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LARGE INDUCTION OF TYPE III DEIODINASE (D3) EXPRESSION AFTER PARTIAL HEPATECTOMY IN THE REGENERATING MOUSE AND RAT LIVER.

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Abstract

The deiodinases type 1 (D1) and type 2 (D2) catalyze the activation of T4 to T3, whereas type 3 deiodinase (D3) catalyzes the inactivation of T3 and T4. D3 plays a key role in controlling thyroid hormone bioavailability. It is highly expressed during fetal development, but also in other processes with increased cell proliferation, e.g. in vascular tumors. Since tissue regeneration is dependent on cellular proliferation and is associated with activation of fetal genes, we evaluated deiodinase activities and mRNA expression in rat and mouse liver as well as the local and systemic thyroid hormone status after partial hepatectomy (PH).

We observed that in rats, D3 activity was increased 10-fold at 20 h and 3-fold at 48 h after PH; D3 mRNA expression was increased 3-fold at 20 h. The increase in D3 expression was associated with maximum 2 to 3-fold decreases of serum and liver T3 and T4 levels at 20 to 24 h after PH. In mice, D3 activity was increased 5-fold at 12 h, 8-fold at 24 h, 40-fold at 36 h, 15-fold at 48 h; and 7-fold at 72 h after PH. In correlation with this, D3 mRNA was highest (6-fold increase) and serum T3 and T4 were lowest at 36 h. Furthermore, as a measure for cell proliferation, BrdU incorporation peaked at 20 to 24 h after PH in rats and at 36 h in mice. No significant effect on D1 activity or mRNA expression was found after PH. D2 activity was always undetectable.

In conclusion, we found a large induction of hepatic D3 expression after PH which was correlated with an increased cellular proliferation and decreased serum and liver T3 and T4 levels. Our data suggest that D3 is important in the modulation of thyroid hormone levels in the regenerating liver, in which a decrease in cellular T3 permits an increase in proliferation.

Introduction

A strict regulation of thyroid hormone levels is essential for normal development of different tissues, as well as for the metabolic function of these tissues. The bioavailability of thyroid hormone is regulated by three iodothyronine deiodinases (D1, D2 and D3). D1 and D2 are thyroid hormone activating enzymes that catalyze the conversion of the prohormone thyroxine (T4) to the active hormone 3,3',5-triiodothyronine (T3). D1 is expressed in liver, kidney, thyroid and pituitary, and plays an important role in the production of serum T3 (1). D2 is mainly expressed in brain, pituitary and brown adipose tissue. In tissues such as brain, this enzyme is important for the local activation of T3 (1). D3 is the major inactivating pathway, catalyzing the degradation of T3 to 3,3'-diiodothyronine (T2) and the conversion of T4 to the inactive metabolite reverse T3 (rT3). It is abundantly present in fetal tissues such as liver and brain, and in placenta, pregnant uterus and umbilical arteries and vein (1, 2). The high expression in the fetal compartment suggests that D3 plays an essential role in the regulation of fetal T3 levels, as a mechanism to protect the fetus from excessive exposure to active thyroid hormone. For instance, in the fetal brain, D3 expression is highly regulated in different regions, protecting the brain from excessive T3 until differentiation is required (3).

In contrast to the high D3 activities in the fetus, its activities are normally undetectable in most adult tissues. This is probably why D3 has long been neglected in studies on the regulation of thyroid hormone bioavailability in the adult. However, recent studies have revealed the reexpression of D3 in different pathophysiological conditions, among which cancer, cardiac hypertrophy, myocardial infarction, chronic inflammation and critical illness (4-9). Just like fetal growth, most of these are processes in which proliferation or cell growth take place.

It is generally believed that T3 stimulates the differentiation of cells and decreases their proliferation. Hernandez et al. recently showed that differentiation of precursor cells to adipocytes was associated with a decrease in D3 expression (10), and Dentice et al. demonstrated that D3 is increased in proliferating keratinocytes (11). Also these findings confirm the concept that in general T3 stimulates cellular differentiation, and suggest a role for D3-mediated low intracellular T3 concentrations in cellular proliferation.

Liver regeneration is also a process dependent on cellular proliferation. Following hepatectomy of 70% of the liver, liver cells switch from a quiescent state to a proliferative state and re-enter the cell cycle (12). In rodents, during the first 4 hours after partial hepatectomy (PH), quiescent hepatocytes enter the cell cycle (G1 phase). This proliferative phase is rather short in rats, but is prolonged in mice, the peak of DNA synthesis being 40-44 h after PH (12, 13). During these processes, many fetal genes, which are not expressed in normal adult liver, are reactivated (12, 14). In the present study we investigated the possible reexpression of D3 in regenerating liver, by determining deiodinase activities and mRNA expression in rat and mouse liver after PH.

Materials and Methods

Materials

[3'-^{125}I]T3 and [3',5'-^{125}I]T4 were obtained from GE Healthcare (Amersham, UK); and [3',5'-^{125}I]rT3 was prepared by radioiodination of 3,3'-T2 as described previously (15). Non-radioactive iodothyronines were purchased from Henning Berlin GmbH (Berlin, Germany); dithiothreitol (DTT) from ICN Biochemicals Inc. (Costa Mesa, CA). 5-Bromo-2'-deoxyuridine (BrdU) and real-time PCR probes and primers were obtained from Sigma (St. Louis, MO), and Eukitt mounting medium from O. Kindler GmbH & Co (Freiburg, Germany).

Partial hepatectomy model

Wistar rats were obtained from the Hubrecht Laboratory, Utrecht, The Netherlands. Mice on the FVB background were obtained from Harlan, Horst, The Netherlands. The animals were allowed free access to food and water. Mice and rats were anesthesized by buprenorphine and then underwent PH by removing 70% of the liver, or only opening of the abdominal skin in the case of sham-treated control animals. Serum and liver tissues were

collected at PH (t=0) and at different time periods after PH. The same region of the liver was isolated from sham-treated controls. All liver samples were immediately frozen in liquid nitrogen and stored at –80 C until further analysis; serum was stored at –20 C. All procedures performed on the animals were approved by the Committee on Animal Experiments of the Utrecht University (protocols 102648-1 and 102648-2).

Determination of BrdU incorporation

Two hours before ending the experiment animals received an intraperitoneal injection with BrdU in PBS, using 30 µg/gram body weight BrdU for mice or 50 µg/gram body weight BrdU for rats. After the animals were sacrificed liver tissues were fixated in 10% formalin and imbedded in paraffin. Slides were stained overnight with the primary mouse anti-BrdU antibody M0744, clone Bu20a (Dako, Heverlee, Belgium), diluted 1:50 for rats and 1:100 for mice. Subsequently, peroxidase-conjugated rabbit anti-mouse Igg was applied and immunoreactivity was detected using the DakoCytomation Envision+ System, HRP (Dako, Heverlee, Belgium) according to the manufacturer's guidelines. Slides were counterstained with Haematoxylin for 30 seconds and mounted in Eukitt mounting medium. Percentages of BrdU positive cells per slide were calculated after counting 1000 nuclei per animal.

Determination of T4 and T3 concentrations in serum and liver samples

Serum T4 and T3 were measured by Vitros ECI technology (Immunodiagnostic System, Ortho-Clinical Diagnostics, Beerse, Belgium). The iodothyronine levels were determined by highly sensitive and specific radioimmunoassays after extraction and purification of the iodothyronines from the liver, as previously described (16).

