Age-Dependent Adaptation of the Liver Thyroid Status and Recovery of Serum Levels and Hepatic Insulin-Like Growth Factor-I Expression in Neonatal and Adult Diabetic Rats

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The effect of treatment with thyroxine (T_4) on the hepatic deiodinase (5′D-I) activity and triiodothyronine (T_3) content and on insulin-like growth factor-I (IGF-I) secretion and mRNA hepatic expression were studied in neonatal and adult diabetic (D) rats and compared with 4 thyroidectomized (Tx) groups: neonatal and adult Tx rats treated or not with T_4. Serum T_3 and T_4 decreased by 92% in both Tx populations and by 80% to 70% in D adults according to the severity of diabetes: −70 mg/kg body weight (BW) (D_{70}) or 50 mg/kg BW (D_{50}) of streptozotocin (STZ) injected, whereas only a 30% to 33% decrease was found in D neonates. A similar decrease of liver 5′D-I activity and T_3 concentrations was found in neonatal and adult Tx rats, whereas a significant reduction in those parameters was observed only in adult diabetics, either D_{70} or D_{50}, but not in D neonates. Serum levels and liver mRNA expression of IGF-I determined by ribonuclease protection assay, plasma and pituitary growth hormone (GH), plasma insulin, and glycemia were also measured in both D populations. A decrease in circulating IGF-I, previously reported for Tx adult rats, was also found in both D populations. T_4 treatment recovered IGF-I and liver T_3 in both Tx groups and D neonates, but not in D adults. These results show an age-dependent adaptation of the liver thyroid economy in diabetes, as hepatic 5′D-I does not respond to diabetes in neonates and IGF-I is insensitive to T_4 treatment in adult diabetics and suggest a positive correlation between hepatic T_3 content and IGF-I expression in conditions of diabetes and Tx.

THE METABOLIC and developmental effects of thyroid hormones are mediated by triiodothyronine (T_3), most of which is produced from thyroxine (T_4) by 5′-deiodination. Type I 5′-deiodinase (5′D-I), predominantly present in liver, kidney, and thyroid, provides most of plasma T_3 in the rat. Low levels of plasma T_3 are associated with decreased or suppressed 5′D-I activity in the liver.[1-3] Diabetes and undernutrition lead to alterations of thyroid hormone status typical of other so called "nonthyroidal illnesses" or "low T_3 syndromes."[4,5] Administration of streptozotocin (STZ) to rats is frequently accomplished to obtain an experimental model for the study of diabetes mellitus.[6] In diabetes, a reduction in the peripheral generation of T_3 from T_4 due to inhibition of 5′D-I is observed.[4,7] In the low T_3 syndromes, thyroid-stimulating hormone (TSH) is reduced[5] in parallel to thyroid hormones, contrary to what happens in hypothyroidism. This distinct TSH adaptation could represent a relevant difference in the thyroid status regulation between hypothyroidism and the low T_3 syndromes, which has been studied mainly in the adult period.[8,9] Previous research has shown that T_4 administration to adult diabetic humans or animals does not restore thyroid hormone levels to normal values,[10,11] contrary to what occurs in hypothyroid rats.[12,13]

Insulin-like growth factors (IGFs) are peptide hormones with endocrine, paracrine, and autocrine modes of action,[14] which are involved in cell growth and differentiation in multiple tissues. IGFs are secreted mostly by the liver and in adults their secretion is regulated by growth hormone (GH) and nutritional status.[15,16] Furthermore, states of nutritional deprivation, such as starvation and protein-caloric undernutrition and type I diabetes mellitus, decrease the liver mRNA expression and serum levels of IGF-I.[16-20] A reduction in serum levels and liver mRNA expression of IGFs is also observed in experimental hypothyroidism[21,22] and hypothyroid patients.[23] Therefore, in the 3 pathological conditions—undernutrition, diabetes, and hypothyroidism—low plasma thyroid hormone levels run in parallel to reduced circulating IGFs. Plasma and pituitary content of GH are regulated by thyroid hormones in vivo[24,25] and in vitro,[26] and these hormones transcriptionally regulate GH gene expression[27]; however, not all the thyroid hormone effects on the IGF system are mediated by GH.[28,29] GH treatment does not restore serum IGF-I in hypothyroid rats,[30] and a direct effect of thyroid hormones on the IGF system has been suggested by studies "in vitro."[2] Moreover, it has also been suggested that the influence of thyroid hormones on the IGFs is age-dependent.[31,32] However, at present, the ultimate mechanism by which reduced levels of thyroid hormones in plasma produce a decrease of IGF secretion as well as the role of the liver thyroid economy (5′D-I activity and T_3 concentrations) in this mechanism remain to be elucidated.

In thyroidectomized (Tx) neonatal and adult rats, insulin and GH respectively seem to mediate the thyroid hormone effects on the IGF system.[18,22] In a previous work[13] on neonatal and adult Tx rats, continual replacement of T_4 by pellets (in doses and way of administration similar to those used in this study for diabetic animals) restored serum levels and liver mRNA expression of IGFs, as well as serum insulin and GH. The thyroid hormone recovery of the IGF system seems to be mediated preferable by insulin in neonatal Tx rats, and by GH in adult Tx rats, but the liver 5′D-I activity and T_3 content were not

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Table 1. BW, Serum T_3 and T_4, Plasma and Pituitary GH Content, Plasma Insulin, and Glycemia of Three Groups of Neonatal (20 days) and Adult (87 days) Rats

