DECREASE IN SERUM THYROXINE LEVEL BY PHENOBARBITAL IN RATS IS NOT NECESSARILY DEPENDENT ON INCREASE IN HEPATIC UDP-GLUCURONOSYLTRANSFERASE

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ABSTRACT:

We have previously reported that there is a poor correlation between increase in the levels of UDP-glucuronosyltransferases, UGT1A1 and UGT1A6, and decrease in the levels of serum total thyroxine (T4) and free T4 in phenobarbital (PB)-treated rats, although the PB-induced decrease in rats is generally thought to occur through induction of the UDP-glucuronosyltransferase (T4-UDP-GT). In the present study, to clarify a relationship between the decrease in serum T4 level and the increase in hepatic T4-UDP-GT activity by PB in rats, we examined the relationship using Gunn rats, a mutant strain of Wistar rats deficient in UGT1A isoforms. Levels of serum total T4, free T4, and total triiodothyronine (T3) were markedly decreased not only in Wistar rats but also in Gunn rats 1 day after the final administration of PB (80 mg/kg i.p., once daily for 4 days), and no significant difference in magnitude of the decrease between Wistar and Gunn rats was observed. On the other hand, the level and activity of T4-UDP-GT were significantly increased by treatment with PB in Wistar rats but not in Gunn rats. Furthermore, significant increase in the activity of hepatic type I iodothyronine deiodinase, which mediates the deiodination of T4 and T3, by PB treatment was observed in both Wistar and Gunn rats. In conclusion, the present results demonstrate that the decrease in serum total T4 level by PB in Gunn rats is not dependent on the increase in hepatic T4-UDP-GT activity and suggest that even in Wistar rats, the PB-induced decrease in serum T4 level does not occur only through increase in hepatic T4-UDP-GT.

Phenobarbital (PB) is well known to decrease the level of serum thyroid hormone and to increase the activities of hepatic drug-metabolizing enzymes in rats and mice (O’Connor et al., 1999; Hood et al., 2003). Furthermore, PB increases levels of serum thyroid-stimulating hormone (TSH) and thyroid gland growth in rats (Hood et al., 1999). As a possible mechanism for the PB-induced decrease in the level of serum thyroid hormone, enhancement of thyroid hormone metabolism by PB is considered (McClain, 1989; Capen, 1997). Especially, the decrease in the level of serum thyroxine (T4) by PB in rats is thought to occur mainly through the induction of T4-UDP-glucuronosyltransferase (T4-UDP-GT), responsible for glucuronidation of T4 (Barter and Klaassen, 1992a; Liu et al., 1995). This hypothesis is supported by the previous reports that a number of T4-UDP-GT inducers, such as polychlorinated biphenyl (PCB), 3-methylcholanthrene, and pregnenolone-16α-carbonitrile, show the ability to decrease serum thyroid hormone (Saito et al., 1991; De Sandro et al., 1992; Barter and Klaassen, 1994). However, the magnitude of decrease in the level of serum total T4 by PB is not necessarily correlated with that of increase in T4-UDP-GT activity (Saito et al., 1991; Hood et al., 2003). Likewise, our preliminary study (Suzuki et al., 2004) has indicated that there is a poor correlation between increase in the levels of UGT1A1 and UGT1A6 and decrease in the levels of serum total T4 and free T4 in PB-treated rats.

In the present study, therefore, we examined a relationship between the decrease in serum total T4 level and the increase in hepatic T4-UDP-GT (UGT1A1 and UGT1A6) by PB using UGT1A-deficient Wistar rats (Gunn rats) and demonstrated that the PB-induced decrease in serum total T4 level in rats was not necessarily dependent on the increase in hepatic T4-UDP-GT activity.

Materials and Methods

Chemicals. PB was purchased from Nakakita Yakuhin Co., Ltd. (Aichi, Japan). The 125I-reverse triiodothyronine (T3r) and [125I]T4, radiolabeled at the 5′-position of the outer ring, was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). All other chemicals were obtained commercially in appropriate grades of purity.

Animal Treatments. Male Wistar rats (160–200 g) and UGT1A-deficient Wistar rats (Gunn rats, 190–260 g) were obtained from Japan SLC., Inc.

ABBREVIATIONS: PB, phenobarbital; ID-I, iodothyronine deiodinase; PCB, polychlorinated biphenyl; T3, triiodothyronine; T4, thyroxine; TTR, transthyretin; TSH, thyroid-stimulating hormone; UDP-GT, UDP-glucuronosyltransferase.
PB-MEDIATED DECREASE IN SERUM \( T_4 \) LEVEL

(Shizuoka, Japan). Male Wistar and Gunn rats were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM-8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%), and handled with humane care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Rats received four consecutive intraperitoneal injections of PB (80 mg/kg) dissolved in 0.9% saline (5 ml/kg). Control animals were treated with a vehicle alone (5 ml/kg). All rats were killed by decapitation 1 day after the final administration. The liver was removed, and hepatic microsomes and cytosols were prepared according to the method of Kato et al. (1995) and stored at −85°C until used. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at −50°C until used.