Deiodinase activity assays

Tissues were homogenized on ice in 10 volumes 0.1 M phosphate (pH 7.2), 2 mM EDTA, containing 1 mm DTT, using a Polytron (Kinematica, Lucerne, Switzerland). The tissue homogenates were stored at -80 C until further analysis. Protein concentrations were determined using the method of Bradford (17), using BSA as standard. D1 activities were determined by incubation of 0.1 µM rT3 (including 200,000 cpm [3',5'-^{125}I]rT3) for 30 min at 37 C with 10 µg protein/ml tissue homogenate in 0.1 ml 0.1 M phosphate (pH 7.2), 2 mM EDTA, 10 mm DTT (PED10). D2 and D3 activities were determined by incubation of 1 nM [3',5'-^{125}I]T4 (200,000 cpm, D2) or 1 nM T3 (including 200,000 cpm [3'-^{125}I]T3, D3) for 60 min at 37 C with 5 mg protein/ml tissue homogenate in 0.1 M PED10. Reactions were stopped by the addition of 0.1 ml ice-cold methanol. After centrifugation, 0.1 ml supernatant was mixed with 0.1 ml 0.02 M ammonium acetate (pH 4.0), and 0.1 ml of the mixture was applied to a 4.6 x 250 mm Symmetry C18 column connected to an Alliance HPLC system (Waters, Etten-Leur, The Netherlands), and eluted with a gradient of acetonitrile in 0.02 M ammonium acetate (pH 4.0) at a flow of 1.2 ml/min. The proportion of acetonitrile was increased linearly from 30 to 44% in 10 min. The radioactivity in the eluate was determined using a Radiomatic A-500 flow scintillation detector (Packard, Meriden, CT).

RNA isolation and quantitative RT-PCR

RNA from rat liver was isolated from 25 mg liver tissue using the high pure RNA tissue isolation kit (Roche Diagnostics, Almere, The Netherlands) according to the manufacturer's guidelines. RNA from mouse liver was isolated using Trizol (Invitrogen, Breda, The Netherlands). Subsequently, 10 µg RNA was treated with 10 U DNAse (Promega, Leiden, The Netherlands) for 30 min at 37 C, and purified using the RNeasy mini kit RNA cleanup protocol (Qiagen). 500 ng RNA was used for cDNA synthesis using TaqMan RT reagents (Roche Diagnostics). RNA samples were verified to be free from genomic DNA by performing negative control cDNA synthesis reactions of 500 ng total RNA using Taqman RT reagents without reverse transcriptase.

Quantitative real-time PCR was carried out using the ABI PRISM 7700 sequence detection system (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Reactions were performed in 25 µl Taqman universal PCR master mix (Roche), containing 20 ng cDNA. mRNA levels are expressed relative to the total

RNA content. Per reaction 200 nM of primers and probe were used for D3, D1 and for the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT). Table 1 shows the sequences of the different primers and probes. Real-time PCR reactions were done for 2 min at 50 C and for 10 min at 95 C, followed by 40 cycles of 15 sec at 95 C and for 1 min at 60 C. Cycle at threshold (Ct) values represent the cycle numbers at which probe-derived absorbance reaches the calculated threshold value. ΔCt values represent the Ct values of the housekeeping gene minus the Ct values of the target gene. Data are expressed as $2^{\Delta Ct}$ or as $2^{\Delta Ct}$ *1000 (relative number of mRNA copies).

Statistics

Results are expressed as means ± SEM. Significance of differences between means was tested using the Student's t test for unpaired observations of the SPSS 10.1 statistical package (SPSS, Inc., Chicago, IL). *P* values ≤ 0.05 were considered significant.

Results

Effects of PH on liver deiodinase expression and BrdU incorporation

We first tested deiodinase activity in rat liver at different time periods after PH (t=0). Figure 1A shows that D3 activity was increased 10-fold at 20 h and 3-fold at 48 h after PH, whereas D1 tended to decrease. D2 activity was absent in all rat liver samples. Next, we determined rat liver deiodinase mRNA levels. D3 mRNA expression tended to increase, whereas D1 mRNA expression tended to decrease at 20 h (Fig. 1B).

We also studied the effects of PH on deiodinase activity and mRNA levels in mice. In this model, D3 activity was increased 5-fold at 12 h, 8-fold at 24 h, 40-fold at 36 h, 15-fold at 48 h, and 7-fold at 72 h after PH compared to t=0, whereas SHAM-treated animals show only a 4-fold increase at 48 h after SHAM surgery (Fig. 2A). D1 activity decreased 2-fold compared to t=0, but was not affected compared to SHAM-treated animals 48 h after SHAM-surgery (Fig. 2A). In all mouse livers D2 activity was absent. Figure 2B shows a 6-fold increase of D3 mRNA at 36 h after PH compared to t=0, and no significant difference in D1 mRNA levels between time periods after PH.

Figure 3 depicts the levels of BrdU incorporation as a measure of cellular proliferation status at different time periods after PH. In correlation with D3 activity, the BrdU level peaked at 20 to 24 h in rats (data not shown), and at 36 h after PH in mice (Fig. 3).

Effects of PH on systemic and local thyroid hormone levels

Finally, we analyzed local and systemic thyroid hormone levels at PH and at different time periods thereafter. In rats, liver and serum T3 and T4 levels decreased markedly, with maximum 2 to 3-fold decreases at 20 to 24 h after PH (Figs. 4A,B). This correlated well with the peak in D3 activity at 20 h after PH in rats (Fig. 1A). In mice, serum T3 and T4 decreased until minimum levels at 36 h after PH (Fig. 5), coinciding with the peak in D3 activity and BrdU incorporation at 36 h after PH (Figs. 2A,3).

Discussion

D3 is highly expressed in tissues of the feto-placental unit, and has therefore long been considered to be mainly important in the regulation of the fetal thyroid hormone status. By inactivating the active thyroid hormone T3 and the prohormone T4, D3 is thought to protect developing organs against excessive exposure to thyroid hormone (18). Although D3 is not expressed in most normal adult tissues, recent studies have demonstrated the reexpression of the enzyme in various disease states. For instance, D3 is induced in liver and skeletal muscle of critically ill patients (6), and high levels of D3 are found in vascular tumors (4, 5). Furthermore, in different animal models, D3 was found to be induced in cardiac hypertrophy, myocardial infarction and chronic inflammation (7-9). The finding that in some of these conditions the accelerated degradation of thyroid hormone causes low serum T3 (6) or even severe hypothyroidism (4, 5) indicates the clinical importance of D3 in the regulation of local and systemic thyroid hormone status.

Not only fetal growth but also the above-mentioned pathophysiological conditions are processes in which cellular growth or

proliferation take place. Therefore, we hypothesized that D3-mediated low intracellular T3 levels are needed for cellular proliferation. Interestingly, Hernandez et al. recently showed high D3 expression in proliferating preadipocytes from brown adipose tissue, and a marked decrease in D3 expression when the cells differentiate into mature adipocytes (10). Additionally, by knocking down D3 in basal cell carcinomas Dentice et al. recently proved that D3 promotes the proliferation of malignant keratinocytes by inhibiting the differentiating action of T3 (11). Liver regeneration is also dependent on cellular proliferation and involves induction of various fetal genes (14). Therefore, in the present study we investigated deiodinase expression and local and systemic thyroid hormone status in rat and mouse liver after PH. We found a 10 to 40-fold induction of hepatic D3 activity after PH which was associated with an increased cellular proliferation and decreased local and circulating T3 and T4 levels. Remarkable in this respect is the tendency to a decrease of D1 in rats at 48 h after PH and the 2-fold decrease of D1 in mice at 48 and 72 h after PH. Since D1 is positively regulated by T3 (1), this may indicate that the slight decrease in D1 is secondary to the decrease in the systemic T3 level.

