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A possible mechanism for the decrease in serum thyroxine level by phenobarbital in rodents

Yoshihisa Kato [a,*], Hiroshi Suzuki [b], Koichi Haraguchi [c], Shinichi Ikushiro [d], Yoshihiko Ito [b], Shinya Uchida [b], Shizuo Yamada [b], Masakuni Degawa [b]

[a] Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Kagawa 769-2193, Japan
[b] School of Pharmaceutical Sciences and Global Center of Excellence Program, University of Shizuoka, Shizuoka 422-8526, Japan
[c] Daiichi College of Pharmaceutical Sciences, Fukuoka 815-8511, Japan
[d] Faculty of Engineering, Toyama Prefectural University, Toyama 939-0398, Japan

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ABSTRACT

Effects of phenobarbital (PB) on the levels of serum thyroid hormones such as total thyroxine (T_4) and triiodothyronine were examined in male mice, hamsters, rats, and guinea pigs. One day after the final administration of PB (80 mg/kg, intraperitoneal, once daily for 4 days), significant decreases in the levels of the serum total T_4 and free T_4 occurred in mice, hamsters, and rats, while a significant decrease in the level of serum triiodothyronine was observed in hamsters and rats among the animals examined. In addition, a significant decrease in the level of serum thyroid-stimulating hormone was observed in only hamsters among the rodents examined. Significant increases in the level and activity of hepatic T_4-UDP-glucuronosyltransferase (UGT1A) after the PB administration occurred in mice, hamsters, and rats, while the increase in the amount of biliary [^{125}I]T_4-glucuronide after an intravenous injection of [^{125}I]T_4 to the PB-pretreated animals occurred only in rats. In mice, rats, and hamsters, but not guinea pigs, PB pretreatment promoted the clearance of [^{125}I]T_4 from the serum, led to a significant increase in the steady-state distribution volumes of [^{125}I]T_4, and raised the concentration ratio (Kp value) of the liver to serum and the liver distribution of [^{125}I]T_4. The present findings indicate that the PB-mediated decreases in the serum T_4 level in mice, hamsters, and rats, but not guinea pigs, occur mainly through an increase in the accumulation level of T_4 in the liver.

Introduction

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, mice, and humans (O'Connor et al., 1999; Hood et al., 2003; Capen 2008; Strolin Benedetti et al, 2005). 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 PB-induced decrease in level of serum thyroid hormone, enhancement of thyroid hormone metabolism through the induction of T_4-UDP-glucuronosyltransferase (T_4-UGT) responsible for glucuronidation of T_4 is considered (Barter and Klaassen, 1992a; Liu et al., 1995; McClain 1989; Capen 2008). This hypothesis appears to be supported by the previous reports that a number of T_4-UGT inducers, such as polychlorinated biphenyl (PCB), 3-methylcholanthrene, and pregnenolone-16α-carbonitrile, show 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 level of serum total T_4 by PB is not necessarily correlated with that of increase in T_4-UGT (UGT1A1 and UGT1A6) activity (Saito et al., 1991; Hood et al., 2003; Lecureux et al., 2009). Furthermore, we have demonstrated that the decrease in serum total T_4 level by PB occurs even in UGT1A-deficient Wistar rats (Gunn rats) (Kato et al., 2005) and, more recently, indicated that decrease in the serum T_4 level in mice, hamsters, rats, and guinea pigs by a commercial PCB mixture Kanechlor-500, which contains PB-type UGT inducers, occurs mainly through an increase in the accumulation level of T_4 in the liver (Kato et al., 2010). To date, however, only limited data to explain the mechanism of the PB-mediated decrease in the level of serum thyroid hormone and its species difference are available.

In the present study, we examined the species differences among mice, hamsters, rats, and guinea pigs in the PB-mediated biological alterations, such as decreases in the levels of serum thyroid hormones, induction of hepatic T_4-UGT, and increase in hepatic accumulation of

Abbreviations: HPLC, high-performance liquid chromatography; ID-I, type-I iodothyronine deiodinase; PB, phenobarbital; PCB, polychlorinated biphenyl; T_3, triiodothyronine; T_4, thyroxine; TBG, thyroxine-binding protein; TSH, thyroid-stimulating hormone; TTR, transthyretin; UGT, UDP-glucuronosyltransferase.