Analysis of Serum Hormones. Levels of serum total \( T_4 \), free \( T_4 \), total \( T_3 \), and TSH were measured by radioimmunoassay using the Total \( T_4 \) and Free \( T_4 \) kit (Diagnostic Products Corporation, Los Angeles, CA), the T-3 · RIABEAD (Abbott Japan Co., Ltd., Tokyo, Japan), and the rTSH \([\text{^125I}]\) Biotrak assay system (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK), respectively.

Hepatic \( T_4 \)-Metabolizing Enzyme Assays. Amounts of proteins of hepatic subcellular fractions, microsomes and cytosols, were determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. The activity of microsomal UDP-GT toward \( T_4 \) was determined by the method of Barter and Klaassen (1992b). The activity of microsomal type I iodothyronine deiodinase (ID-I) was determined by the method of Hood and Klaassen (2000). The activity of cytosolic sulfotransferase toward \( T_4 \) was determined by the method of Kaptein et al. (1997).

Western Blot Analysis. Monoclonal antibody against the common region of UGT1A isoforms and specific antibodies against UGT1A1, UGT1A6, and UGT2B1 were used (Ishii et al., 1995, 1997). Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita et al. (2001). Isolated proteins corresponding to UGT1A1, UGT1A6, and UGT2B1 were detected using chemical luminescence (ECL detection kit; Amersham Biosciences Inc., Piscataway, NJ), and the level of each protein was determined densitometrically with LAS-1000 (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Analysis of \([\text{^125I}]T_4\) Binding to Serum Proteins. At 1 day after a consecutive 4-day treatment with PB, rats were anesthetized with 50 mg/ml sodium pentobarbital combined 1:1 with 1 mg/ml potassium iodide at 2 mg/ml. The femoral artery was cannulated (polyethylene tube SP31; Natume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml). Fifteen minutes later, rats were given 1 ml of \([\text{^125I}]T_4\) i.v., at 15 μCi/ml in 10 mM NaOH saline (+1% normal rat serum. After the administration of \([\text{^125I}]T_4\), a portion (0.3 ml) of blood was sampled from the artery at the indicated time, and serum was collected and stored at −50°C for assay. Serum was diluted in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels PAG Mid “Daiichi” 4/20 (Daiichi Pure Chemicals Co., Tokyo, Japan). Proteins corresponding to UGT1A1, UGT1A6, and UGT2B1 were detected using chemical luminescence (ECL detection kit; Amersham Biosciences Inc., Piscataway, NJ), and the level of each protein was determined densitometrically with LAS-1000 (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Serum Hormone Levels. Constitutive levels of serum total \( T_4 \), free \( T_4 \), total \( T_3 \), and TSH were more than 1.8-fold higher in Gunn rats than in Wistar rats. Effects of PB on levels of serum thyroid hormones were next examined in Wistar and Gunn rats (Fig. 1). In both Wistar and Gunn rats, PB treatment resulted in decreases of serum total \( T_4 \), free \( T_4 \), and total \( T_3 \), and the magnitude of the decrease in each serum thyroid hormone was almost the same in both rats. On the other hand, no significant change in the level of serum TSH by PB treatment was observed in either Wistar or Gunn rats.

Hepatic \( T_4 \)-Metabolizing Enzyme Activities. It has been reported that \( T_4 \) glucuronidation is primarily mediated by UGT1A enzymes, UGT1A1 and UGT1A6, in the rat liver (Visser, 1996). Therefore, we examined effects of PB on hepatic microsomal \( T_4 \)-UDP-GT activity in Wistar and Gunn rats. Constitutive activity of \( T_4 \)-UDP-GT was more than 2.1-fold higher in Wistar rats than in Gunn rats. PB treatment resulted in a significant increase of \( T_4 \)-UDP-GT activity in Wistar rats but not in Gunn rats (Fig. 2).

Hepatic microsomal ID-I activity in both Wistar and Gunn rats was significantly decreased by PB (Fig. 3). On the other hand, no significant change in activity of hepatic sulfotransferase by PB treatment was observed in either PB-treated Wistar or Gunn rats (data not shown).
Immunoblot Analysis for UGT1As. Levels of immunoreactive proteins responsible for UGT1A isoforms, UGT1A1 and UGT1A6, were increased by PB in Wistar rats but not in Gunn rats (Figs. 4 and 5). In addition, no constitutive expression of the UGT1A isoforms was confirmed in Gunn rats. On the other hand, the level of UGT2B1 was significantly increased by PB in both Wistar and Gunn rats, and the magnitude of the increase was higher in Gunn rats than in Wistar rats (Figs. 4 and 5).