	BW (g)	Serum T_3 (ng/dL)	Serum T_4 (μg/dL)	Plasma GH (ng/mL)	Pituitary GH (ng/mg)	Plasma insulin (μU/mL)	Glycemia (ng/dL)
Neonatal							
Diabetic (D)	39.26 ± 1.72*	59.26 ± 30.8*	4.16 ± 0.62*	3.37 ± 0.14	8.86 ± 0.92*	23.26 ± 1.22*	270.29 ± 10.45*
Controls (C)	52.66 ± 0.86	90.50 ± 1.84	5.85 ± 0.58	3.42 ± 0.71	12.47 ± 0.39	52.42 ± 1.61	133.58 ± 2.38
D+T_4	46.27 ± 0.65*†	98.10 ± 6.89†	6.61 ± 1.32†	89.67 ± 15.04*†	15.97 ± 2.09*†	28.55 ± 4.70*	199.12 ± 11.64*
Adults							
D_{70}+T_4	156.13 ± 10.36*	51.89 ± 8.58*†	3.71 ± 0.91*†	28.31 ± 6.12*†	18.20 ± 0.98*	11.36 ± 3.55*	454.82 ± 4.49*
Diabetic (D_{70})	146.10 ± 6.03*	11.93 ± 0.21*	1.03 ± 0.15*	15.80 ± 3.52*	14.19 ± 1.26*	10.68 ± 2.36*	490.47 ± 4.81*
Controls (C)	213.47 ± 3.33	94.53 ± 4.46	5.25 ± 0.63	90.84 ± 11.27	37.96 ± 6.46	70.31 ± 9.27	133.05 ± 1.89
Diabetic (D_{50})	167.51 ± 9.89*	31.65 ± 2.11*	1.19 ± 0.33*	17.04 ± 4.65*	28.28 ± 7.83*	16.89 ± 5.19*	273.53 ± 19.29*
D_{50}+T_4	159.89 ± 17.81*	45.55 ± 11.66*	3.05 ± 0.67*†	20.12 ± 2.52*†	28.55 ± 4.19*	16.39 ± 8.55*	170.38 ± 22.15*†

NOTE. Populations: D, diabetic neonates; C, controls; D+T_4, diabetic neonates treated with T_4; D_{70} and D_{50}, diabetic adults 70 mg STZ/kg BW or 50 mg STZ/kg BW, respectively; D_{70}+T_4 and D_{50}+T_4, diabetic adults treated with T_4. See Fig 1 and Methods for further details. Values are mean ± SD of 8–10 animals.

*$P < .05$ relative to C rats.

†$P < .05$ relative to D rats.

determined in those conditions.[13] The fact that both mediating factors (insulin and GH) are reduced in diabetes led us to investigate the IGF-I response to T_4 treatment, as well as the liver thyroid economy before and after T_4 treatment in neonatal and adult diabetic rats. Accordingly, it seemed appropiate to complete our previous results on Tx rats treated with T_4[13] by studying whether the recovery of serum thyroid hormones, GH, insulin, and IGFs previously observed is followed by recovery of liver T_3 and T_4 content and 5′D-I activity in both neonatal and adult Tx populations. Our goal was to compare the changes of 5′D-I and T_3 liver content in neonatal and adult rats in the absence of insulin (diabetes) or in its presence (Tx), 2 situations in which circulating thyroid hormones are low.

The aim of the present experiments was to study the adaptation of liver thyroid status in the adult and neonatal diabetes, while trying to explain why the treatment with T_4 did not improve the thyroidal status of adult diabetics and to consider the possibility that this is also found in the neonatal period. In addition, we set out to determine in both situations, diabetes and Tx, whether a positive correlation between liver thyroid status and hepatic IGF-I expression is present.

MATERIALS AND METHODS

Animals

Wistar rats bred in our laboratory with controlled temperature and artificial dark-light cycle (6 AM to 6 PM) were used throughout the study. After birth, the number of pups in each litter was evened out to 8, and males and females were used in equal numbers for the neonatal populations, while only males were used for the adult populations to avoid sex-linked differences in adaptative responses. Animals were fed a standard laboratory diet ad libitum. Tx and implantation of T_4 pellets for continuous T_4 replacement were performed under ether anesthesia, which seems to be the most suitable method for thyroid replacement.[13] Pellets were implanted once in each animal to deliver 1.5 or 1.75 μg/100 g body weight (BW) per day. Control rats were sham-operated. Although no signs of calcium deficiency were observed in the rats at any stage, total calcium concentration was measured in serum using the o-cresolphthalein complexone kit (Biomerieux, Marcy L'Etoile, France) and no significant differences between control and Tx sera were found in either the neonatal or adult rats. However, a possible undetected hypocalcemia due to potential loss of parathyroid glands during Tx was prevented by addition of 1% calcium lactate in the drinking water of experimental and control rats. Blood was harvested from the trunk after decapitation and plasma or serum was stored at −80°C until assayed. Livers and pituitaries were frozen in liquid N_2. Diabetes was induced by a single intraperitoneal injection of STZ (70 mg/kg BW) in 0.05 mol citrate buffer/L, pH 4.5, and was confirmed by the determination of glycemia and insulinemia (Table 1) at the time of killing. Control rats were sham-injected with citrate buffer. To achieve a milder diabetic condition in adult rats, similar to that of neonatal rats, which show a well-known reported resistance to STZ,[33] a group of adult rats was injected with lower doses of STZ (50 mg/kg BW), a dose that ensured a degree of hyperglycemia similar to that of neonatal rats treated with 70 mg/kg BW L-thyroxine and mercapto-1-methylimidazole (MMI) were obtained from Sigma Chemical (St Louis, MO). L-Thyroxine pellets were obtained from Innovative Research of America (Sarasota, FL).