* Corresponding author. Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan. Fax: +81 87 894 01 81.
E-mail address: kato@kph.bunri-u.ac.jp (Y. Kato).

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T_4. On the basis of the obtained results, a mechanism underlying the PB-mediated decrease in serum T_4 level was discussed.

Materials and methods

Chemicals

PB was purchased from Nakakita Yakuhin Co., Ltd. (Aichi, Japan). The [^{125}I]-reverse triiodothyronine (T_3) (greater than 95% radiochemical pure as determined by high-performance liquid chromatography (HPLC), specific activity: 959 μCi/μg T_3) and [^{125}I]T_4 (greater than 95% radiochemical pure as determined by HPLC, specific activity: 150 μCi/μg T_4), radiolabeled at the 5′-position of the outer ring, were obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA). All the other chemicals used were obtained commercially at the highest grade of purity.

Animal treatments

Male ddy mice (30–46 g), Syrian hamsters (85–146 g), Wistar rats (163–235 g), and Hartley guinea pigs (416–701 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). They 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 to 8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1 °C; humidity, 55 ± 5%), and handled with animal care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Animals were received four consecutive intraperitoneal injection of PB (80 mg/kg) dissolved in 0.9% saline (5 ml/kg). Control animals were treated with a vehicle alone (5 ml/kg).

In vivo study

All animals were killed by decapitation 1 day after the final administration of PB or a vehicle alone. The liver was removed, and hepatic microsomes were prepared according to the method of Kato et al. (1995) and stored at −85 °C until use. 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 use.

Analysis of serum hormones. Levels of total T_4, free T_4, total T_3, and TSH were measured by radioimmunoassay using a Total T4 and Free T4 kit (Diagnostic Products Corporation, Los Angeles, CA), T-3 RIABEAD (Dainabot Co., Ltd., Tokyo, Japan), and the rTSH [^{125}I] Biotrak assay system (GE Healthcare, Little Chalfont, Buckinghamshine, UK), respectively.

Hepatic microsomal T_4-metabolizing activity. 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 UGT toward T_4 (T_4-UGT activity) was determined by the methods 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 methods of Kaptein et al. (1997).

Western blot analysis. Western blot analyses for microsomal UGT isoforms in various rodents were performed by the method of Luquita et al. (2001) using polyclonal anti-peptide antibodies against the common region of rat UGT1A isoforms and specific antibodies against UGT1A1, UGT1A6, and UGT2B1 (Ikushiro et al., 1995, 1997). It is well known that there are orthologues of UGT1A isoforms and UGT2B1 in mammal including rats and mice (Mackenzie et al., 2005). These antibodies against rat UGTs were used in the analysis of Western blot for mice, hamsters, and guinea pigs. The separated bands corresponding to UGT1A1, UGT1A6, and UGT2B1 in a nitrocellulose sheet were detected using chemical luminescence (ECL detection kit, GE Healthcare UK, Ltd.), and the level of each UGT protein was determined densitometrically with LAS-1000 (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Ex vivo study

At 1 day after consecutive 4-day treatment with PB, the animals were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP8, SP10 and SP31, Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 U/ml). The bile duct was cannulated, and then the animal's body was warmed to 37 °C. Fifteen minutes later, the animals were given intravenously [^{125}I]T_4 (15 μCi/ml) dissolved in saline containing 10 mM NaOH and 1% normal animal serum. The doses of [^{125}I]T_4 were 0.1 ml for mice, 0.6 ml for hamsters, 1 ml for rats, and 2 ml for guinea pigs, respectively. The doses of [^{125}I]T_4 administered to the animals were calculated on the base of the dose used for rats by Vansell and Klaassen (2001).

Clearance of [^{125}I]T_4 from serum. Clearance of [^{125}I]T_4 from serum was measured according to the method of Oppenheimer et al. (1968). In brief, after the administration of [^{125}I]T_4, a portion (0.1–0.3 ml) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at −50 °C until use. Two aliquots (15 μl each) of each serum were used for determination of the level of [^{125}I]T_4 by a gamma counter (Cobra II Auto-Gamma 5002, Perkin Elmer Life and Analytical Sciences).