Serum Protein Binding of \([^{125}\text{I}]\text{T}_4\). The effect of PB on the binding of \(\left[{^{125}\text{I}}\right]\text{T}_4\) to serum proteins, TTR and albumin, was examined in Wistar rats. No significant change in the binding level of \(\left[{^{125}\text{I}}\right]\text{T}_4\) to each serum protein by PB treatment was observed with the exception of a decrease in the level of \(\left[{^{125}\text{I}}\right]\text{T}_4\)-TTR complex at 120 min after \(\left[{^{125}\text{I}}\right]\text{T}_4\) administration (Fig. 6).

Discussion

In the present study, we found that treatment with PB resulted in a drastic decrease in serum total \(\text{T}_4\) and free \(\text{T}_4\) levels in both Wistar and Gunn rats, although significant increase in the activity of \(\text{T}_4\)-UDP-GT occurred only in Wistar rats and not in Gunn rats. The present findings demonstrate that in Gunn rats, PB-induced decrease in the level of serum \(\text{T}_4\) occurs in a hepatic \(\text{T}_4\)-UDP-GT-independent fashion. In addition, constitutive levels of serum total \(\text{T}_4\) and \(\text{T}_3\) were more than 1.8-fold higher in Gunn rats than in Wistar rats, suggesting that the deficit of \(\text{T}_4\) glucuronidation results in higher \(\text{T}_4\) serum levels. Similar results and suggestions have been obtained by Benathan et al. (1983).

In general, \(\text{T}_4\)-UDP-GT inducers, including PB, clobazam, PCB, 3-methylcholanthrene, and pregnenolone-16α-carbonitrile, have been considered to decrease a level of serum \(\text{T}_4\) through an increase in hepatic \(\text{T}_4\)-UDP-GT (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Miyawaki et al., 2003). However, it has been reported that the difference between rats and mice in the magnitude of decrease in the level of serum total \(\text{T}_4\) by an inducer of \(\text{T}_4\)-UDP-GT is not well
correlated with that of the increase in activity of T4-UDP-GT (Craft et al., 2002; Hood et al., 2003). Furthermore, we have reported previously that the PCB-induced decrease in serum T3 level might occur not only through the increase in hepatic T4-UDP-GT activity but also via formation of hydroxylated PCB metabolites in rats (Kato et al., 2004) and that in PB-treated rats, there was a poor correlation between the increase in the levels of UGT1A1 and UGT1A6 and the decrease in the levels of serum total T4 and free T3 (Suzuki et al., 2004). These previous reports strongly support a possibility that the decrease in serum total T4 level by PB does not occur only through an increase in hepatic T4-UDP-GT activity.

As possible mechanisms for the PB-induced decrease in serum T4 level, changes in hepatic ID-I activity and serum TSH level might be considered. However, a significant decrease in activity of hepatic ID-I, which mediates the deiodination of T3 and T4, by PB treatment was observed in both Wistar and Gunn rats. Similar results have been reported in PB-treated Sprague-Dawley rats (O’Connor et al., 1999; Hood and Klaassen, 2000). Accordingly, the PB-induced decrease in serum T4 level is thought to occur through an ID-I-independent pathway. Furthermore, in the present study, levels of serum TSH in both Wistar and Gunn rats were not significantly changed by PB, with the exception of the slight displacement in Wistar rats at 120 min after PB treatment. Accordingly, the decrease in the level of serum T4 in PB-treated rats occurs in a TTR-independent pathway. In addition, no change in activity of hepatic sulfotransferase, which efficiently catalyzes the sulfation of iodothyronines (Kester et al., 1999), by PB treatment was observed in either Wistar or Gunn rats.

In conclusion, the present findings demonstrate that the decrease in serum total T4 level by PB in Gunn rats occurs without increases in hepatic T4-metabolizing enzymes (T4-UDP-GT, ID-I, and sulfotransferase), the binding of PB to serum TTR, and the level of serum TSH, although an exact mechanism for the PB-induced decrease remains unclear. In Wistar rats, PB-induced T4-UDP-GT might also contribute, in part, to the decrease in serum T4 level. To clarify the exact mechanisms for the PB-induced decrease in serum thyroid hormones, further studies involving T4 transporters, nonhepatic T4-UDP-GT, and exchangeable thyroid hormone pools in nonhepatic tissues would be necessary.

References
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