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The Nuclear Receptor Constitutively Active/Androstane Receptor Regulates Type 1 Deiodinase and Thyroid Hormone Activity in the Regenerating Mouse Liver

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ABSTRACT

We observed that the level of reverse triiodothyronine (rT3) was significantly increased after partial hepatectomy (PH) in both wild-type and constitutively active/androstane receptor (CAR) knockout (KO) mice, and treatment with phenobarbital (PB), a CAR activator, after PH decreased rT3 to restore its original level only in wild-type mice. On the other hand, no significant changes in the level of total T3 or free T3 in the serum were observed in either wild-type or CAR KO mice after PH or treatment with PB. Type 1 deiodinase (D1) activity and expression were significantly reduced by PH and up-regulated by PB in a CAR-dependent manner. In addition, known T3-regulated genes [tyrosine aminotransferase (TAT) and basic transcription element binding protein (BTEB)] were also significantly decreased by PH and induced by PB. Injection of rT3 into normal mice revealed that rT3 is capable of repressing the known thyroid hormone-regulated genes *Tat*, *Bteb*, and *Cpt-1* in the liver. Our results suggest that PH decreases D1 activity leading to increased rT3 level, resulting in the repression of T3 target genes. Subsequent treatment with PB decreases rT3 in a CAR-dependent manner through the up-regulation of the D1 gene.

Phenobarbital (PB) is a sedative used to treat patients with ailments such as epilepsy and jaundice. In addition, PB has been suggested to affect thyroid hormone signaling through the nuclear receptor constitutively active/androstane receptor (CAR) (Sueyoshi et al., 1999; Maglich et al., 2004; Qatanani et al., 2005). Thyroid hormone has a well established role in liver regeneration and energy usage and regulates genes involved in gluconeogenesis and fatty acid oxidation (Giralt et al., 1991; Park et al., 1997; Jansen et al., 2000; Boelaert and Franklyn, 2005). Recently it was reported that mice after partial hepatectomy (PH) or mice treated with the PB-like inducer TCPOBOP (a potent CAR activator) regulate the same subset of immediate early genes in their respective livers involved in suppression of apoptosis and alteration of signal transduction (Locker et al., 2003). Based on the similarity between TCPOBOP response and liver regeneration, we performed microarray analysis to examine whether CAR regulates gene expression in the liver after PH using wild-type and CAR knockout (KO) mice. From these arrays, we identified the thyroid hormone-metabolizing enzyme *Dio1* as a CAR-regulated gene in the liver after PH. Whereas CAR has been suggested to regulate thyroid hormone, there have been no reports concerning the potential for CAR regulation of the biosynthesis of thyroid hormones. Therefore, we examined whether CAR regulates the synthesis of thyroid hormone in the liver after PH.

PB elicits pleiotropic effects on liver functions, including drug metabolism, energy metabolism, and cell growth by regulating hepatic gene expression (Honkakoski and Negishi, 1998). Of the genes identified on these arrays as regulated by PB, roughly half are regulated by CAR in the liver of PB-treated mice, including various cytochromes P450 (P450s), phosphoenolpyruvate carboxykinase, and carnitine palmitoyl transferase 1 (CPT-1) (Ueda et al., 2002). CAR is a member of the NR1I subfamily of nuclear hormone receptors (Laudet et al., 1999) and was originally found to regulate the CYP2B family of drug-metabolizing enzymes (Honkakoski et

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ABBREVIATIONS: PB, phenobarbital; CAR, constitutive active/androstane receptor; PH, partial hepatectomy; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; KO, knockout; P450, cytochrome P450; CPT-1, carnitine palmitoyl transferase 1; T4, tetraiodothyronine; D1, type 1 deiodinase; D2, type 2 deiodinase; D3, type 3 deiodinase; T3, triiodothyronine; rT3, reverse triiodothyronine; PCR, polymerase chain reaction; TAT, tyrosine aminotransferase; BTEB, basic transcription element binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; TSH, thyroid-stimulating hormone.

al., 1998; Sueyoshi et al., 1999). The CAR KO mouse has confirmed that CAR is the primary regulator of this subfamily of P450s in the presence of CAR activators such as PB or PB-like inducers like TCPOBOP (Wei et al., 2000; Ueda et al., 2002). In addition to P450s, CAR is also known to regulate organic anion transporters, such as organic anion transporting polypeptide 2 and multidrug resistance gene 3 (MDR3), which aid in the removal of bile salts, bilirubin, and hormones, including thyroid hormone, from the blood (Friesema et al., 1999; Guo et al., 2003; Saini et al., 2004; Wong et al., 2005). Recent reports also suggest that CAR is involved in gluconeogenesis through the regulation of genes such as phosphoenolpyruvate carboxykinase (Kodama et al., 2004). Studies have also linked CAR to tumor development in the liver after chronic PB exposure, suggesting a role for the receptor in the promotion of hepatocyte proliferation (Bouzahzah et al., 1998; Yamamoto et al., 2004; Huang et al., 2005).

