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SHORT COMMUNICATION

The Effect of Triiodothyronine on Changes of Membrane Fluidity in Regenerating Rat Liver

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Summary

The increase of the membrane fluidity during the early phase of liver regeneration after partial hepatectomy was described in literature in plasma membrane and in microsomes. We found similar changes also in isolated mitochondria and in crude total membrane fraction of the liver homogenate. The administration of triiodothyronine to rats before partial hepatectomy diminished the increase of the membrane fluidity in the regenerating liver by 50 %. Triiodothyronine effect is explained by hormonal modification of lipid metabolism in the regenerating liver.

Key words

Liver regeneration • Membrane fluidity • Triiodothyronine • Mitochondria

It has repeatedly been shown that changes of microsomal and plasma membrane fluidity occur during the early phase of liver regeneration after partial hepatectomy (Bruscalupi *et al.* 1980, Koshlukova *et al.* 1992, Yoshida *et al.* 1993, Macho *et al.* 1994).

The highest increase of plasma membrane fluidity was found 6-24 h after partial hepatectomy which was followed by full recovery of the original fluidity after 48-72 h. This increase of membrane fluidity correlates with liver steatosis and an increase of triacylglycerols in the serum (Tijburg *et al.* 1991, Červinková *et al.* 1998). However, the mechanism and physiological significance of this process is not yet fully understood.

We have previously found that triiodothyronine affects many metabolic processes occurring in the regenerating liver including changes of triacylglycerols (Svátková *et al.* 1997, Červinková *et al.* 1998). In the present report, we tested to what extent the increase of membrane fluidity after partial hepatectomy can be modified by triiodothyronine administration.

Changes of membrane fluidity in the regenerating liver were measured in isolated plasma membranes (Bruscalupi *et al.* 1980, Yoshida *et al.* 1993, Macho *et al.* 1994) and also in isolated microsomes (Koshlukova *et al.* 1992). These findings indicated that these changes are more general and could be detected in all cell membranes. We have therefore tested the changes

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of membrane fluidity after partial hepatectomy in isolated mitochondria, where this process has not been hitherto evaluated. We have also tested the changes of membrane fluidity after partial hepatectomy in crude preparations of whole liver cell membranes.

In our experiments, we used male albino Wistar rats (Velaz, Prague, Czech Republic) with an initial body mass of 180-200 g housed at 23 ± 1 °C, in a 12:12 hour light-dark regime. The animals had free access to standard laboratory rat chow (DOS 2B Velaz, Prague, Czech Republic) and tap water. Partial hepatectomy was performed under mild ether anesthesia according to Higgins and Anderson (1931). Most of liver mass (65-70 %) was removed comprising the left and medial lobe of the liver. Surgery was performed between 07:00-09:00 h. The animals were kept under the above described conditions after the operation and decapitated 24, 48, and 72 h after partial hepatectomy. Triiodothyronine (Gedeon Richter, Hungary) dissolved in physiological saline (20 μg/ml) was applied by stomach tube in three portions: 48 and 24 h before partial hepatectomy and immediately after the surgery in a dose of 200 μg/kg body weight. Control animals received equivalent amounts of a physiological saline solution. The excised liver tissue was homogenized at 0 °C in 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4 using a glass Teflon homogenizer. Aliquots of 10 % homogenates were used for isolation of mitochondria (Svátková *et al.* 1996). The remaining part of the homogenate was sonicated three-times for 30 s at maximal output of the MSE sonicator. The suspension was centrifuged for 60 min at 100 000 x *g* at 0 °C using a Beckman L 90 ultracentrifuge. Particle sediment was suspended in 0.154 M KCl, 10 mM Tris-HCl, pH 7.4 and stored at −70 °C. Diphenylhexatriene steady-state fluorescence anisotropy was determined and calculated as described by Kuneš *et al.* (1994). Frozen-thawed membranes were labeled with 2 x 10^{-7} M diphenylhexatriene (Molecular Probes, Eugene, Oregon, USA) at 30 °C for 20 min. The fluorescence measurements were carried out in 5 x 5 mm quartz microcuvettes at 37 °C using a Perkin Elmer luminescence spectrometer LS 50 B equipped with polarizers. The excitation and emission wavelengths were 350 and 450 nm, respectively. Proteins were determined according to Lowry *et al.* (1951). All values in Figure 1 and Table 1 represent averages from 6 animals ± S.E.M. values. Statistical significance was calculated using Student's t-test.

Table 1. Changes of diphenylhexatriene steady-state fluorescence anisotropy in rat liver mitochondria during regeneration after partial hepatectomy

Time after hepatectomy (hours)	Fluorescence anisotropy	%	
0	0.126±0.002	100	
24	0.112±0.002	88	(p<0.001)
48	0.125±0.002	99	n.s
72	0.121±0.002	96	n.s

Data represent means ± S.E.M. values from six animals.