The European Community regulations for the use of animals for experimental models and other scientific purposes were followed. All experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health (NIH; Bethesda, MD) guide for care and use of experimental animals.

Experimental Groups

Diabetic populations. Two populations of rats were analyzed: neonatal and adult rats (Fig 1). Neonatal rats were divided into 3 groups: diabetic (D), D+T_4, and controls (C). D rats were injected intraperitoneally with STZ (70 mg/kg BW) in 0.05 mol citrate buffer/L (pH 4.5) on day 10 of life. The suppression of insulin secretion was confirmed 3 days later by the determination of glycemia and insulinemia. They were killed on day 20. In D+T_4 rat, after STZ injection on day 10 they were implanted on day 15 with T_4 pellets ensuring a daily delivery of 1.5 μg T_4/100 g BW and killed at 20 days of life. C rats were sham-injected with citrate buffer. Adult rats were divided into 5 groups: D_{50}, D_{50}+T_4, D_{70}, D_{70}+T_4, and controls (C). D_{50} and D_{70} rats were injected intraperitoneally with STZ on day 75 of life as above with 50 or 70 mg/kg BW, respectively. The suppression of insulin secretion was confirmed 3 days later by the determination of insulin and glycemia and the rats were killed at 87 days of life. D_{50}+T_4 and D_{70}+T_4 rats were injected intraperitoneally with STZ on day 75 of life as described above and implanted on day 82 of life with T_4 pellets ensuring a daily delivery of 1.75 μg T_4/100 g BW and killed at 87 days of life. T_4 doses

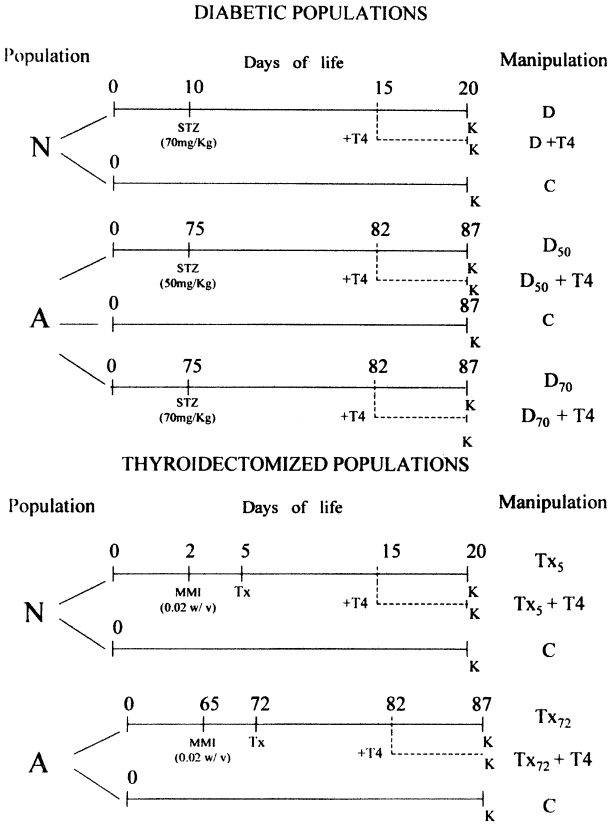

Fig 1. Diabetic populations. Two groups of rats were used: neonatal (N) and adults (A). N rats were divided into 3 groups: D (diabetic), D+T_4 (diabetic treated with T4) and C (control). A rats were divided into 5 groups: D_{50} (diabetic 50 mg STZ/kg BW), D_{70} (diabetic 70 mg STZ/kg BW), D_{50}+T_4 (diabetic 50 mg STZ/kg BW treated with T_4), D_{70} + T_4 (diabetic 70 mg STZ/kg BW treated with T_4), and C (control). See Methods for further details. Thyroidectomized (Tx) populations. N and A populations as above. N rats were divided into 3 groups: Tx_5 (Tx at 5 days and killed at 20 days), Tx_5+T_4 (Tx at 5 days and treated with T_4), and C (control). A rats were divided into 3 groups: Tx_{72} (Tx at 72 days and killed at 87 days), Tx_{72}+T_4 (Tx at 72 days and treated with T4), and C (control). See Methods for further details.

were greater in adults than in neonates because of the smaller sensitivity to T_4 in adults. C rats were sham-injected with citrate buffer. These 2 groups of diabetic adult rats (D_{50} and D_{70}) with different levels of hyperglycemia and serum insulin were studied to investigate whether the potential differences between neonatal and adult diabetic rats were due to age, or to the degree of the diabetic condition. Due to the well-known resistance of neonatal pancreatic islets to destruction by STZ,[33] the above protocol was established in order to avoid the use of very high doses of STZ, which could be extremely toxic in neonatal rats.

TX populations. Two populations of rats were analyzed: neonatal and adult Tx rats (Fig 1). Neonatal rats were divided into 3 groups: Tx_5, Tx_5+T_4, and controls (C). Tx_5 rats received MMI (0.02% [wt/vol] added to the drinking water of mother) from day 2 of life; they underwent Tx on day 5 and were killed on day 20 of life. A small quantity of MMI was given from day 2 of life to ensure that T_3 and T_4 were adequately reduced. Tx_5+T_4 rats underwent Tx as above and were implanted on day 15 of life with T_4 pellets, ensuring a daily delivery of 1.5 μg T_4/100 g BW during 5 days, and were killed at 20 days of life. C rats were sham-operated. Adult rats were divided into 3 groups: Tx_{72}, Tx_{72}+T_4, and controls (C). Tx_{72} rats received MMI (0.02% [wt/vol] added to the drinking water) starting on day 65 of life. They underwent Tx on day 72 and were killed on day 87 of life. Tx_{72}+T_4 rats underwent Tx as above and were implanted on day 82 with T_4 pellets, ensuring a daily delivery of 1.75 μg T_4/100 g BW during 5 days, and were killed at 87 days of life. C rats were sham-operated.