Biliary excretions of total [^{125}I]T_4 and [^{125}I]T_4 glucuronide. After the administration of [^{125}I]T_4, bile was collected in glass tube on ice for 2 h at 30-min intervals. Bile volume was determined gravimetrically. For analysis of biliary total [^{125}I]T_4 level, two aliquots (10–30 μl each) of each bile sample were used for determination of [^{125}I]T_4 level by a gamma counter (Cobra II Auto-Gamma 5002). The amount of biliary [^{125}I]T_4 glucuronide was determined with HPLC as described by Vansell and Klaassen (2001). In brief, a portion (10–20 μl) of bile was added to 2 volumes of methanol and kept at −20 °C for 1 h to precipitate protein. After 12,000×*g* centrifugation of the mixture at 4 °C for 10 min, the resultant supernatant was collected for HPLC analysis. The HPLC analysis was performed using a ChromSpher C18 column (10×0.3 cm) (Chrompack, Inc., Raritan, NJ) in combination with both a ChromSep reverse-phase guard column (10×2 mm) (Chrompack, Inc.) and Adsorbosphere C18 reverse-phase guard column (7.5×4.6 mm) (Alltech Associates, Deerfield, IL). Then 0.02 M ammonium acetate, pH4.0, containing 16% to 45% of acetonitrile solution was used for elution of [^{125}I]T_4 glucuronide; 16% of acetonitrile was used as the initial solution for 6 min, and then the concentration of acetonitrile in elution solution was changed by a linear increase to 27% over 12 min, held for 4 min, followed by a linear increase to 45% over 5 min and held for 11 min. The levels of biliary [^{125}I]T_4 glucuronide were determined by a Radioisotope Detector 171 (Beckman Coulter, Fullerton, CA).

To further identify [^{125}I]T_4 glucuronides, the disappearance of a peak responsible for [^{125}I]T_4 glucuronides by treatment with β-glucuronidase was examined. A portion (100 μl) of bile was incubated for 4 h at 37 °C with β-glucuronidase (250 U) in 100 mM phosphate buffer (100 μl, pH 6.8), and the reaction was stopped by addition of 50 μl methanol and cooling on ice. After the reaction mixture was centrifuged at 12,000×*g* and 4 °C for 10 min, the resultant supernatant was collected and used for the HPLC analysis of [^{125}I]T_4 derivatives.

Analysis of [^{125}I]T_4 bound to serum proteins. The levels of serum [^{125}I]T_4-thyroxine-binding protein (TBG), [^{125}I]T_4-albumin, and [^{125}I]T_4-transthyretin ([^{125}I]T_4-TTR) complexes were determined according

to the method of Davis et al. (1970). In brief, serum was diluted in 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and the diluted serum was subjected to electrophoresis on 4% to 20% gradient native polyacrylamide gels (PAG Mid "Daiichi" 4/20, Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The electrophoresis was performed at 4 °C for 11 h at 20 mA in 0.025 M Tris buffer, pH 8.4 containing 0.192 M glycine. The human albumin and TTR incubated with [^{125}I]T_4 were also applied on the gel as references. After the electrophoresis, the gel was dried and autoradiographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd.). The levels of [^{125}I]T_4-TBG, [^{125}I]T_4-albumin, and [^{125}I]T_4-TTR in serum were determined by counting the corresponding gel fractions identified with the Bio Imaging Analyzer (BAS-2000II IP Reader; Fuji Photo Film Co., Ltd.).

Tissue distribution of [^{125}I]T_4. Tissue distribution of [^{125}I]T_4 was assessed according to the modified method of Oppenheimer et al. (1968). In brief, at 5 min after administration of [^{125}I]T_4 to PB-pretreated animals, blood was sampled from the abdominal aorta. Then the cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, and cecum were removed and weighed. Radioactivities in serum and the tissues were determined by a gamma counter (Cobra II Auto-Gamma 5002), and amounts of [^{125}I]T_4 in the tissues were calculated as ratios to the amount in serum.

Statistics

The data obtained were statistically analyzed according to the Student's *t*-test or Dunnett's test after analysis of variance. In addition, clearance of [^{125}I]T_4 from the serum, amounts of biliary total [^{125}I]T_4 and [^{125}I]T_4 glucuronide, and the binding level of [^{125}I]T_4 to serum proteins were statistically analyzed according to the Newman–Keuls test after analysis of variance. The pharmacokinetic parameters of [^{125}I]T_4 were estimated with noncompartmental methods as described previously (Tabata et al., 1999).