It should be noted that D1 activity was 2-fold decreased and D3 activity was 4-fold increased in SHAM-treated mice compared to t=0. Therefore, although D1 activity after PH is 2-fold decreased compared to t=0, it is not significantly altered compared to SHAM-treated controls. However, when D3 activity after PH is compared to SHAM-treated controls, it remains highly induced after PH. The mechanisms involved in the decrease of D1 and the increase of D3 activity after SHAM-treatment are unknown. The combined upregulation of D3 and downregulation of D1 by the SHAM operation may be caused by illness (6) and/or inflammation (9). Also the possible influence of the used anesthetics on deiodinase expression should be considered, since it is well known that some anesthetics alter thyroid function. We used the opiate buprenorphine. Baumgartner et al. showed an acute downregulation of D2 and upregulation of D3 in rat brain after administration of the mu-opiate agonist etonitazene (19).

We measured tissue and serum T3 and T4 levels, and found a significant decrease of local and systemic thyroid hormone levels at 20 h after PH in rat and at 36 h after PH in mice, which correlated well with the increase in D3 at these time periods. Tien et al. also studied rT3 levels in mouse liver after PH, and reported on a significant increase in the rT3 level after PH compared to SHAM-treated controls at 36 h after PH, which was correlated to a significant decrease in the T3-regulated genes tyrosine aminotransferase (TAT) and basic transcription element binding protein (BTEB) (20). In agreement with our data, they found 50% decreased D1 at 36 h after PH compared to SHAM-treated controls. However, because of nearly undetectable D3 mRNA expression levels, they did not determine D3 activities (20).

Previous cell culture studies have shown that 12-*O*-tetradecanoylphorbol-13-acetate (TPA), growth factors such as EGF, FGF and TGFβ, cyclic AMP, T3, and retinoids are able to induce D3 activity (21-28). However, the molecular mechanisms responsible for the reactivation of D3 in the regenerating liver is still poorly understood. In the proliferating skin hedgehog signalling is involved in the induction of Dio3 expression (11). However, gene expression microarray analyses of regenerating liver after PH do not indicate that the sonic hedgehog/gli protein pathway is involved in this process (29-32).

As for the TGFβ/Smad signalling pathway, TGFβ1 expression does increase after PH, with a peak level 24-48 h after operation (33-35). However, this increase in TGFβ is accompanied by a downregulation of the TGFβ receptors and by an increase of the transcriptional repressors SnoN and Ski (33-35), and TGFβ does not increase but suppresses hepatocyte proliferation through direct effects on the hepatocytes (36).

Another mechanism involved in the induction of D3 after PH might be cellular hypoxia. Interestingly, Simonides et al. recently showed that hypoxia induces D3 expression via a hypoxia inducible factor (HIF)-dependent pathway (37). Since PH is well recognized as a hypoxic-ischemic injury, and HIF-1α is induced after PH (38), the HIF-dependent pathway may well contribute to the induction of D3 after PH at the transcriptional level.

Regulation of D3 expression is complex, because the *Dio3* gene is imprinted, with preferential expression from the paternal allele (28). The gene is located in an imprinted region on human chromosome 14, mouse chromosome 12 and rat chromosome 6. One of the other imprinted genes in this region is called *Dio3os*, and is transcribed from the opposite DNA strand compared to *Dio3* (28). Interestingly, Hernandez et al. demonstrated an inverse correlation of the paternally imprinted *Dio3* gene with the maternally imprinted *Dio3os* gene (10). This *Dio3os* gene partially overlaps the coding region of the *Dio3* gene, and may thus interfere with the translation of the Dio3 transcript.

In summary, we found a large induction of D3 expression in regenerating liver which was correlated with an increased cellular proliferation and decreased local and systemic T3 and T4 levels. Future research should elucidate the roles of hypoxia and *Dio3os* in the regulation of Dio3 transcription and translation. Our findings support the concept that D3-mediated low cellular T3 is needed for cellular proliferation. Future studies, e.g. using conditional D3 knock-out mice, should further unravel the role of D3 in this process.

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References

1. **Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR** 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38-89
2. **Huang SA, Dorfman DM, Genest DR, Salvatore D, Larsen PR** 2003 Type 3 iodothyronine deiodinase is highly expressed in the human uteroplacental unit and in fetal epithelium. J Clin Endocrinol Metab 88:1384-8
3. **Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D**, **Howatson A**, **Visser TJ**, **Hume R**, **Morreale de Escobar G**. 2004 Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab 89:3117-28
4. **Huang SA, Tu HM, Harney JW, Venihaki M**, **Butte AJ**, **Kozakewich HP**, **Fishman SJ**, **Larsen PR**. 2000 Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. N Engl J Med 343:185-9
5. **Huang SA, Fish SA, Dorfman DM, Salvatore D, Kozakewich HP, Mandel SJ, Larsen PR.** 2002 A 21-year-old woman with consumptive hypothyroidism due to a vascular tumor expressing type 3 iodothyronine deiodinase. J Clin Endocrinol Metab 87:4457-61
6. **Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ, Van den Berghe G** 2003 Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. J Clin Endocrinol Metab 88:3202-11
7. **Wassen FW, Schiel AE, Kuiper GG, Kaptein E, Bakker O, Visser TJ, Simonides WS.** 2002 Induction of thyroid hormone-degrading deiodinase in cardiac hypertrophy and failure. Endocrinology 143:2812-5
8. **Olivares EL, Marassi MP, Fortunato RS, da Silva AC, Costa-e-Sousa RH, Araújo IG, Mattos EC, Masuda MO, Mulcahey MA, Huang SA, Bianco AC, Carvalho DP.** 2007 Thyroid function disturbance and type 3 iodothyronine deiodinase induction after myocardial infarction in rats a time course study. Endocrinology 148:4786-92
9. **Boelen A, Kwakkel J, Alkemade A, Renckens R**, **Kaptein E**, **Kuiper G**, **Wiersinga WM**, **Visser TJ**. 2005 Induction of type 3 deiodinase activity in inflammatory cells of mice with chronic local inflammation. Endocrinology 146:5128-34
10. **Hernandez A, Garcia B, Obregon MJ** 2007 Gene expression from the imprinted Dio3 locus is associated with cell proliferation of cultured brown adipocytes. Endocrinology 148:3968-76
11. **Dentice M, Luongo C, Huang S, Ambrosio R**, **Elefante A**, **Mirebeau-Prunier D**, **Zavacki AM**, **Fenzi G**, **Grachtchouk M**, **Hutchin M**, **Dlugosz AA**, **Bianco AC**, **Missero C**, **Larsen PR**, **Salvatore D**. 2007 Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes. Proc Natl Acad Sci U S A 104:14466-71
12. **Fausto N** 2000 Liver regeneration. J Hepatol 32:19-31
13. **Palmes D, Spiegel HU** 2004 Animal models of liver regeneration. Biomaterials 25:1601-11
14. **Tanimizu N, Miyajima A** 2007 Molecular mechanism of liver development and regeneration. Int Rev Cytol 259:1-48
15. **Visser TJ, Docter R, Hennemann G** 1977 Radioimmunoassay of reverse tri-iodothyronine. J Endocrinol 73:395-6
16. **Morreale de Escobar G, Calvo R, Escobar del Rey F, Obregon MJ** 1994 Thyroid hormones in tissues from fetal and adult rats. Endocrinology 134:2410-5
17. **Bradford MM** 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-54
18. **Darras VM, Hume R, Visser TJ** 1999 Regulation of thyroid hormone metabolism during fetal development. Mol Cell Endocrinol 151:37-47
19. **Baumgartner A, Pinna G, Hiedra L, Bauer F, Wolf J, Eravci M, Prengel H, Brödel O, Schmidt G, Meinhold H.** 1998 Effects of acute administration of ethanol and the mu-opiate

agonist etonitazene on thyroid hormone metabolism in rat brain. Psychopharmacology (Berl) 135:63-9