Determination of Serum Glucose and Plasma Insulin and GH

Glucose was determined with a Reflolux IIM (Boehringer Mannheim, Leverkusen, Germany) glucose analyzer. Plasma immunoreactive insulin was estimated with purified rat insulin as standard (Novo Nordisk, Bagsvaerd, Denmark), antibody to porcine insulin, which cross-reacted similarly with pork and rat insulin standards, and monoiodinated ^{125}I-labeled human insulin. The minimal detectable dose was 0.04 ng/mL, with a coefficient of variation within and between assays of 10%. Plasma and pituitary GH were determined using the reagents generously supplied by the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases, NIH (rGH Standard RP-2). The minimal detectable dose in pituitary homogenates and serum was 0.03 ng/mL of GH. To prevent as much as possible circadian variations of GH in blood, samples were obtained from 6 to 8 animals at the same hour (between 10 AM and noon) each day.

Determination of 5′D-I Activity and T_3 and T_4 Content in the Liver and Serum T_3 and T_4

5′D-I activity (pmol/min/mg protein) was assayed in liver homogenates as previously described.[34,35] Plasma T_3 and T_4 were determined at Instituto de Investigaciones Biomédicas using highly specific radioimmunoassays previously described [36] and modified for rat plasma and tissues.[35] Liver T_3 and T_4 concentrations were determined as above after extraction and purification of the tissues. The limits of detection were 2.5 pg T_4 and 0.7 pg T_3 per assay tube.

Iodination, Purification, and Determination of Serum IGF-I and IGF-II

Recombinant human IGF-I was labeled using a modified chloramine T method.[20,37] The specific activity achieved was approximately 90 to 175 μCi/μg. Prior to IGF-I determination, serum IGF binding proteins (IGFBPs) were removed by standard acid gel filtration. This method has proved to be the most reliable one for rat serum.[20,37]

The radioimmunoassay for IGF-I was performed as previously described.[20,37] The coefficients of variation within and between assays were 8.0%. Recombinant human IGF-I (Boehringer Mannheim) was used for iodination.

Preparation of RNA

Total RNA was prepared by homogenization of livers in guanidinium thiocyanate as originally described.[38] Samples were electrophoresed using 1% agarose and 2.2 mol formaldehyde/L gels and stained with ethidium bromide in order to visualize the 28S and 18S ribosomal RNA and thereby to confirm the integrity of the RNA. pT7 RNA S18 antisense control template (Ambion, Austin, TX) was used to normalize the quantity of RNA in the different lanes.

Riboprobes

Rat IGF-I cDNA was kindly provided by Drs C. T. Roberts and D. LeRoith (NIH). Riboprobes for IGF-I were prepared as described previously.[22] S18 cDNA was incubated with T7 RNA polymerase to produce a 109-nucleotide runoff transcript, 80 nucleotides of which are complementary to human 18S ribosomal RNA. The above riboprobe

was synthesized with (^{32}P)uridine triphosphate (UTP), purchased from ICN (Nuclear Iberica, Madrid, Spain). Riboprobe Gemini II Core System (Promega, Madison, WI) was used for the generation of RNA probe.

Solution Hybridization/Ribonuclease Protection Assay

Solution hybridization/ribonuclease (RNase) protection assays were performed as previously described.[20,37] Autoradiography was performed at −70°C against a Hyperfilm MP film (Amersham, Pharmacia-Biotech, Barcelona, Spain) between intensifying screens. Bands representing protected probe fragments were quantified using a Molecular Dynamics (Sunnyvale, CA) scanning densitometer and accompanying software. RNAse A and RNAse T1 were also purchased from Boehringer Mannheim.

Statistical Analysis

All data are presented as means ± SD. Statistical comparisons were performed by 1-way analysis of variance (ANOVA), followed by the protected least significant difference test.[39]

RESULTS

BWt, Serum GH, T_3, T_4, Glycemia, Insulin, and Pituitary GH Content in Neonatal and Adult Diabetic Rats Before and After T_4 Treatment and Plasma T_3 and T_4 in Tx Neonatal and Adult Rats Before and After T_4 Treatment

Table 1 shows the decrease of BW (20% to 30% *v* controls) in both diabetic populations, neonatal and adult. A decrease in plasma T_3 and T_4 was also observed, ie, 34% and 28% reduction for T_3 and T_4, respectively, in diabetic neonates, 87% and 80% in D_{70} adults, and 68% to 77% in D_{50} adults. As expected, the smaller decrease of thyroid hormones found in diabetic neonates induced no changes in plasma GH, whereas an 80% decrease of plasma GH was observed in adult diabetic rats (D_{70} and D_{50}). In agreement with this, treatment of neonatal diabetics with T_4 (1.5 μg T_4/100 g BW per day) increased plasma T_3 and T_4 above control values (8% and 13%, respectively), while T_4 treatment (1.75 μg T_4/100 g BW per day) of adult diabetic rats also induced an increase of plasma T_3 and T_4, but the values remained below those of controls. Moreover, the smaller increase of thyroid hormones in adult diabetics, after treatment with a greater dose of T_4 (1.75 *v* 1.5 μg/100 g BW), consequently produced a smaller increase of plasma and pituitary GH content observed in adult as compared to neonatal diabetic rats. In addition, the T_4-induced BW increase was smaller in diabetic adults (7% to 5% in D_{70} and D_{50}) than in neonates (17%). These results agree with the already reported greater sensitivity to T_4 showed by the neonatal diabetic rats, which explains why higher T_4 doses were given to the adult than to the neonatal rats.