The deiodinases are the enzymes that convert thyroxine (3–5-3′-5′-tetraiodothyronine, T4) into the various forms of thyroid hormone. Three isoenzymes are known: type 1 deiodinase (D1, gene name *Dio1*), type 2 (D2, *Dio2*), and type 3 (D3, *Dio3*) (Kohrle, 2000). D1 and D2 are responsible for the conversion of T4 into 3–3′-5-triiodothyronine (T3) by outer ring deiodination where the 5′ iodine is removed. D1 and D3 catalyze the conversion of T4 into the lesser active 3–3′-5′-triiodothyronine (reverse T3, rT3) through inner ring deiodination, which removes the 5 position iodine. Clearance of thyroid hormone is accomplished through processes including sulfation and glucuronidation for excretion through the bile or urine (Visser, 1994; Visser et al., 1998). Thus, a balance between synthesis and clearance can control the level of thyroid hormone in the serum.

We observed that PH elicits a large increase in the level of rT3 in the serum but no significant change in the levels of total or free T3. A significant down-regulation of D1 activity correlates with the increase of rT3 after PH. Treatment with PB after PH did not affect the level of total T4 but significantly increased the expression and activity of D1 and returned the serum content of rT3 to nearly normal levels in wild-type but not in CAR KO mice. We present experimental results consistent with the hypothesis that CAR and PB activate the *Dio1* gene to alter rT3 level in the regenerating liver, modulating the effect of thyroid hormone regulating its target genes.

Materials and Methods

Chemicals. PB, T3, rT3, and all of the other chemical reagents were purchased from Sigma (St. Louis, MO) unless otherwise specified.

Animal Surgery and Treatments. Female wild-type and CAR KO mice on the C3H/HeNCrIBR genetic background (age 6–10 weeks) were used for these studies. All of the animal handling was performed in accordance with institution ethical guidelines with regard to humane care and treatment. Animals were fed standard mouse chow and allowed free access to food and water. Animals were maintained in a temperature-controlled environment with 12-h light and dark cycles. Mice were anesthetized by vapor inhalation of isoflurane (AErrane, Baxter Pharmaceutical Products, Deerfield, IL) and then underwent PH according to the Higgins and Anderson method (Higgins and Anderson, 1931). Mice were allowed to recover from anesthesia for 24 h, injected i.p. with 100 mg/kg PB or phosphate-buffered saline, and euthanized 12 h postinjection. This experimental design was used for subsequent PH/PB studies to reproduce the microarray data and for consistency. Serum and liver tissue were harvested and analyzed. The same region of the liver was isolated from the sham-treated mice compared with PH mice. For rT3 experiments, female wild-type and CAR KO mice (age 6–10 weeks) were treated with 100 μg/kg rT3 or phosphate-buffered saline via single i.p. injection. After 36 h, mice were euthanized, and serum and liver tissue were isolated and analyzed. The treatment time of 36 h for rT3 injection experiments was chosen to reflect the time frame of the PH/PB studies in which mice underwent surgery and were allowed to recover for 24 h, injected with PB, and euthanized 12 h later (36 h total).

RNA Preparation, Reverse Transcription, and Real-Time Polymerase Chain Reaction. Total RNA was isolated from a small portion (100 mg) of tissue using TRIzol reagent (Invitrogen, Carlsbad, CA) according to manufacturer protocol. RNA was quantitated, and 2 μg was used for reverse transcription-polymerase chain reaction (PCR). Reverse transcription was performed using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) according to the manufacturer protocol. Real-time PCR was performed using TaqMan PCR Master Mix (Applied Biosystems) with TaqMan primer-probe sets. TaqMan primer-probe sets for D1 (assay ID: Mm00839358_m1), tyrosine aminotransferase (TAT) (assay ID: Mm00455392_m1), basic transcription element-binding protein (BTEB) (assay ID: Mm00495172_m1), Cpt-1 (custom, forward: 5′-CCCTGGGCATGATTGCAA-3′; reverse: 5′-AAGAGGACGCCACTCACGAT-3′; probe: 5′-6FAM-ACCCTAGACACCACTGGCCGCATGTTAMRA-3′), and Spot14 (assay ID: Mm01273967_m1) were analyzed as multiplex PCR using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as endogenous control (Rodent GAPDH Control Reagent Kit; Applied Biosystems). Thermocycling reactions were carried out using the ABI Prism 7900 Sequence Detection System. Data were analyzed using S.D.S. 2.2.1 (Applied Biosystems), and values for target genes were corrected for endogenous control (GAPDH). A previous report suggested that GAPDH is significantly regulated by hepatectomy (Iwasaki et al., 2004); however, when GAPDH levels were presented in graphical format, no significant alterations in response to PB or hepatectomy were observed (data not shown).