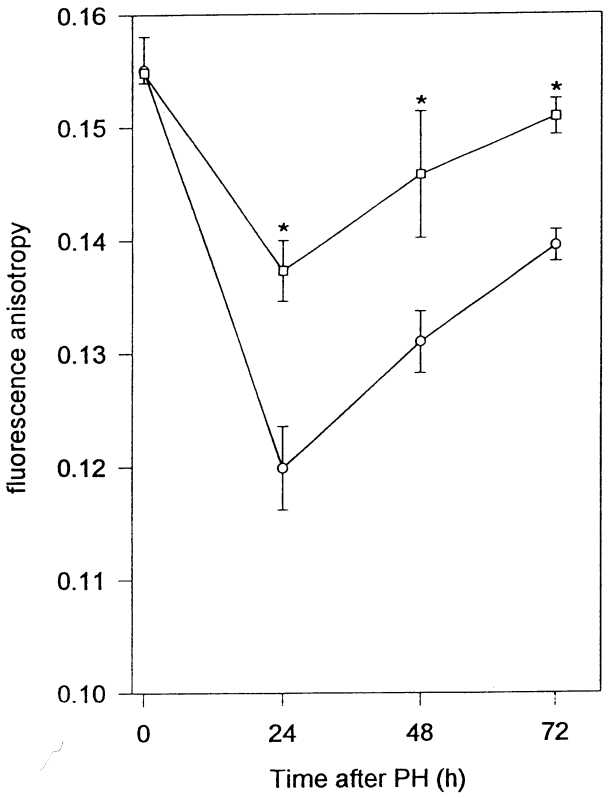

Fig. 1. *Changes of diphenylhexatriene steady-state fluorescence anisotropy in total liver membranes of control (circles) and triiodothyronine-treated (3 x 200 μg triiodothyronine/kg body weight) rats (squares) during liver regeneration after partial hepatectomy (PH). All values represent means ± S.E.M. n=6. Asterisks indicate the significant (p< 0.05) difference between control and triiodothyronine-treated group.*

As is demonstrated in Table 1, the mitochondrial membrane fluidity changes are less marked but parallel to those described in plasma membranes and microsomes (Bruscalupi *et al.* 1980, Koshlukova *et al.* 1992, Yoshida *et al.* 1993, Macho *et al.* 1994). The maximal decrease of fluorescence steady-state anisotropy, indicating an increase of membrane fluidity, occurred 24 h after partial hepatectomy. The values steady-state fluorescence anisotropy found 48 and 72 h after partial hepatectomy were the same as in the normal liver.

The findings presented in Table 1 support the idea that the changes of membrane fluidity after partial hepatectomy are more general and are thus related to all cell membrane lipoprotein structures. Therefore, on the basis of these findings we used crude liver membrane fractions prepared from liver homogenates (100 000x*g* sediment) in our further experiments and we found similar changes (Fig. 1) as those described in plasma membranes (Bruscalupi *et al.* 1980, Yoshida *et al.* 1993, Macho *et al.* 1994), microsomes (Koshlukova *et al.* 1992) and mitochondria (Table 1). Contrary to our results with isolated mitochondria, we found a significant decrease of steady-state fluorescence anisotropy also 48 and 72 h after partial hepatectomy. This indicates that the extent of fluorescence steady-state anisotropy decreases and its rate of recovery might be different in various cell membrane fractions.

We also used crude membrane preparations for detecting the membrane fluidity changes after partial hepatectomy in triiodothyronine-treated rats (Fig. 1). We found that triiodothyronine application diminishes the decrease of membrane fluidity induced by partial hepatectomy by 50 %. Our data have shown that triiodothyronine can not completely eliminate the changes in steady-state fluorescence anisotropy occurring during the regeneration process, but these changes are less expressed and the rate of recovery is higher in comparison with the group of non-treated rats (Fig. 1).

The mechanism of the triiodothyronine effect on changes of membrane fluidity during the early phase of liver regeneration after partial hepatectomy could be explained by interaction of the hormone with cell lipid metabolism. Two mechanisms participating in the membrane fluidity changes could be involved: (a) modification of phospholipid composition (Bangur *et al.* 1995) or changes of the cholesterol to phospholipid ratio (Koshlukova *et al.* 1992), (b) modification of the increase of liver triacylglycerols content (Tijburg *et al.* 1991, Červinková *et al.* 1998) and/or an increase of free fatty acids (Lai and Chen 1995), known as the factors increasing membrane fluidity (Amler *et al.* 1986).

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