As expected, increased glycemia was found in both diabetic populations versus controls, which remained elevated after T_4 administration. As shown in Table 1, a dose of 70 mg STZ/kg BW induced a 2-fold increase in glycemia in diabetic neonates, whereas the same dose induced a 3.7-fold increase in diabetic adult rats. Accordingly, the same STZ dose induced a greater decrease of insulin in D_{70} adults (83%) than in neonates (55%). In order to ensure a similar degree of diabetes (similar hyperglycemia and hypoinsulinemia) in neonatal and adult populations, a dose/response screening with STZ was performed in adult animals prior to the depicted experiments. Table 1 shows that adult rats treated with 50 mg STZ/kg BW had a 2-fold increase of glycemia versus controls, similar to that of neonates treated with 70 mg STZ/kg BW However, the adult rats (D_{50}) presented a 75% decrease of serum insulin as compared to a 55% reduction found in the neonates. The reduced degree of diabetes in adult D_{50} rats was accompanied, as expected, by less severe changes in all parameters as compared to those observed in adult D_{70} rats, namely, a smaller decrease of serum T_3, pituitary GH concentration, plasma insulin, and BW.

Table 2. Serum T_3 and T_4 of Neonatal and Adult Rats

	Serum T_3 (ng/dL)	Serum T_4 (μg/dL)
Neonatal		
Controls	98.50 ± 6.00	6.00 ± 0.50
Tx_5	8.00 ± 0.02*	2.00 ± 0.50*
Tx_5+T_4	40.00 ± 5.00*†	3.50 ± 0.80*
Adults		
Controls	93.50 ± 5.00	5.00 ± 0.60
Tx_{72}	8.10 ± 0.30*	0.50 ± 0.06*
$Tx_{72}+T_4$	98.00 ± 5.00†	7.00 ± 0.10†

NOTE. Neonatal rats (20 days): thyroidectomized (Tx_5), intact controls (C), and thyroidectomized treated with T_4 (1.5 μg T_4/100 g BW per day) (Tx_5+T_4). Adult rats (87 days): thyroidectomized (Tx_{72}), intact controls (C), and thyroidectomized treated with T_4 (1.75 μg T_4/100 g BW per day) ($Tx_{72}+T_4$). See Fig 1 for further details. Mean ± SD of 8–10 animals.

*$P < .05$ relative to C rats.

†$P < .05$ relative to T rats.

Table 2 shows a similar decrease in serum T_3 in Tx_5 and Tx_{72} (~92%), while T_4 decreased less in neonates. Following T_4 treatment, the levels of serum T_3 and T_4 in the adult rats increased beyond those of the controls. The neonatal rats after T_4 treatment presented a lesser increase according to the higher requirement of thyroid hormones in the neonatal stage. So, after Tx, a similar decrease in serum T_3 was observed in neonates and adult rats (with a higher decrease in serum T_4 in adult rats), while diabetes showed a higher decrease in serum thyroid hormones in adults than in neonates.

Hepatic 5′D-I Activity and T_3 and T_4 Content in Liver of Tx and D Neonatal and Adult Rats Before and After T_4 Treatment

In agreement with the serum changes described above, neonatal and adult rats undergoing Tx at 5 and 72 days of life, respectively, and killed 15 days later, showed a reduction of liver 5′D-I activity (Fig 2A). T_4 administration by pellets to Tx_5 (1.5 μg T_4/100 g BW per day for 5 days) and Tx_{72} (1.75 μg T_4/100 g per day for 5 days) increased 5′D-I activity to values higher than those of controls. Hepatic T_3 increased (Fig 2B), in agreement with these changes in hepatic 5′D-I activity,

In contrast, in diabetic neonates no changes in 5′D-I activity were observed versus controls, and 5′D-I activity remained unchanged after T_4 treatment (Fig 3A). Using similar doses to that used in Tx rats (Fig 3A), an equally diminished 5′D-I activity was found in the liver of D_{70} and D_{50} adults, which further decreased after T_4 administration. The lack of changes