Microarray Analysis. RNA to be used in microarray analysis was isolated as described and then further purified using RNEasy Midi Kit (Qiagen, Valencia, CA). Gene expression analysis was conducted using Agilent Mouse Oligo arrays (Agilent Technologies, Palo Alto, CA). Total RNA was amplified using the Agilent Low RNA Input Fluorescent Linear Amplification Kit protocol. Starting with 500 ng of total RNA, Cy3- or Cy5-labeled cRNA was produced according to manufacturer's protocol. For each two-color comparison, 750 ng of each Cy3- and Cy5-labeled cRNA was mixed and fragmented using the Agilent in situ hybridization kit protocol. Hybridizations were performed for 17 h in a rotating hybridization oven using the Agilent 60-mer oligo microarray processing protocol. Slides were washed as indicated in this protocol and then scanned with an Agilent Scanner. Data were obtained using the Agilent Feature Extraction software (version 7.1) using defaults for all of the parameters. Hybridizations, scanning, and data analysis were performed by the National Institute of Environmental Health Sciences Microarray Group (http://dir.niehs.nih.gov/microarray/).

Serum Biochemistry. To isolate serum, blood was collected and allowed to coagulate at room temperature for 25 min. Serum was isolated by centrifugation in Microtainer Serum Separation Tubes (Becton Dickson, Franklin Lakes, NJ) according to manufacturer's protocol and analyzed via radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). Serum analysis was performed by Dr. Ralph Wilson at the National Institute of Environmental Health Sciences. Levels of total T4, total T3 (Diagnostic Products Corp., Los Angeles, CA), thyroid-stimulating hormone (TSH) (Amersham Life Science, Buckinghamshire, England), and rT3 (Animal Laboratory Products Company, Windham, NH) were determined using radioimmunoassay according to the manufacturer protocol. In brief, samples

or calibrator samples were added to tubes coated with total T4 or total T3 antibody. Labeled (^{125}I) total T4 or total T3 (1 ml) was added to each tube and gently vortexed. Samples were incubated at 37°C (total T4, 1 h; total T3, 2 h). Liquid was removed from each tube, and tubes were counted for 1 min in a gamma counter (Cobra Quantum Gamma Counter, PerkinElmer Life and Analytical Sciences, Boston, MA). Amounts of total T4 or total T3 were calculated by determining the percentage bound to the antibody in each tube (net counts/net max binding counts) compared with standard curve samples. The rT3 assay was performed in a similar manner with a few variations. The rT3 antibody was added to each tube, and samples with antibody were incubated for 20 h at room temperature. Antibody bound to antigen was precipitated out of solution using secondary antibody conjugated to polyethylene glycol resin and centrifugation. The supernatant was removed, and the radioactivity in the tube was counted. Levels of free T3 were determined by colorimetric ELISA assay according to the manufacturer protocol (Animal Laboratory Products Company). In brief, 50 μl of serum was mixed with 100 μl of conjugated hormone competitor. Samples were incubated for 1 h at room temperature and then washed three times with distilled water. Samples were visualized by colorimetric assay by addition of hydrogen peroxide/tetramethylbenzidine and incubated for 20 min at room temperature in the dark. Visualization was stopped by addition of 3N HCl and mixed thoroughly. Absorbance was measured at 450 nm within 30 min of adding HCl. Measurements for all of the assays were performed in triplicate. To measure TSH, standard curve samples were diluted according to manufacturer instructions. Equal amounts of unknown sample and antiserum (100 μl) were pipetted into tubes along with 100 μl of tracer. Tubes were vortexed, capped tightly, and allowed to incubate at room temperature for 24 h. After incubation, 400 μl of second antibody reagent (from kit) was added to tubes and vortexed to mix. Reactions were incubated at room temperature for another 10 min. Samples were centrifuged for 10 min at 1500*g* at 4°C. The resulting supernatant was removed, and tubes were inverted to allow excess liquid to drain. Samples were then counted on a gamma counter for 1 min/sample.

Deiodinase Activity Assay. Whole liver microsomes were isolated from mouse tissue (approximately 800 mg) by homogenization in 30 ml of 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), and 1 mM EDTA. Homogenate was centrifuged at 15,000*g* for 20 min at 4°C. Supernatant was transferred to a fresh tube and centrifuged again at 100,000*g* for 90 min at 4°C. Resulting pellet was washed three times with 15 ml of 50 mM Tris-HCl (pH 7.5) and 1 mM EDTA, resuspended in 3 ml of wash buffer, aliquoted, and stored at −80°C until use. Protein concentration was determined by Bradford Assay (Bio-Rad, Hercules, CA). Activity assay was performed by mixing 300 μg of whole liver microsomes with 1 μCi of [^{125}I]T4 (GE Healthcare, Piscataway, NJ) to a final volume of 300 μl in Tris-EDTA buffer and 25 mM DTT and incubating for 1 h at 37°C. Cation exchange columns (SP Sepharose, Amersham Biosciences) were prepared with 2 ml of 50% slurry resin. Column was washed with 10 column volumes of Tris-EDTA buffer. The entire volume of the microsome reaction was applied to the column and allowed to drain by gravity, and the flow-through was collected. Activity was measured on a Cobra Quantum Gamma Counter (PerkinElmer Life and Analytical Sciences). Activity was calculated as picomole of ^{125}I liberated per milligram of microsomal protein per hour.

Statistical Analyses. Statistical analysis was performed as one-way analysis of variance of the values using JMP 5.0.1 (SAS, Cary, NC). Error bars are represented as standard errors. Designations for the level of confidence are noted in the legend for each figure.