20. **Tien ES, Matsui K, Moore R, Negishi M** 2007 The nuclear receptor constitutively active/androstane receptor regulates type 1 deiodinase and thyroid hormone activity in the regenerating mouse liver. J Pharmacol Exp Ther 320:307-13
21. **Huang SA, Mulcahey MA, Crescenzi A, Chung M, Kim BW, Barnes C, Kuijt W, Turano H, Harney J, Larsen PR.** 2005 TGF-{beta} promotes inactivation of extracellular thyroid hormones via transcriptional stimulation of type 3 iodothyronine deiodinase. Mol Endocrinol
22. **Esfandiari A, Courtin F, Lennon AM, Gavaret JM, Pierre M** 1992 Induction of type III deiodinase activity in astroglial cells by thyroid hormones. Endocrinology 131:1682-8
23. **Esfandiari A, Gagelin C, Gavaret JM, Pavelka S, Lennon AM, Pierre M, Courtin F.** 1994 Induction of type III-deiodinase activity in astroglial cells by retinoids. Glia 11:255-61
24. **Courtin F, Liva P, Gavaret JM, Toru-Delbauffe D, Pierre M** 1991 Induction of 5-deiodinase activity in astroglial cells by 12-O-tetradecanoylphorbol 13-acetate and fibroblast growth factors. J Neurochem 56:1107-13
25. **Kester MH, Kuiper GG, Versteeg R, Visser TJ** 2006 Regulation of type III iodothyronine deiodinase expression in human cell lines. Endocrinology 147:5845-54
26. **Hernandez A, Obregon MJ** 1995 Presence of growth factors-induced type III iodothyronine 5-deiodinase in cultured rat brown adipocytes. Endocrinology 136:4543-50
27. **Hernandez A, St Germain DL, Obregon MJ** 1998 Transcriptional activation of type III inner ring deiodinase by growth factors in cultured rat brown adipocytes. Endocrinology 139:634-9
28. **Hernandez A, Fiering S, Martinez E, Galton VA, St Germain D** 2002 The gene locus encoding iodothyronine deiodinase type 3 (Dio3) is imprinted in the fetus and expresses antisense transcripts. Endocrinology 143:4483-6
29. **Jochheim-Richter A, Rudrich U, Koczan D, Hillemann T, Tewes S, Petry M, Kispert A, Sharma AD, Attaran F, Manns MP, Ott M.** 2006 Gene expression analysis identifies novel genes participating in early murine liver development and adult liver regeneration. Differentiation 74:167-73
30. **Togo S, Makino H, Kobayashi T, Morita T, Shimizu T, Kubota T, Ichikawa Y, Ishikawa T, Okazaki Y, Hayashizaki Y, Shimada H.** 2004 Mechanism of liver regeneration after partial hepatectomy using mouse cDNA microarray. J Hepatol 40:464-71
31. **Lai HS, Chen Y, Lin WH, Wu HC, Chang CJ, Lee PH, Chang KJ, Chen WJ.** 2005 Quantitative gene expression analysis by cDNA microarray during liver regeneration after partial hepatectomy in rats. Surg Today 35:396-403
32. **Xu CS, Chang CF** 2007 Expression profiles of the genes associated with metabolism and transport of amino acids and their derivatives in rat liver regeneration. Amino Acids
33. **Bissell DM, Wang SS, Jarnagin WR, Roll FJ** 1995 Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. J Clin Invest 96:447-55
34. **Chari RS, Price DT, Sue SR, Meyers WC, Jirtle RL** 1995 Down-regulation of transforming growth factor beta receptor type I, II, and III during liver regeneration. Am J Surg 169:126-31; discussion 131-2
35. **Macias-Silva M, Li W, Leu JI, Crissey MA, Taub R** 2002 Up-regulated transcriptional repressors SnoN and Ski bind Smad proteins to antagonize transforming growth factor-beta signals during liver regeneration. J Biol Chem 277:28483-90
36. **Romero-Gallo J, Sozmen EG, Chytil A, Russell WE, Whitehead R, Parks WT, Holdren MS, Her MF, Gautam S, Magnuson M, Moses HL, Grady WM.** 2005 Inactivation of TGF-beta signaling in hepatocytes results in an increased proliferative response after partial hepatectomy. Oncogene 24:3028-41
37. **Simonides WS, Mulcahey MA, Redout EM, Muller A**, **Zuidwijk MJ**, **Visser TJ**, **Wassen FW**, **Crescenzi A**, **da-Silva WS**, **Harney J**, **Engel FB**, **Obregon MJ**, **Larsen PR**, **Bianco AC**,

Huang SA. 2008 Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. J Clin Invest 118:975-83

38. **Maeno H, Ono T, Dhar DK, Sato T, Yamanoi A, Nagasue N** 2005 Expression of hypoxia inducible factor-1alpha during liver regeneration induced by partial hepatectomy in rats. Liver Int 25:1002-9

Legends

Fig. 1. Effect of partial hepatectomy in rats on D1 and D3 activity (A) and mRNA level relative to HPRT (B). Reaction conditions for the D3 activity assay were 1 nM ^{125}I-labeled T3, 5 mg protein/ml tissue homogenate, and 1 h incubation. Reaction conditions for the D1 assay were 100 nM ^{125}I-labeled rT3, 10 µg protein/ml tissue homogenate, and 1 h incubation. Results are the means±SEM (n=2-4). Significance of differences is indicated as follows: ***: $P<0.001$ *vs.* control (0).

Fig. 2 Effect of partial hepatectomy in mice on D1 and D3 activity (A) and mRNA level relative to HPRT (B). Reaction conditions for the D3 activity assay were 1 nM ^{125}I-labeled T3, 5 mg protein/ml tissue homogenate, and 1 h incubation. Reaction conditions for the D1 activity assay were 100 nM ^{125}I-labeled rT3, 10 µg protein/ml tissue homogenate, and 1 h incubation. Results are the means±SEM (n=3). Significance of differences is indicated as follows: ***: $P<0.001$ *vs.* control (0); **: $P<0.01$ *vs.* control (0); *: $P<0.05$ *vs.* control (0); ++: $P<0.01$ *vs.* control (48 SHAM); +: $P<0.05$ *vs.* control (48 SHAM).

Fig. 3 Effect of partial hepatectomy in mice on BrdU incorporation in mice. Results are the means±SEM (n=3). Significance of differences is indicated as follows: ***: $P<0.001$ *vs.* control (0).

Fig. 4 Effect of partial hepatectomy on liver T3 and T4 levels in rats (A) or on serum T3 and T4 levels in rats (B). Results are means±SEM (n=2-5). Significance of differences is indicated as follows: ***: $P<0.01$ *vs.* control; **: $P<0.01$ *vs.* control. (0); *: $P<0.05$ *vs.* control (0).

Fig. 5 Effect of partial hepatectomy on serum T3 and T4 levels in mice. Results are the means±SEM (n=3-5). Significance of differences is indicated as follows: **: $P<0.01$ *vs.* control. (0); *: $P<0.05$ *vs.* control (0).

Table 1. Oligonucleotide primers used for real-time PCR.