Results

Serum hormone levels

We carried out the preliminary experiments of the dose-response (PB: 80, 100, 125, 150 and 200 mg/kg, intraperitoneal, once daily for 4 days) and time-course (PB: 80 mg/kg, intraperitoneal, once daily for 4, 5, 6 and 7 days). On the basis of the results, we determined the suitable dose and time (PB: 80 mg/kg, intraperitoneal, once daily for 4 days). Effects of PB on levels of serum thyroid hormones, total T_4, free T_4, total T_3, and TSH in mice, hamsters, rats, and guinea pigs were examined. Serum total T_4 levels in the PB-treated mice, hamsters, and rats were decreased to 40%, 42%, and 60% of the corresponding controls, respectively. Likewise, serum-free T_4 levels in the PB-treated mice, hamsters, and rats were decreased to 25%, 49%, and 50% of the corresponding controls, respectively (Fig. 1). Serum total T_3 levels in PB-treated hamsters and rats were decreased to 70% and 47% of the corresponding controls, respectively. Significant decrease (69% of control) in serum TSH level by PB occurred in only hamsters among the rodents examined (Fig. 2). In addition, no such decreases in the levels of all the serum hormones examined were observed in guinea pigs.

Hepatic T_4-metabolizing enzyme activities

The effect of PB on hepatic microsomal T_4-UGT activity was examined in mice, hamsters, rats, and guinea pigs. A significant increase in the activity of hepatic T_4-UGT by the treatment with PB (80 mg/kg, intraperitoneal, once daily for 4 days) was observed in all the rodents examined, with the exception of guinea pigs (Fig. 3).

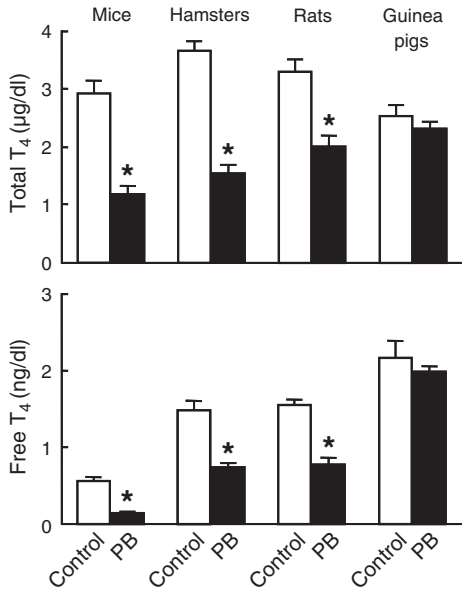

Fig. 1. Effects of PB on the levels of serum total T_4 and free T_4. Levels of serum thyroid hormones in the individual animals treated with PB or vehicle alone (control) were measured using a commercial RIA kit. Each column represents the mean ± SE (vertical bars) for four to seven animals. *P<0.01, significantly different from each control.

Hepatic ID-I activity was significantly decreased by PB in hamsters and rats (Fig. 4). On the other hand, no significant change in the activity of hepatic T_4-sulfotransferase by PB was observed in any species of the animals examined (Fig. 4).

Immunoblot analysis for UGT1As

Levels of immunoreactive proteins responsible for UGT1A isoforms, UGT1A1 and UGT1A6, were increased by PB (80 mg/kg, intraperitoneal, once daily for 4 days) in only rats, but not in other

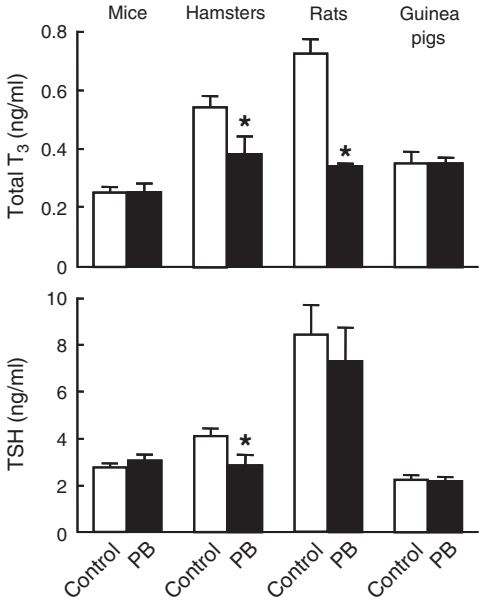

Fig. 2. Effects of PB on the levels of serum total T_3 and TSH. Levels of serum thyroid hormones in the individual animals treated with PB or vehicle alone (control) were measured using a commercial RIA kit. Each column represents the mean ± SE (vertical bars) for four to seven animals. *P<0.05, significantly different from each control.