Results

Increased Serum rT3 Levels by PH. As shown in Fig. 1A, treatment with only PB resulted in no statistically significant change in total T4 level. After PH, however, there was a statistically significant decrease in total T4 of approximately 20% in wild-type and 40% in CAR KO mice, respectively. Subsequent treatment with PB after PH did not further alter total T4 level. Because the effect elicited by PH on total T4 occurred in both wild-type and CAR KO mouse, this alteration is CAR-independent. The level of total T3 (Fig. 1B) and TSH (Fig. 1E) showed no statistically significant change in the wild-type and CAR KO mice after either PH or PB treatment or after both treatments. Total T3 is a measure of free T3 and T3 conjugated to carrier proteins (bound T3), and free T3 is considered the biologically active form. As shown in Fig. 1C, free T3, similar to total T3, was not significantly altered by PH and/or PB treatment.

Conversely, PH was observed to markedly increase rT3 in both wild-type and CAR KO mice more than 3- and 6-fold, respectively (Fig. 1D). Treatment with PB after PH reduced the level of rT3 only in the wild-type mice to a level comparable with that in sham mice. The rT3 level in sham wild-type mice after PB treatment was also similar to sham CAR KO mice. These results suggested that CAR regulates the decrease of rT3 levels by PB in both sham and PH mice.

Decrease in D1 Activity after PH. The levels of thyroid hormone can be regulated by three types of deiodinases: D1, D2, and D3. We first examined expression of all three in the liver in response to PH and PB using real-time PCR. Consistent with a previous report showing that D2 is not expressed in the liver (Connor et al., 2005), we did not detect D2 mRNA in our experiments (data not shown). D3 expression in the liver was nearly undetectable, which is also in agreement with other reports and was not regulated by CAR or hepatectomy (data not shown). In sharp contrast, D1 mRNA was highly expressed in the liver and was significantly repressed after PH in both the wild-type and CAR KO mice by approximately 80%, suggesting a CAR-independent mechanism of repression (Fig. 2A). Treatment with PB reversed this repression of D1 in only wild-type mice, indicating that this effect is CAR-dependent.

To examine whether the CAR-regulated increase of D1 mRNA was reflected at the level of enzyme activity, liver microsomes were prepared to measure outer ring deiodinase activity using ^{125}I-labeled T4 as a substrate. The T4 used was labeled at the 3′ and 5′ positions and ensured that the activity being measured was only the activity of D1. Because D3 does not remove either 3′ or 5′ iodine and because D2 is not expressed in the liver, D1 is the only enzyme in the liver that can remove 5′ iodine. As shown in Fig. 2B, PH reduced microsomal D1 activity by approximately 40% in both wild-type and CAR KO mice. Treatment with PB after PH resulted in a significant increase (2.9-fold) in D1 activity in only the wild-type mice. Interestingly, however, PB increased D1 enzymatic activity in wild-type PH animals to levels higher than might be expected from the increase in D1 mRNA, although the -fold increase in D1 mRNA and enzymatic activity after PH and PB are similar compared with PH alone (2.5-fold for mRNA, 2.9-fold for activity). Regardless, D1 enzymatic activity followed the same general pattern as D1 mRNA expression, suggesting that alterations in D1 mRNA expression are reflected in the activity of D1.

Role of rT3 in Expression of Thyroid Hormone-Targeted Genes after PH. Although the level of total and free T3 remained unchanged, rT3 was altered by PH and PB treatment. Therefore, we examined whether the alterations