Gene	Sense primer	Antisense primer	Probe
D3	5'-TTCATGGCGCGGATGAG-3'	5'-GATGATAAGGAAGTCAACGTCGC-3'	5'-FAM-TTCCAGCGCCTGGTCACCAAGTACC-TAMRA-3'
D1	5'-ATTTGACCAGTTCAAGAGACTCGTAG-3'	5’-CCACGTTGTTCTTAAAAGCCCA-3’	5’-FAM-TCATTTACATTGAAGAAGCTCACGCCACAGA-3’
HPRT	rat 5'-TATCAGACTGAAGAGCTACTGTAATGACC-3'	5'-TTACCAGTGTCAATTATATCTTCAACAATC-3'	5'-FAM-TGAGAGATCATCTCCACCAATAACTTTTATGTCCC-TAMRA-3'
	mouse 5’-TATCAGACTGAAGAGCTACTGTAATGATC-3'		

Fig. 1A

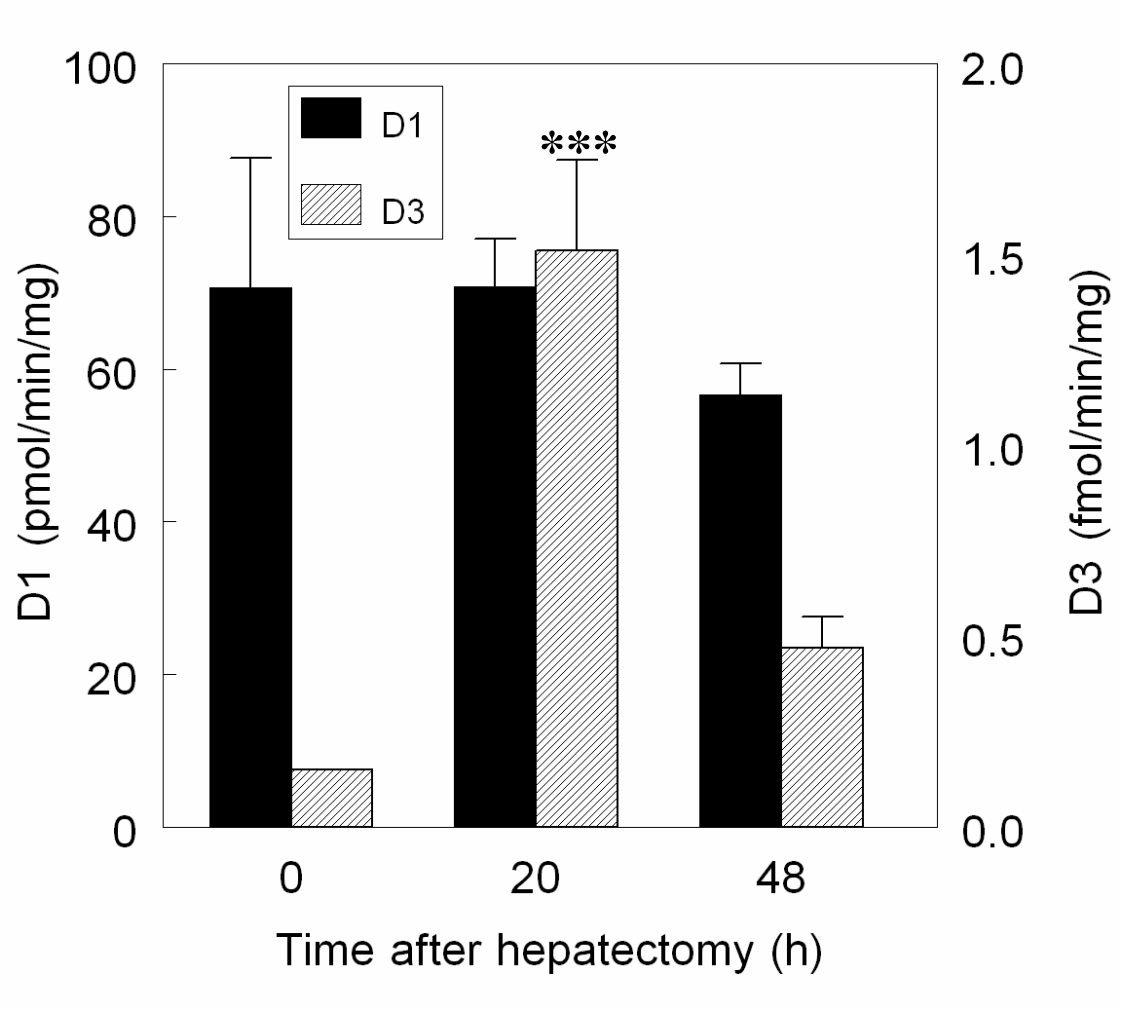

100
80
60
40
20
0
2.0
1.5
1.0
0.5
0.0
D1
D3
***
D1 (pmol/min/mg)
D3 (fmol/min/mg)
0
20
48
Time after hepatectomy (h)

Fig. 1B

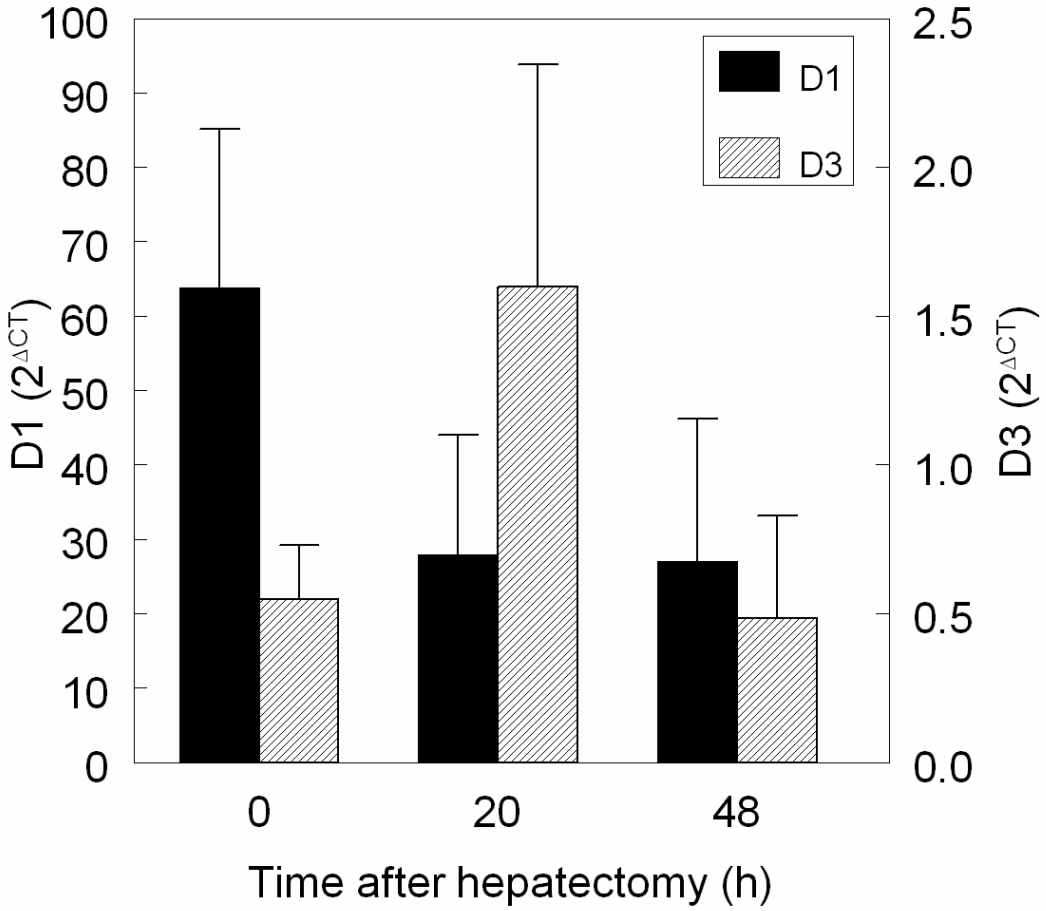

D1
D3
D1 (2^ΔCT)
D3 (2^ΔCT)
100
90
80
70
60
50
40
30
20
10
0
2.5
2.0
1.5
1.0
0.5
0.0
0
20
48
Time after hepatectomy (h)