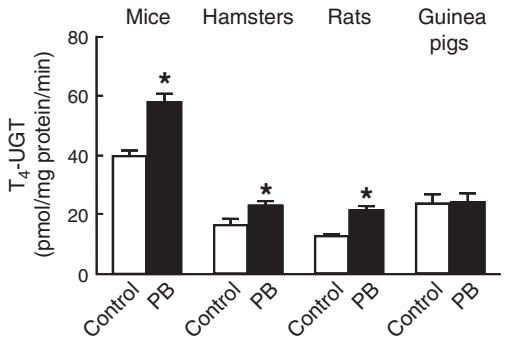

Fig. 3. Effect of PB on hepatic microsomal T_4-UGT activity. T_4-UGT enzyme assays were performed using hepatic microsomes from the individual animals treated with PB or vehicle alone (control). Each column represents the mean ± SE (vertical bars) for five animals. *$P<0.05$, significantly different from each control.

species of the animals examined (Figs. 5 and 6). PB treatment also resulted in significant increases in level of UGT1As in mice, hamsters, and rats, but not in guinea pigs. On the other hand, level of UGT2B1 was significantly increased by PB in all the rodents examined, and magnitude of the increase was higher in hamsters and rats than in mice and guinea pigs (Figs. 5 and 6).

Biliary excretion of [^{125}I]T_4 and [^{125}I]T_4 glucuronide

Effects of PB pretreatment (80 mg/kg, intraperitoneal, once daily for 4 days) on the biliary excretion of T_4 and T_4-glucuronide were examined in mice, hamsters, rats, and guinea pigs. After intravenous injection of [^{125}I]T_4 to the PB-pretreated animals, biliary excretion levels of T_4 and T_4-glucuronide were measured. Significant increases in the excretion levels were observed only in rats among the rodents examined (Figs. 7 and 8).

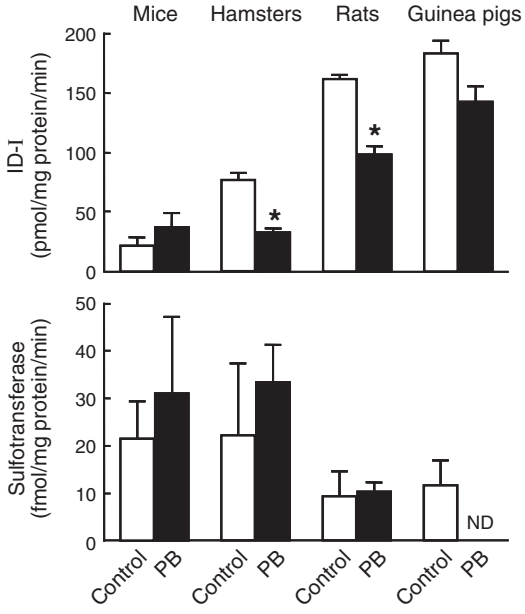

Fig. 4. Effects of PB on the activities of hepatic microsomal ID-I and cytosolic sulfotransferase. The ID-I and sulfotransferase enzyme assays were performed using hepatic microsomes and cytosols from the individual animals treated with PB or vehicle alone (control). Each column represents the mean ± SE (vertical bars) for three to five animals. *$P<0.001$, significantly different from each control. ND, not detectable.