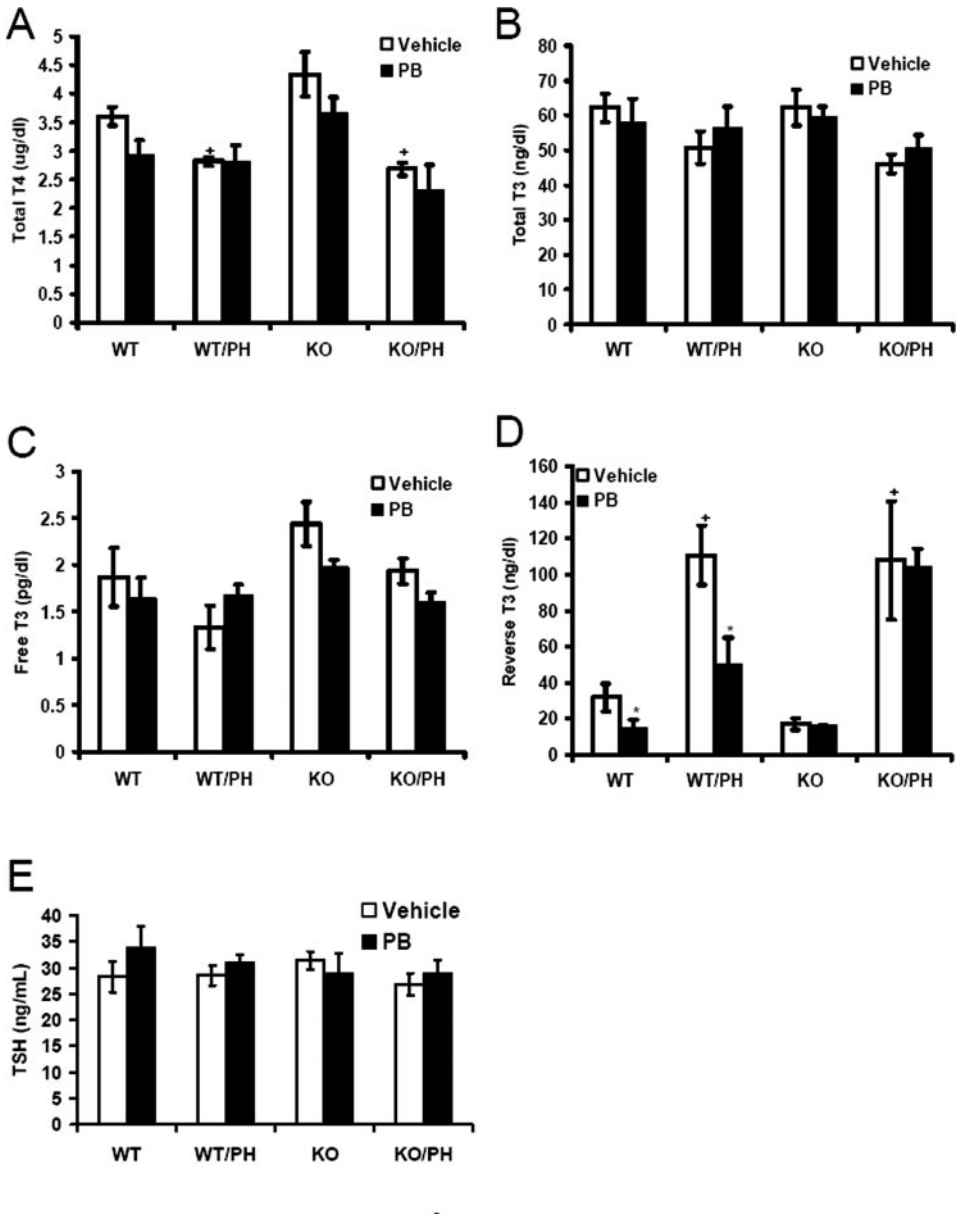

Fig. 1. Serum biochemistry for wild-type and CAR KO mice after PH. Wild-type and CAR KO mice underwent PH or sham operation and were then treated with PB or control treatment. Serum was isolated and analyzed for various forms of thyroid hormone. A, total T4 as measured by radioimmunoassay. B, total T3 as measured by radioimmunoassay. C, free T3 as measured by colorimetric ELISA. D, rT3 as measured by radioimmunoassay. E, TSH as measured by radioimmunoassay. *, $p < 0.05$ comparing PB-treated bar with control bar for same genotype animal and same surgery type (sham or PH). +, $p < 0.05$ comparing PH to sham bar for same genotype animal and same treatment type (PB or control).

of rT3 correlated with the expression of genes that are regulated by thyroid hormone. For this purpose, the expression of two known thyroid hormone-regulated genes, BTEB and TAT, were measured in the liver after PH. As shown in Fig. 3, A and B, BTEB and TAT were both significantly down-regulated in both wild-type and CAR KO mice after PH. BTEB was reduced by 60% in both mouse types, and TAT was reduced by 55% in both mouse types. Similar to D1 activity and correlating with the decrease of rT3, both BTEB and TAT were significantly up-regulated by PB after PH, but only in wild-type mice. These results indicated that the high levels of rT3 are associated with the repression of these genes.

We tested whether rT3 could repress the expression of T3-regulated genes in liver in vivo. Injection of 100 μg/kg rT3 via i.p. injection resulted in an rT3 level of approximately 75 ng/dl, which is comparable with the approximately 100 ng/dl level seen in both wild-type and CAR KO mice after PH and significantly higher than the rT3 level seen in sham mice. As shown in Fig. 4, A, B, and D, mice treated with a single injection of rT3 exhibited significantly reduced mRNA expression of TAT, BTEB, and CPT-1, all known thyroid hormone-regulated genes. All of these mRNA were down-regulated by at least 40% after treatment with rT3 in both wild-type and CAR KO mice. Interestingly, D1 was not repressed by injection of rT3, even though D1 is regulated by T3 (Fig. 4C). These findings clearly showed that rT3 can regulate the expression of some thyroid hormone target genes, such as *Tat*, *Bteb*, and *Cpt-1*, but not *Dio1*.