Fig. 2A

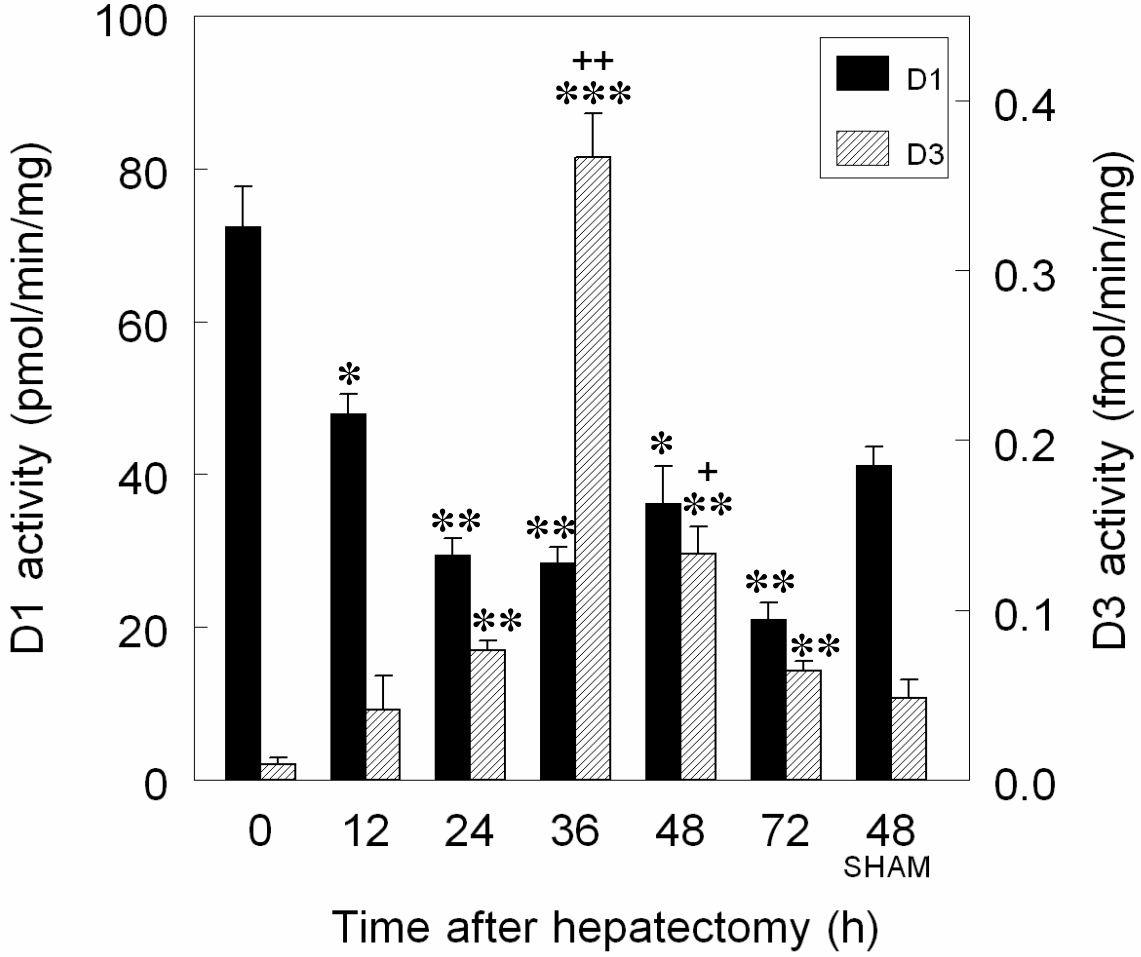

D1
D3
D1 activity (pmol/min/mg)
D3 activity (fmol/min/mg)
100
80
60
40
20
0
0.4
0.3
0.2
0.1
0.0
0
12
24
36
48
72
48
SHAM
Time after hepatectomy (h)

Fig. 2B

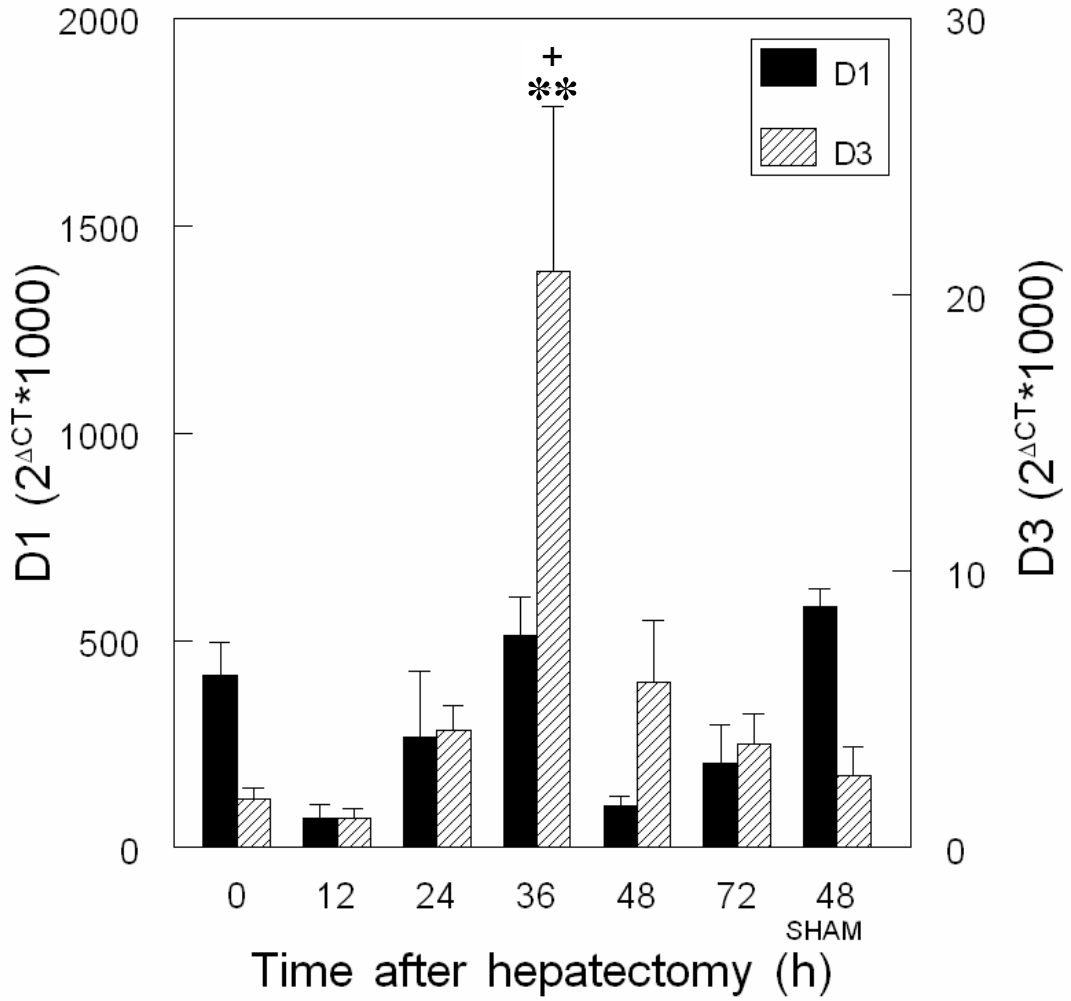

2000
1500
1000
500
0
30
20
10
0
D1
D3
+
**
D1 (2^ΔCT*1000)
D3 (2^ΔCT*1000)
0
12
24
36
48
72
48
SHAM
Time after hepatectomy (h)