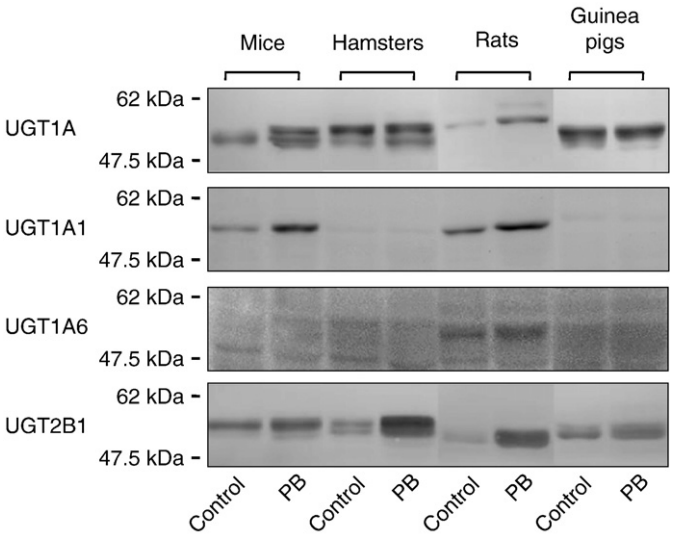

Fig. 5. Representative Western blot profiles for hepatic microsomal UGT isoforms in the PB-treated animals. Western blot analyses were performed using the hepatic microsomes from the individual animals treated with PB or vehicle alone (control), and the separated bands on a nitrocellulose sheet were detected using chemical luminescence (ECL detection kit).

Clearance of [^{125}I]T_4 from serum

After an intravenous administration of [^{125}I]T_4 to the PB (80 mg/kg, intraperitoneal, once daily for 4 days)-pretreated mice, hamsters, rats, and guinea pigs, serum concentrations of [^{125}I]T_4 in the animals were measured at the indicated times (Fig. 9). PB pretreatment clearly enhanced the clearance of [^{125}I]T_4 from the serum in the animals, with the exception of guinea pigs. Within 5 min after the administration of [^{125}I]T_4, concentrations of serum [^{125}I]T_4 in mice, hamsters, and rats were 77%, 80% and 80% of the corresponding control levels, respectively, and the decreases remained up to 120 min later.

The serum pharmacokinetic parameters of the [^{125}I]T_4 estimated from the data in Fig. 9 were summarized in Table 1. The mean total body clearance of [^{125}I]T_4 and steady-state volume of distribution in the PB (80 mg/kg, intraperitoneal, once daily for 4 days)-pretreated mice, hamsters, and rats increased, as compared with the corresponding control animals. The steady-state volumes of distribution in the PB-pretreated mice, hamsters, and rats increased to 1.6, 1.7, and 1.6 times over the corresponding control animals, respectively (Table 1).

Tissue distribution of [^{125}I]T_4

Effects of PB (80 mg/kg, intraperitoneal, once daily for 4 days) pretreatment on the tissue-to-serum concentration ratio (Kp value) and the distribution level of [^{125}I]T_4 in various tissues were examined in mice, hamsters, rats, and guinea pigs. In all the species of control animals examined, the liver, thyroid gland, and kidney, but not other extrahepatic tissues, including the pituitary gland had Kp values over 0.2 (Fig. 10). Pretreatment with PB resulted in significant increases in Kp values of the liver in all the animals examined, with the exception of guinea pigs (Fig. 10). In addition, such significant increases were observed in the thyroid gland of mice and in the kidney of hamsters.

In PB-untreated (control) animals, accumulation level of [^{125}I]T_4 was the highest in the liver among the tissues examined (Fig. 11). In all the animals examined, with the exception of guinea pigs, pretreatment with PB (80 mg/kg, intraperitoneal, once daily for 4 days) resulted in significant increase in the accumulation level of [^{125}I]T_4 in the liver. More than 48%, 39%, and 36% of the [^{125}I]T_4 doses were accumulated in the liver in the PB-pretreated mice, hamsters, and rats, respectively (Fig. 11). In addition, the accumulation level per gram of liver was increased in the PB-pretreated mice, as compared