It is well known that PB can induce hepatomegaly in mice (Huang et al., 2005). It is also well established that thyroid hormone can increase the growth of the liver after PH because injection of T3 into mice after PH increases the rate of liver regeneration (Moro et al., 2004; Alisi et al., 2005). It does not appear that CAR directly regulates the levels of T3, although ability of CAR to regulate the levels of rT3 may be akin to regulation of T3 itself. We treated wild-type and CAR KO mice with a single injection of PB after PH and observed

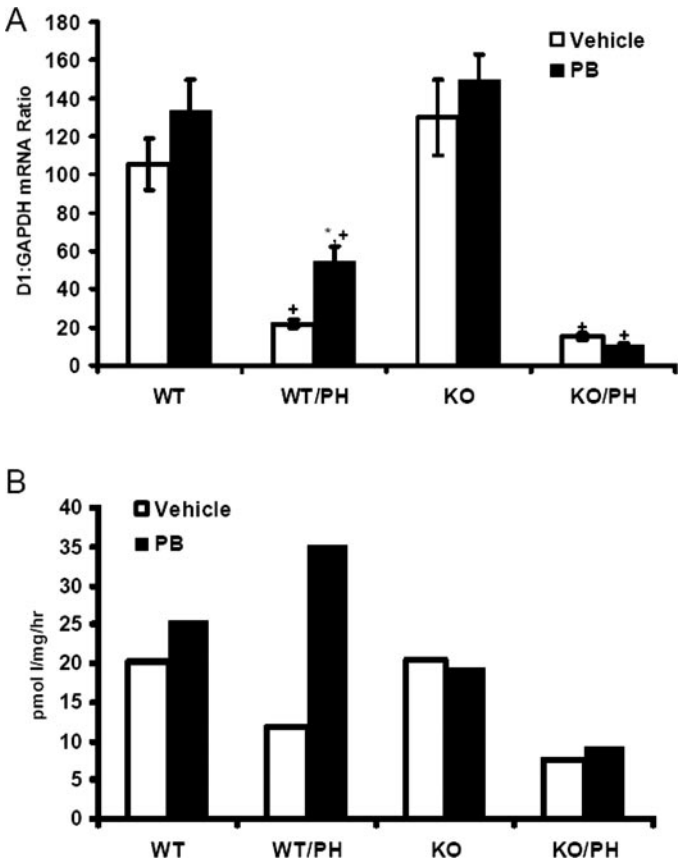

Fig. 2. D1 expression and activity in the liver after PH. Wild-type and CAR KO mice underwent PH or sham operation and were then treated with PB or control treatment. A, total RNA was isolated from mouse livers, and D1 mRNA expression was determined by real-time PCR. *, $p < 0.05$ comparing PB-treated bar to control bar for same genotype animal and same surgery type (sham or PH). +, $p < 0.05$ comparing PH with sham bar for same genotype animal and same treatment type (PB or control). B, mouse liver microsomes were isolated from mice treated as above and were incubated with ^{125}I-labeled T4 and passed over a cation exchange column. Flow-through was analyzed for presence of free radioactive iodine. Enzyme activity was calculated in terms of picomole of free iodine per milligram microsomal protein per hour. Graph is representative of three independent experiments.

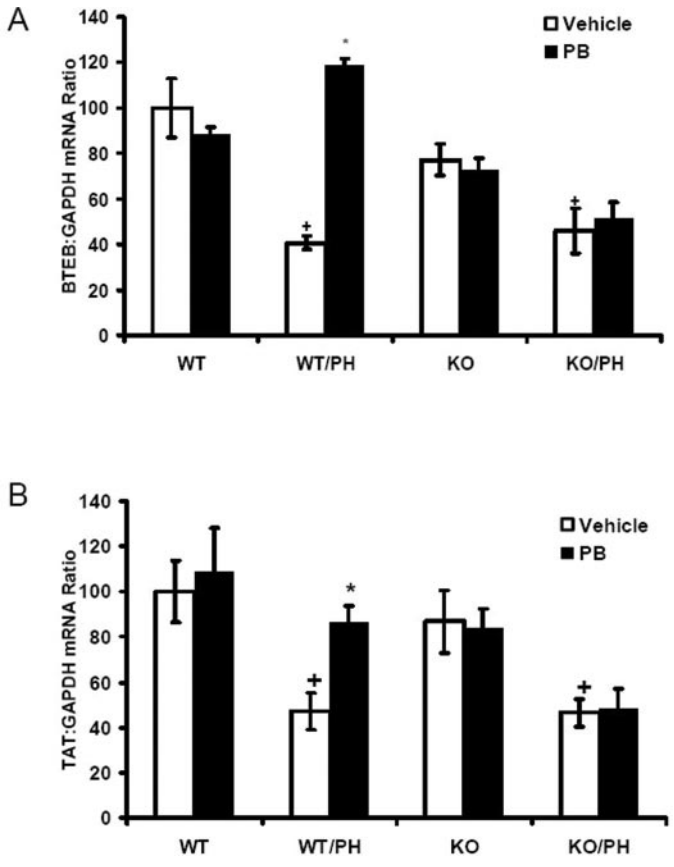

Fig. 3. Analysis of T3-regulated gene expression after PH. Wild-type and CAR KO mice underwent PH or sham operation and were then treated with PB or control treatment. Total mRNA was isolated from mouse livers and reverse transcribed for analysis by real-time PCR. A, BTEB real-time PCR. B, TAT real-time PCR. *, $p < 0.05$ comparing PB-treated bar to control bar for same genotype animal and same surgery type (sham or PH). +, $p < 0.05$ comparing PH with sham bar for same genotype animal and same treatment type (PB or control).

significantly increased liver weight gain only in wild-type mice, suggesting that PB can positively influence the regenerative rate of the liver through activation of CAR (Fig. 5). This result is consistent with a previous report suggesting CAR activation plays a positive role in bile acid-induced liver regeneration (Huang et al., 2006).