Fig. 3

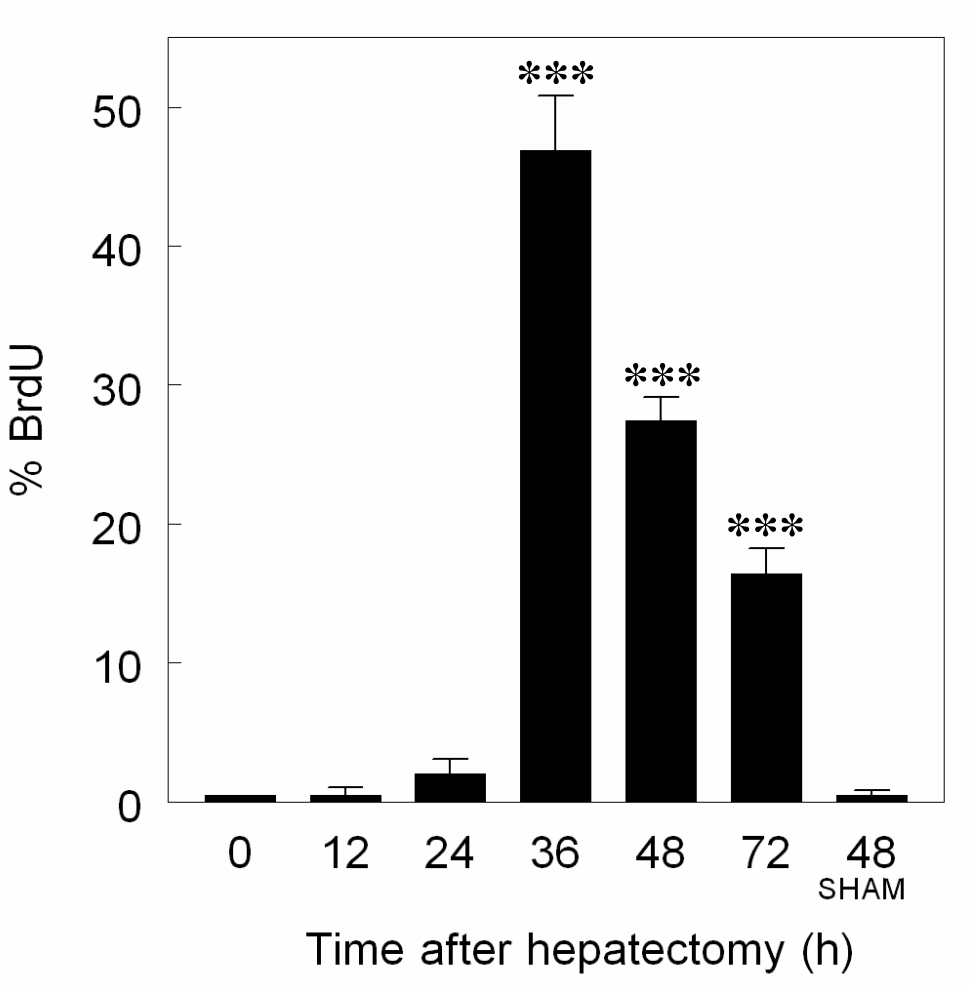

% BrdU
50
40
30
20
10
0
***
***
***
0
12
24
36
48
72
48
SHAM
Time after hepatectomy (h)

Fig. 4A

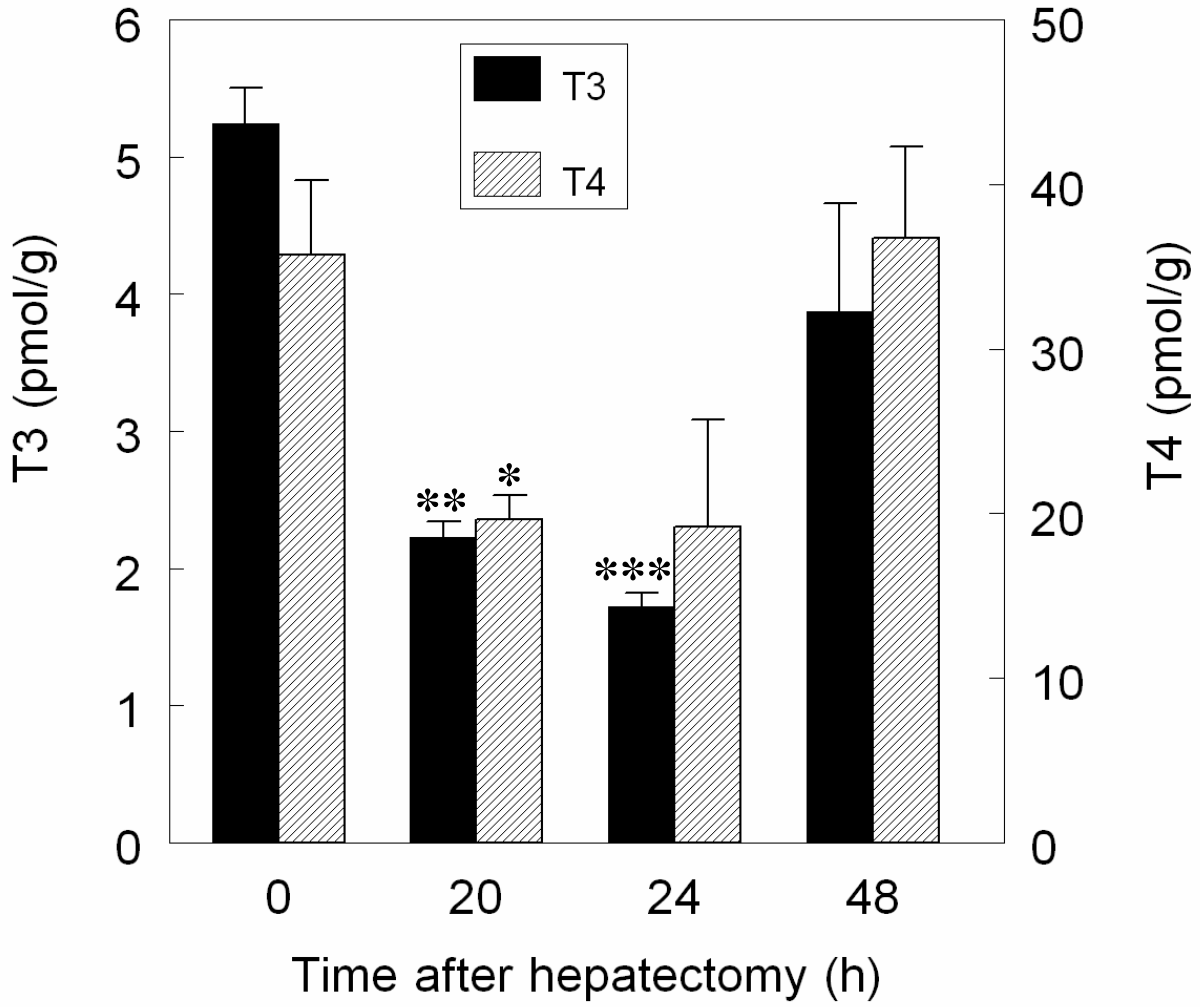

T3
T4
T3 (pmol/g)
T4 (pmol/g)
0
1
2
3
4
5
6
0
10
20
30
40
50
**
*
***
0
20
24
48
Time after hepatectomy (h)