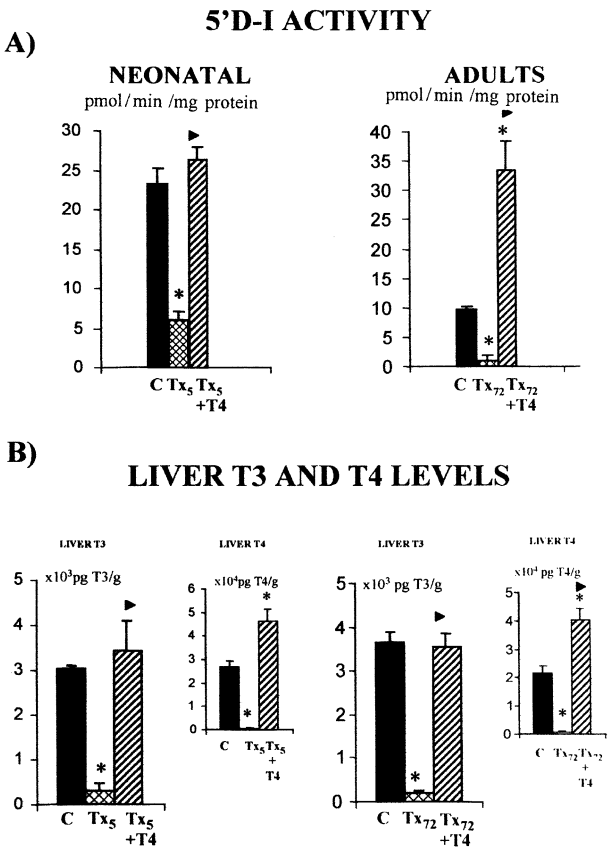

Fig 2. (A) rT_3 5′D-I activity (pmols/min/mg protein) in liver homogenates of neonatal and adult Tx populations. Tx neonates were divided into 3 groups: Tx_5 (Tx at 5 days and killed at 20 days), Tx_5+T_4, and C (control). See Fig 1. Tx adults were divided into 3 groups: Tx_{72} (Tx at 72 days and killed at 87 days), $Tx_{72}+T_4$, and C (control). See Fig 1. Assays were run in duplicate. See Methods for further details. (B) Liver T_3 and T_4 levels in neonatal and adult Tx populations. Rat groups as in (A). Assays were run in duplicate. Data are mean ± SD. Number of animals: 8-10. *$P < .05$ relative to C group. ►$P < .05$ relative to Tx group.

in deiodinase activity in the liver of diabetic neonates (Fig 3A) clearly favored an increased hepatic T_3 and T_4 content after T_4 treatment (Fig 3B). However, the reduced 5′D-I activity in the liver of diabetic adults and its further decrease after T_4 administration (Fig 3A) produced severe decreases in hepatic T_3 content in both adult populations, D_{70} and D_{50} (Fig 3B), indicating that the inhibition of liver thyroid status was independent of the severity of diabetes. Furthermore, the decrease of liver T_3 content observed in diabetic adults after T_4 administration could be favored by the fact that treatment with T_4 did not increase liver T_4 content (Fig 3B, right panel).

In summary, Tx induced similar adaptive responses of liver 5′D-I activity to T_4 treatment in neonatal and adult Tx rats, while different adaptive responses were found in neonatal and in adult diabetic rats. Moreover, the lack of insulin did not decrease the hepatic 5′D-I activity in neonates, while it had an important effect in the adult diabetic rats.

Serum Levels and Liver mRNA Expression of IGF-I in Neonatal and Adult Diabetic Rats Before and After T_4 Administration

Figure 4A shows a decrease of serum IGF-I. It also shows that the decrease of circulating IGF-I was greater in adult D_{70} than in adult D_{50} rats, in parallel to a greater severity of diabetes and, consequently, to lower levels of plasma insulin and GH in D_{70} than in D_{50} (Table 1). T_4 treatment induced an increased serum IGF-I in diabetic neonates but not in diabetic adults (Fig 4A), whereas insulin remained unchanged after T_4 treatment in both groups (Table 1). The parallel changes of liver mRNA expression as compared to those of serum levels of IGF-I shown in Fig 4B suggest a transcriptional regulation of the genes at the hepatic level. It is important to point out that in Tx neonatal rats, an increase was observed in serum IGF-I and mRNA expression that paralleled the increases in insulin and GH, while Tx adult rats had a decrease in insulin, GH, and IGF-I.[13,22] In addition to this, treatment of both Tx groups with T_4 doses, similar to those used in the present study in diabetic rats, restored all the alterations both in neonates and adults, with an increase (determined in the present study) of the hepatic thyroid status (5′D-I activity and T_3 content, Table 2 and Fig 2).

DISCUSSION

Experimental hypothyroidism may be accompanied, in different animal species, by normal, slightly increased or decreased basal plasma insulin[40] and a comparable variability was shown by the insulinogenic index.[41] However, the changes induced by diabetes in liver thyroid economy, including 5′D-I activity and T_3 and T_4 content, have been less studied,[42] and only in the adult animal.[8,9] In this work, the circulating thyroid hormones, in diabetic neonatal rats, decreased less (30%) than in diabetic adult rats (80% to 87%). Accordingly, no changes in hepatic 5′D-I activity and T_3 content were found in diabetic neonates, whereas a severe decrease of both parameters was observed in diabetic adult rats, independent of the degree of hyperglycemia (greater in D_{70} than in D_{50}) in accordance with the described regulation of hepatic 5′D-I activity by insulin in adults rats.[42-44] The lack of decrease in hepatic 5′D-I activity in the neonatal diabetic rats had not been reported previously. This points to a different regulation of hepatic 5′D-I, which is unaffected by diabetes during the neonatal period, either due to the immaturity of the enzymatic system, to a different regulation by other hormones, or due to the capacity of regeneration and proliferation of the β islets in this early period.[33] This difference is also apparent throughout treatment with T_4, which restores the liver T_3 content in neonatal diabetic rats but not in adult rats. The fact that level of plasma insulin as 20% higher in neonatal diabetic rats than in diabetic adult rats (Table 1) does not explain this different response, unless the threshold of 5′D-I for insulin requirement is different at both ages.