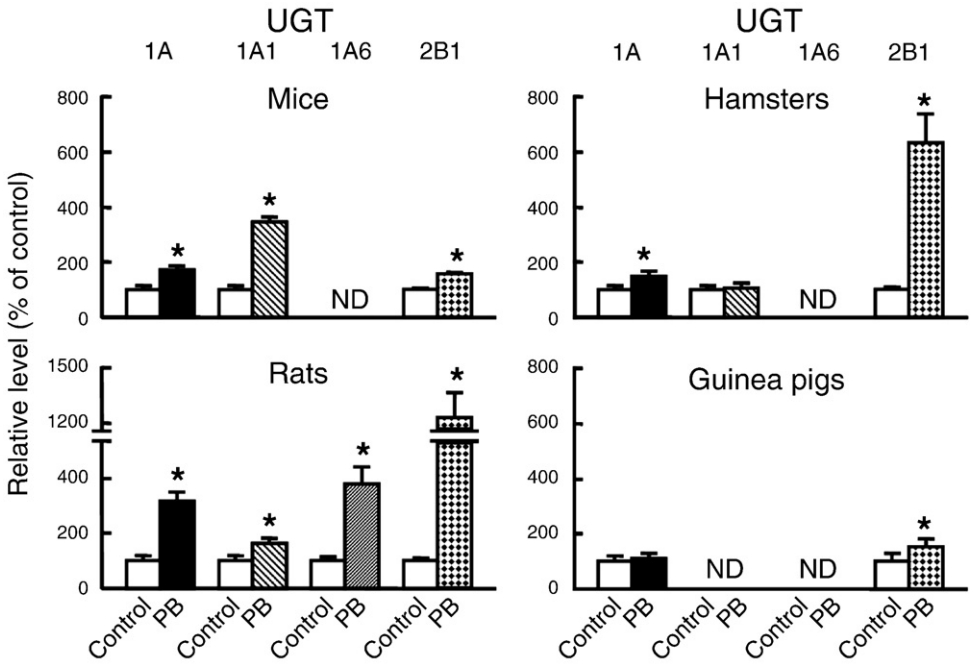

Fig. 6. Effects of PB on the levels of hepatic microsomal UGT isoforms. The separated bands responsible for the UGT isoforms, which are shown in Fig. 5, were densitometrically quantified. The data are represented as the mean ± SE (vertical bars) for four animals. *$P<0.05$, significantly different from each control. ND, not detectable.

with the control animals (Table 2), although PB-mediated increases in the liver weight occurred in rats and hamsters, but in neither mice nor guinea pigs (Table 3). Incidentally, in all the animals examined, no significant increases in the accumulation levels of [^{125}I]T_4 by PB pretreatment were observed in all the extrahepatic tissues examined, with the exception of the thyroid gland in mice.

Serum proteins bound to [^{125}I]T_4

The effects of pretreatment with PB (80 mg/kg, intraperitoneal, once daily for 4 days) on the binding of [^{125}I]T_4 to serum proteins, such as albumin and TTR, were examined in mice, hamsters, rats, and guinea pigs (Fig. 12). No PB-mediated change in the binding level of [^{125}I]T_4 to each serum protein, with the exception of decrease in the level of [^{125}I]T_4-TTR complex 120 min after [^{125}I]T_4 administration to rats, was observed in any species of the animals examined (Fig. 12). No significant change in the binding level of [^{125}I]T_4 to TBG was also observed in PB-pretreated mice (data not shown).

Discussion

In the present study, we demonstrated that in all the animals examined, with the exception of guinea pigs, treatment with PB promoted accumulation of T_4 in several tissues, especially the liver,

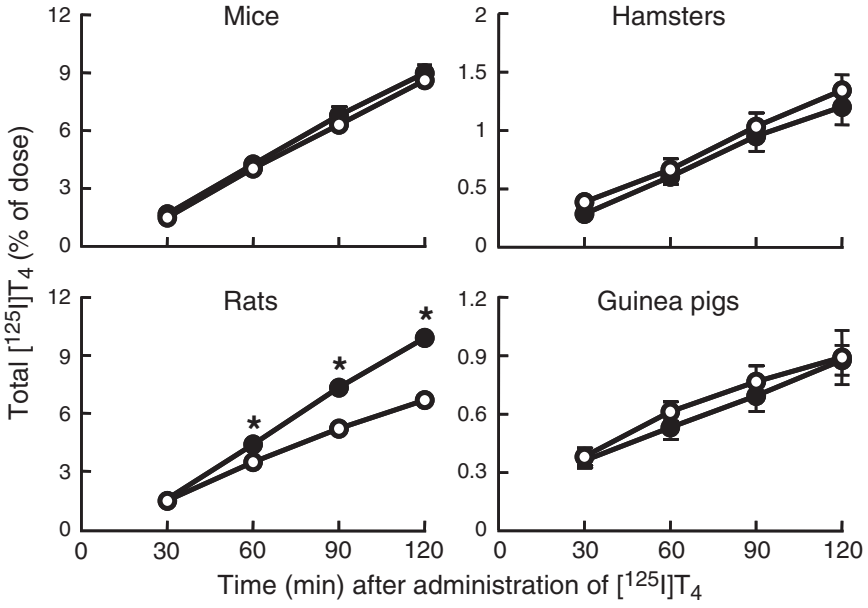

Fig. 7. Effect of PB pretreatment on the amount of biliary total [^{125}I]T_4. A portion of [^{125}I]T_4 (15 μCi/ml) was intravenously administered to the animals pretreated with PB or vehicle alone (control). Bile was collected at 30-min intervals after the intravenous administration of [^{125}I]T_4, and the amount of total [^{125}I]T_4 in a bile was measured, as described in Materials and methods. Each point represents the mean ± SE (vertical bars) for three to five animals. *$P<0.05$, significantly different from each control. —○—, Control; —●—, PB.