Discussion

In the studies described here, we observed a large increase in the level of rT3 after PH, which was correlated with a repression of D1 activity. Treatment with the antiseizure drug PB after PH decreased the level of rT3 and increased the expression of D1 in a CAR-dependent manner. These alterations of D1 and rT3 levels were directly reflected on the expression of thyroid hormone-regulated genes *Bteb* and *Tat*. Moreover, CAR regulation of D1 expression suggests control of thyroid hormone activity via the alteration of the level of rT3.

Reverse T3 has very low hormone activity, and reports have begun to suggest that rT3 may decrease T3 activity by competing with T3 for binding to lipids, transporters, and receptors, affecting the activity of hormone in cells (Benvenga et al., 1993; Mitchell et al., 1999). That rT3 may function as an inhibitor of T3 offers an interesting new regulatory pathway in the thyroid hormone signaling cascade. In support of this hypothesis, injection of rT3 into mice revealed that rT3 is capable of significantly decreasing the expression of multiple T3-regulated genes (*Bteb*, *Tat*, and *Cpt-1*) in the liver.

T4 is converted to rT3 by the activity of D3 (reviewed in Kohrle, 2000). Thus, the extremely low hepatic expression of D3 means that the liver is not the primary site of rT3 production. Rather, rT3 production is primarily carried out in extrahepatic tissues where D3 is highly expressed. In our studies, we observed that the liver is sensitive to circulating rT3 because the injection of exogenous rT3 can repress T3-targeted genes. To counter the repressive activity of rT3, the liver uses D1 to convert rT3 to T2 for excretion. In regenerating liver, down-regulation of D1 may decrease conversion of rT3 to T2, disrupting this rT3 clearance mechanism, allowing rT3 to accumulate in the circulation and resulting in inhibition of hepatic thyroid hormone responsive gene expression. PB treatment increases the D1 activity in the CAR-dependent manner, reopening the clearance of rT3 by converting it to T2 and decreasing rT3 level. This hypothesis is

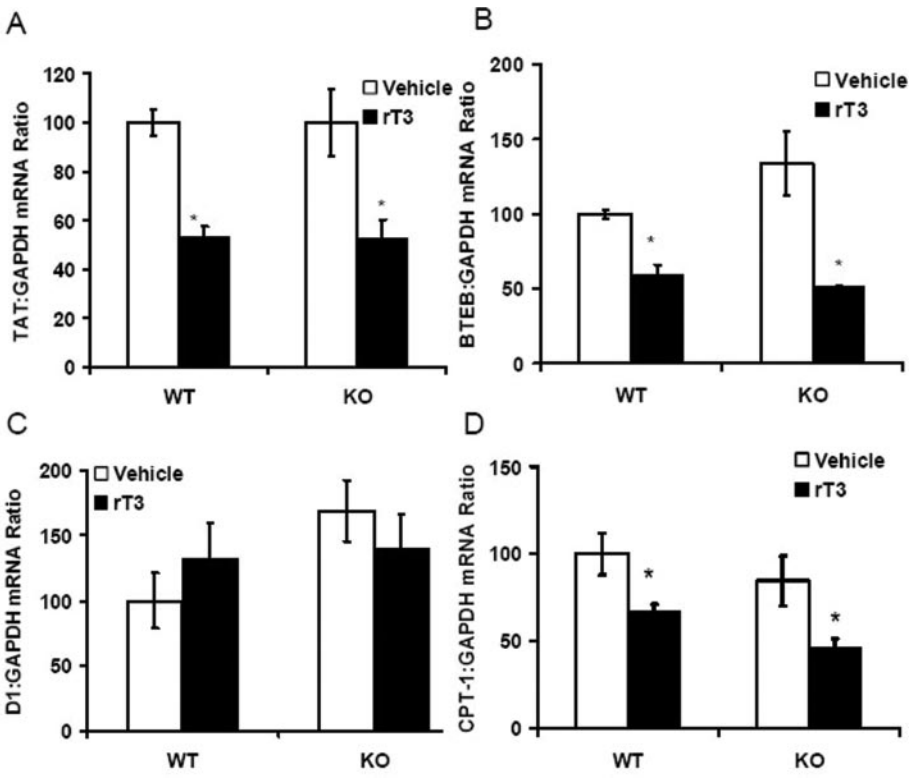

Fig. 4. rT3 inhibition of T3 target gene expression. Wild-type mice were injected with 100 μg/kg of rT3 or control treatment. Thirty-six hours after injection, livers were isolated. Total RNA was isolated and reverse-transcribed for analysis by real-time PCR. A, TAT real-time PCR. B, BTEB real-time PCR. C, D1 real-time PCR. D, CPT-1 real-time PCR. *, $p < 0.05$ compared with control treatment bar for the same genotype mouse (wild-type or CAR KO).