Tx increases serum insulin, IGF-I, and GH in neonatal rats, but decreases these parameters in adult rats,[13,22] which implies a different response to the lack of thyroid hormones depending on the age of the animal. The lack of decrease in liver 5′D-I activity and T_3 content and consequently the smaller decrease in circulating thyroid hormones in neonatal diabetic rats, as

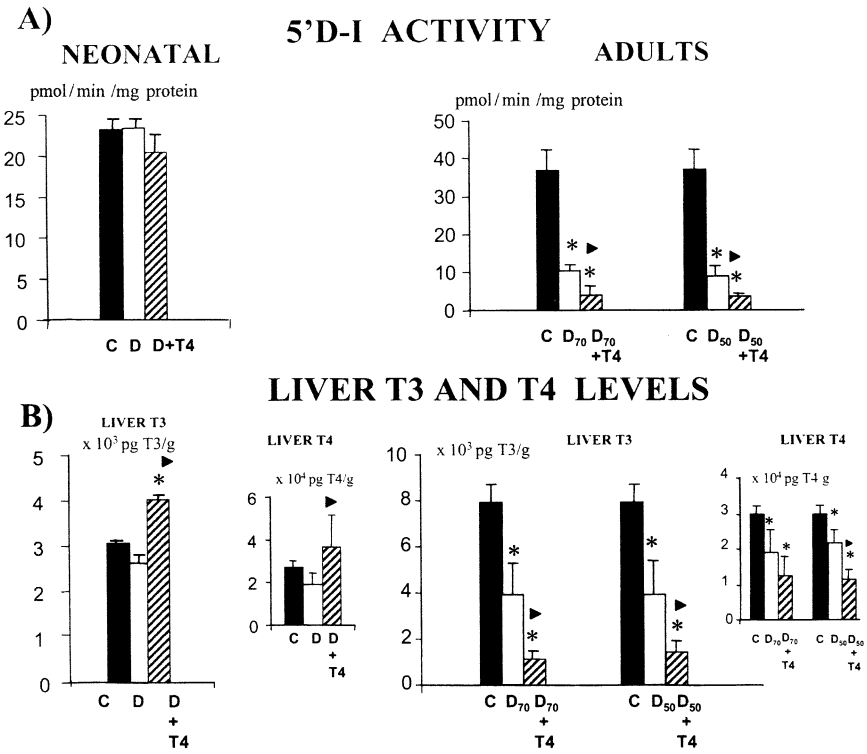

Fig 3. (A) rT$_3$ 5′D-I activity in liver homogenates of neonatal (D) and adult diabetic (D$_{70}$ and D$_{50}$) populations. See populations in Fig 1 and in Methods. Assays were run in duplicate. (B) Liver T$_3$ and T$_4$ levels in neonatal and adult D populations (see Fig 1). Assays were run in duplicate. Data are mean ± SD. Number of animals: 8-10. *P < .05 relative to C group. ▶P < .05 relative to D neonatal and adult groups (D$_{70}$ or D$_{50}$).

well as the insulin increase in Tx neonatal rats, could have biological significance to compensate the decrease in insulin or thyroid hormones in diabetic and Tx neonatal rats, respectively. Both hormones are crucial regulators of growth during immature stages. Furthermore, we previously reported that the increase of IGF-I, insulin, and GH observed in Tx neonatal rats[13] was not found in neonatal rats made hypothyroid by adequate doses of MMI (0.05% wt/vol) in drinking water of the mother.[13,22] This is probably due to the described reduction of stomach milk content found in these animals,[13,45] which was probably caused by the sour taste of milk produced by MMI. This undernourished condition would prevent the increase of insulin and IGF-I. All the above shows that Tx is the most adequate model of hypothyroidism for this kind of studies.

In this work, a 92% reduction of serum T$_3$ was found in neonatal and adult rats after Tx, followed by a large decrease in hepatic T$_3$ content and 5′D-I activity, in accordance with the fact that circulating T$_3$ results mostly from 5′-deiodination of T$_4$ in the liver.[4,46] However, contrary to what happens in the Tx adult rats after T$_4$ (Tx$_{72}$+T$_4$), in the neonatal Tx rats treated with T$_4$, the recovery of serum T$_3$ and T$_4$ is incomplete; this may be due to an immaturity of the hepatic 5′D-I that prevents a complete recovery or to higher demands of T$_4$ and T$_3$; our previous results showed that the recovery of serum T$_3$ and the normalization of serum TSH is only observed with a dose of 3 μg T$_4$/100 g BW per day in Tx neonatal rats.[13]

Thus this work shows the different adaptation of liver thyroid status to diabetes between neonatal and adult rats, as opposed to the similar adaptation found in both Tx neonatal and adult rats, which could be modulated by the different adaptative pattern at the neuroendocrine level between diabetic and Tx adults rats. The diabetic adult rats present a reduced hypothalamic thyrotropin-releasing hormone (TRH) content[47] and reduced release,[48] resulting a decreased TSH as opposed to the increase found after Tx of TSH. Depression of circulating TSH levels, with or without a decline in TRH release, has been recognized as one of the primary causal factors of decreased levels of both serum T$_3$ and serum T$_4$ in the diabetic state.[8,9,49] It is possible that the hypothalamic TRH may be reduced less in neonatal diabetic rats than in adult rats due to the immaturity of the neuroendocrine system, in agreement with the suggestion that 5′D-I is not the only factor responsible for the regulation of T$_3$ content in the liver of mice.[42] But all this remains a subject for future research. Likewise, the diabetes-induced reduction of the hepatic thyroid status in adult rats is not restored by T$_4$ in any of the diabetic adult groups studied, and such treatment aggravates the situation, contrary to what happened after Tx.[13] However, the present results also show that, thyroid gland deiodination could account for the 3-fold increase in serum T$_3$ in adult diabetic rats after T$_4$ treatment vs. the levels found in diabetic adult rats (Table1), because there is not a complete correspondence between serum and hepatic thyroid hormones; although the liver thyroid status is essential in the regulation of serum T$_3$ levels.

