We used the data of cytochrome aa_3 for evaluation of glycerolphosphate cytochrome *c* reductase activity per nmol cytochrome aa_3. These calculations showed that values of glycerophosphate cytochrome *c* reductase per nmole of cytochrome aa_3 in control animals are 63.6 and 67.4 nmol of cytochrome *c* oxidized per min per nmole of cytochrome aa_3 before PH and 72 h after PH, respectively. In hormone-treated rats, we found 100.5 and 132.7 nmol of cytochrome *c* oxidized per min per nmol cytochrome aa_3 before PH and 72 h after PH, respectively. These data further confirm that both glycerophosphate cytochrome *c* reductase and cytochrome *c* oxidase activity are higher in hormone-pretreated animals during the regeneration process. Our data thus show that the increase of mGPDH activity is relatively higher than that of cytochrome c oxidase. This could be explained by the fact that the activity of cytochrome *c* oxidase, a terminal enzyme of mitochondrial respiratory chain, is in high excess when compared with activities of all respiratory chain-linked oxidases. Therefore, an increase of mGPDH activity is not necessary for compensating a parallel increase of cytochrome *c* oxidase.

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Reprint requests

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