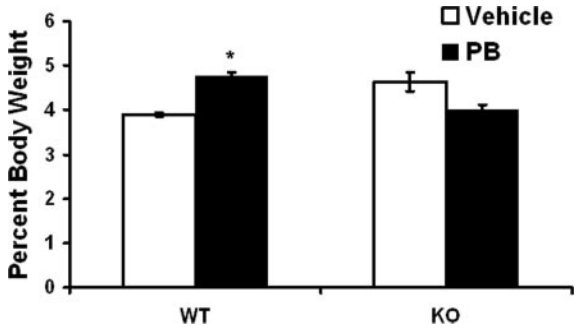

Fig. 5. PB treatment enhances liver regrowth after PH. Wild-type or CAR KO mice underwent PH and 24 h later were injected once with 100 mg/kg PB. Seventy-two hours postinjection, total body weight and liver weight were measured. Liver mass is shown as percentage of total body weight. *, $p < 0.05$ compared with control treatment bar for the same genotype mouse (wild-type or CAR KO).

supported by the fact that the level of D1 activity is inversely related to the level of rT3 as shown in the recently described *Dio1* KO mouse. This mouse exhibited a very similar phenotype as observed in our work: abrogated D1 activity and an increased level of rT3 in the circulation (Schneider et al., 2006). In our study, the CAR-dependent increase in D1 activity was higher than that of D1 mRNA in the PB-treated PH livers when corrected to wild-type sham vehicle-treated control values. This difference of induction is intriguing and raises the question that post-transcriptional regulation such as translational regulation, protein stabilization and degradation, and enzyme activation may also be involved in the PB induction of D1 enzyme. These post-transcriptional events would also be regulated by CAR (because these PB effects are absent in the CAR KO mouse) and present interesting subjects for future investigations.

Both wild-type and CAR KO mice exhibit elevated rT3 and down-regulated D1 after PH, meaning that these effects do not involve CAR. Interestingly, rT3 is unable to inhibit expression of D1, even though D1 is known to be regulated by T3 (Toyoda et al., 1992). This suggests that the increase in rT3 seen after PH is not responsible for the decrease in D1 expression. It is unknown at this point what signaling pathways evoke this D1 repression, although recent reports suggest that cytokines such as interleukin 6 may be involved in the repression of D1 in the liver during nonthyroidal illness, a condition in which rT3 is also elevated (Yu and Koenig, 2006). It is entirely possible that such a signal is at work after PH. The lack of inhibition of D1 by rT3 also suggests that rT3 does not affect all of the T3-regulated genes in the same manner, and more work is needed to determine how this occurs.

Thus, in the PH model system, the decrease in D1 activity brought about by PH may adversely affect the conversion of rT3 into T2, allowing rT3 to accumulate. D1 is also responsible for the conversion of T4 into T3, but a PH-induced decrease in hepatic D1 did not significantly affect T3 levels, suggesting that the conversion of T4 into T3 is rather unaffected and that extrahepatic tissues are able to compensate for the lost T3 production in the liver through the activity of D2. This situation is also in agreement with the *Dio1* KO mouse, which also showed no significant difference in T3 level between wild-type and KO mice (Schneider et al., 2006).

Total T4 levels were slightly decreased after PH, regardless of CAR status. This effect is possibly linked to the increase in rT3. One function of rT3 is to facilitate the removal of excess T4. Increased rT3 after PH indicates that removal of rT3 is not functioning properly, thus prompting the decreased production of T4 by the thyroid gland. Qatanani et al. (2005) suggested that a decrease of T4 level in response to PB results in a hypothyroid state leading to an increase of TSH

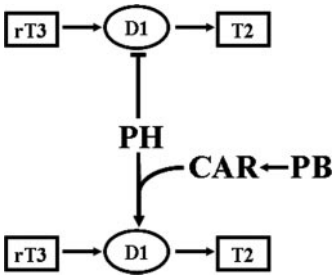

Fig. 6. Proposed model of the role of CAR/PB and D1 in the regulation of rT3 after PH.

secretion by the pituitary gland. In our studies, however, no alteration in the level of TSH was observed after PH or PB treatment. In addition, PB treatment alone or after PH did not affect the level of total T4, which is inconsistent with previous reports (Maglich et al., 2004; Qatanani et al., 2005). Such a difference may be because the mice used in our studies were bred on the C3H background and not on the C57BL/6 background used in other studies. There are many known differences between these two mouse strains, especially concerning the promotion of hepatocyte proliferation and tumor formation (Diwan et al., 1986). The difference in response to PB with regard to thyroid hormone may be yet another facet of this strain difference.

Our results presented here suggest that CAR is a regulator of thyroid hormone activity in the liver after PH. We propose a model whereby PH represses D1 in a CAR-independent manner, resulting in reduced conversion of rT3 into T2 and thereby allowing rT3 to accumulate and repress target gene expression in the liver. PB treatment after PH increases D1 in a CAR-dependent manner, increasing conversion of rT3 into T2 and relieving the repressive effects of rT3 (Fig. 6). Thus, CAR activators such as PB may provide us with a way to modulate liver regeneration after insults and injuries such as transplantation, chemical exposure, or physical injury/resection.

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