Fig. 4B

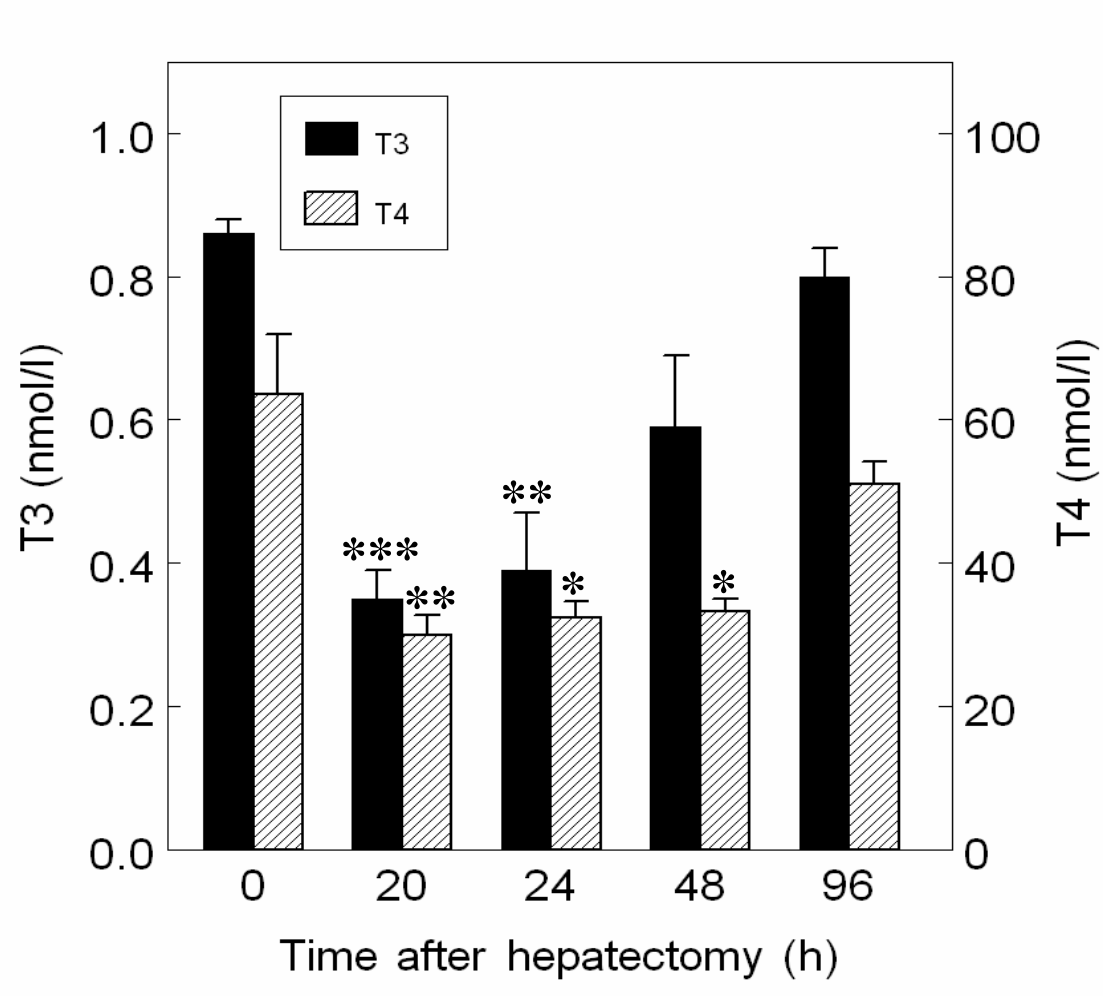
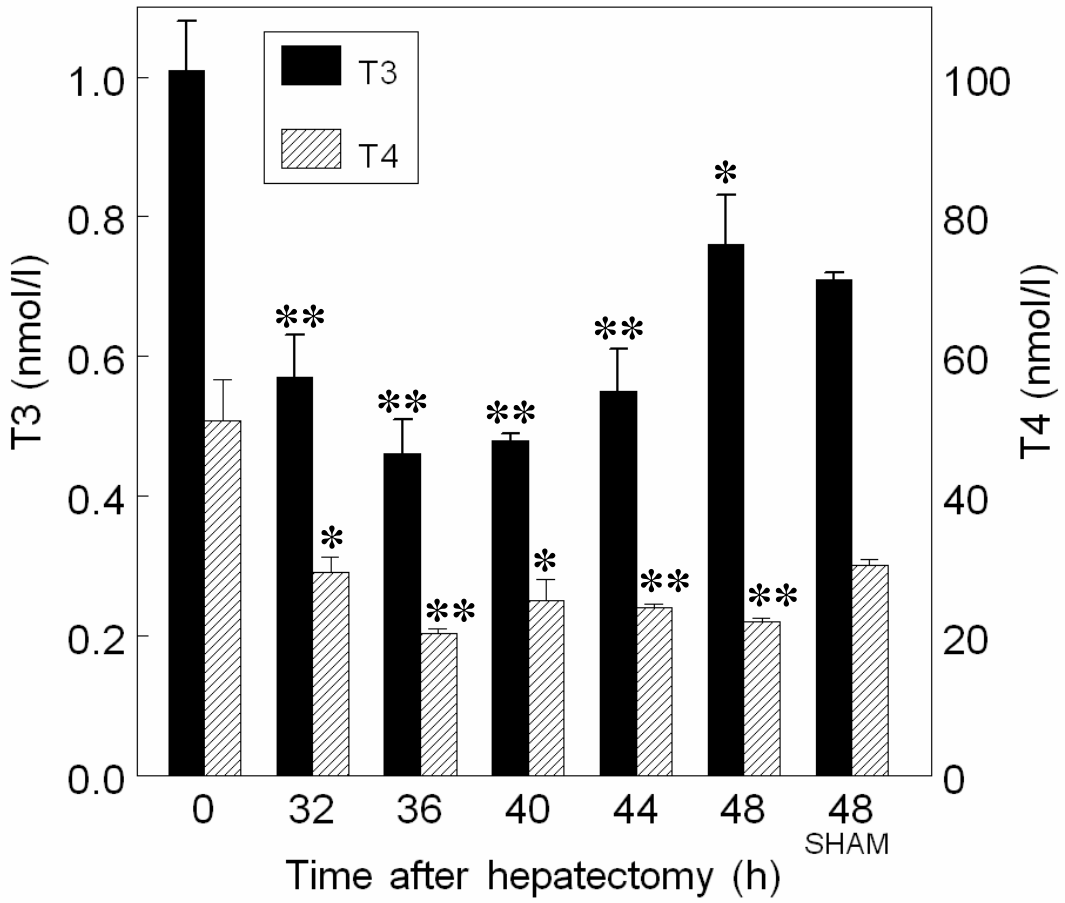

T3
T4
1.0
0.8
0.6
0.4
0.2
0.0
100
80
60
40
20
0
T3 (nmol/l)
T4 (nmol/l)
***
**
**
*
*
0
20
24
48
96
Time after hepatectomy (h)

Fig. 5


T3
T4
T3 (nmol/l)
T4 (nmol/l)
1.0
0.8
0.6
0.4
0.2
0.0
100
80
60
40
20
0
0
32
36
40
44
48
48
SHAM
Time after hepatectomy (h)