On the other hand, serum IGF-I and its bioavailability is decreased in hypothyroidism[12] and in nonthyroidal illnesses,

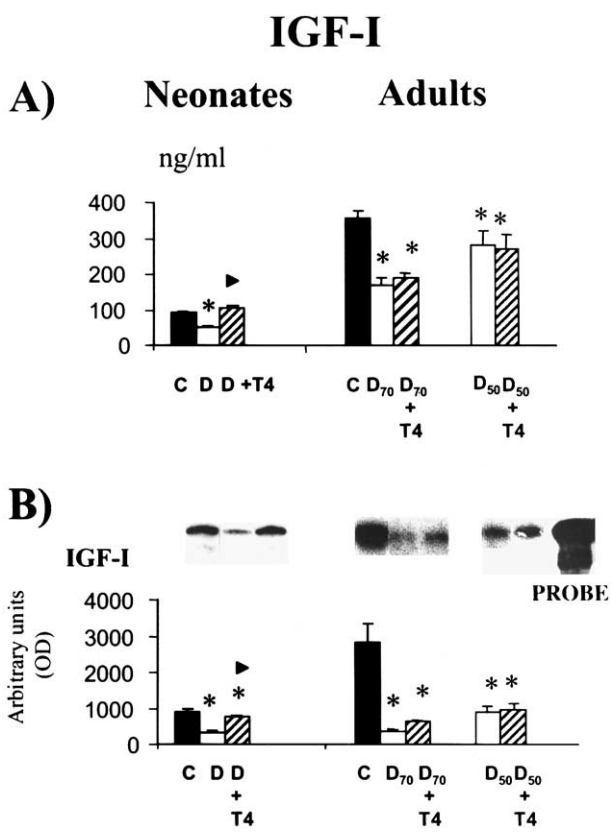

Fig 4. (A) Serum IGF-I in diabetic (D) neonates (killed on day 20 of life) and D adult (D_{70} and D_{50} killed on day 87) rats. See populations in Fig 1 and in Methods. (B) Rat liver mRNA expression of IGF-I in neonates and in adults (see populations in Fig 1). RNA expression of IGF-I was determined by RNase protection assay, and S18 ribosomal antisense assayed in the same samples was used for lane loading control. Representative bands are depicted in the figure and the densitometric quantification of 8-12 samples from different animals is shown below the bands. Data are mean ± SD. Number of animals: 8-10. *$P < .05$ relative to C group. ►$P < .05$ relative to D neonatal and adult groups (D_{70} and D_{50}).

such as undernutrition[15,16] and diabetes mellitus,[19,20] conditions in which reduced circulating thyroid hormones result from decreased peripheral T_4 deiodination to T_3.[7,46,50] A positive correlation between serum levels of T_3 and T_4 and those of IGF-I in neonatal and adult Tx rats[13] has recently been established. However, the potential relationship between the hepatic T_3 content and 5′-D-I activity and the reduced liver IGF-I secretion has not been studied in Tx nor in diabetic animals.

Since neonatal diabetes does not change liver 5′D-I activity in our experiments, T_4 treatment induces an increase in hepatic T_3 content. Consequently, serum T_3 and GH also increase above control values; thus, serum levels and liver mRNA expression of IGF-I are restored to control values.

T_4 administration to diabetic adult rats induces a further decrease in hepatic 5′D-I and, consequently, in liver T_3 concentrations, thus preventing the recovery of serum GH levels and, therefore, of the GH-dependent serum levels and liver mRNA expression of IGF-I.[16,22] A similar situation of reduced serum GH, insulin, and IGF-I was found in adult Tx rats prior to T_4 treatment.[13] Unchanged levels of liver T_4 after T_4 treatment of diabetic adults (Fig 3, right panel), indicate an impaired hepatic uptake of T_4, suggesting that insulin has a positive effect on hepatic thyroid hormone uptake in adulthood, as has been envisaged.[51]

Interestingly, the parallel changes found between IGF-I levels and liver T_3 content, observed in the present study in both neonatal and adult diabetic rats, support the idea of a possible direct effect of T_3 liver concentration on the IGFs secretion in the liver, previously suggested in "in vitro" studies[28] and "in vivo" in neonatal and adult Tx rats treated with T_4.[13] In fact, treatment with T_4 of adult diabetic rats (D_{70} or $D_{50}+T_4$) leads to an increase of serum GH levels to values above those of untreated diabetic rats but below those of controls. In this situation IGF-I levels remain unchanged, which shows for the first time that adult diabetic rats also need adequate T_3 levels for IGF-I secretion, regardless of plasma GH levels. A similar effect can be observed in Tx rats, treated with T_4, since T_4 induces an increase in hepatic 5′D-I activity and in T_3 content, and consequently in circulating thyroid hormones, which contribute to the reported recovery of IGF-I.[13]

This absence of IGF-I recovery in adult diabetic rats treated with T_4 doses had previously been reported in studies in which liver thyroid status was not determined.[52] Most important, our results give a fair explanation of the clinical fact that T_4 treatment does not improve the condition of adult patients with nonthyroidal illnesses.[10,53] Moreover, the present data are in agreement with the lack of increase in maternal T_3 pool in diabetic rat mothers after T_4 administration, and consequently this treatment was harmful to the outcome of pregnancy.[11] According to the present results, the thyroidal status of the adult animal deteriorates because of the further decrease of liver 5′D-I activity and T_3 content induced by T_4 administration and the blunted liver uptake of T_4, which is probably due to the reduced insulin. These results show, for the first time, that in the neonatal period the lack of insulin does not block T_4 liver uptake and does not decrease 5′D-I, contrary to what is found in adult diabetic rats. These experiments with diabetic and Tx neonatal and adult rats support the positive correlation between the liver thyroid economy (liver 5′D-I activity and T_3 content) and the secretion of IGF-I.

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