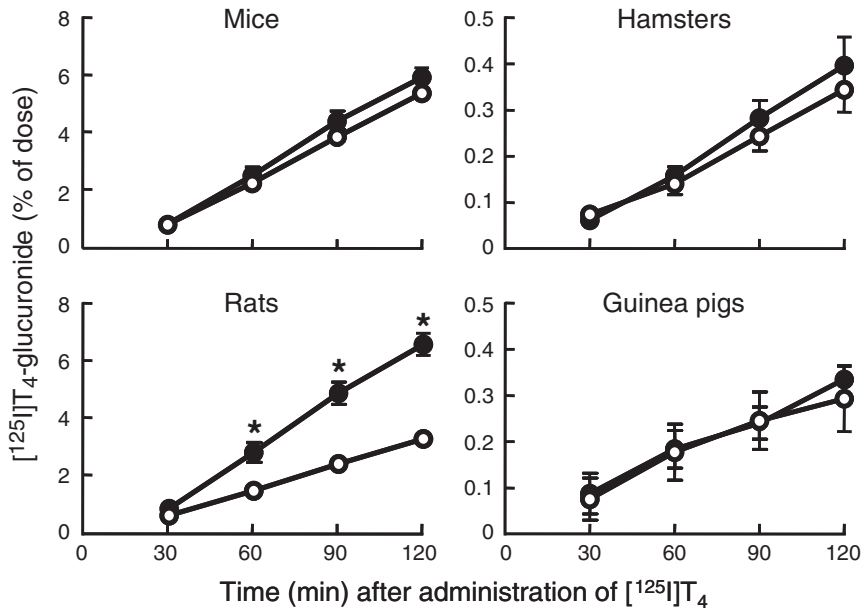

Fig. 8. Effect of PB pretreatment on the amount of biliary [^{125}I]T_4-glucuronide. A portion of [^{125}I]T_4 (15 μCi/ml) was intravenously administered to the animals pretreated with PB or vehicle alone (control). Bile was collected at 30-min intervals after the intravenous administration of [^{125}I]T_4, and the amount of [^{125}I]T_4-glucuronide in a bile was measured as described in Materials and methods. Each point represents the mean ± SE (vertical bars) for three to five animals. *P<0.01, significantly different from each control. —○—, Control; —●—, PB.

and resulted in drastic decreases in the levels of serum total T_4 and free T_4. Thus, species difference in PB-mediated decrease in the serum T_4 level between guinea pigs and other rodents was found for the first time in the present experiments.

As a possible explanation for the PB-induced decrease in serum thyroid hormones, a hepatic T_4-UGT-dependent mechanism is generally considered because T_4-UGT inducers, such as PB, polychlorinated biphenyl (PCB), 3-methylcholanthrene, and pregnenolone-16α-carbonitrile, show strong activities for decreasing the levels of serum total thyroid hormones in rats (Saito et al., 1991; De Sandro et al., 1992; Barter and Klaassen, 1994; Van Birgelen et al., 1995; Capen, 2008). However, between the mice and rats treated with T_4-UGT inducers including PB, difference in the decrease of serum total T_4 level is not necessarily dependent on that in the increase of hepatic T_4-UGT activity (Craft et al., 2002; Hood et al., 2003; Kato et al., 2003; Lecureux et al., 2009). More recently, we have demonstrated that PB treatment resulted in significant decreases in the level of serum total T_4 not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2005) and further indicated that the Kanechlor-500 (a commercial PCB mixture)-mediated decrease occurred through an increase in the accumulation of T_4 in several tissues, especially the liver, rather than through an increase in hepatic T_4-UGT activity in mice, hamsters, rats, and guinea pigs (Kato et al., 2007, 2010). In addition to the previous results, we herein showed

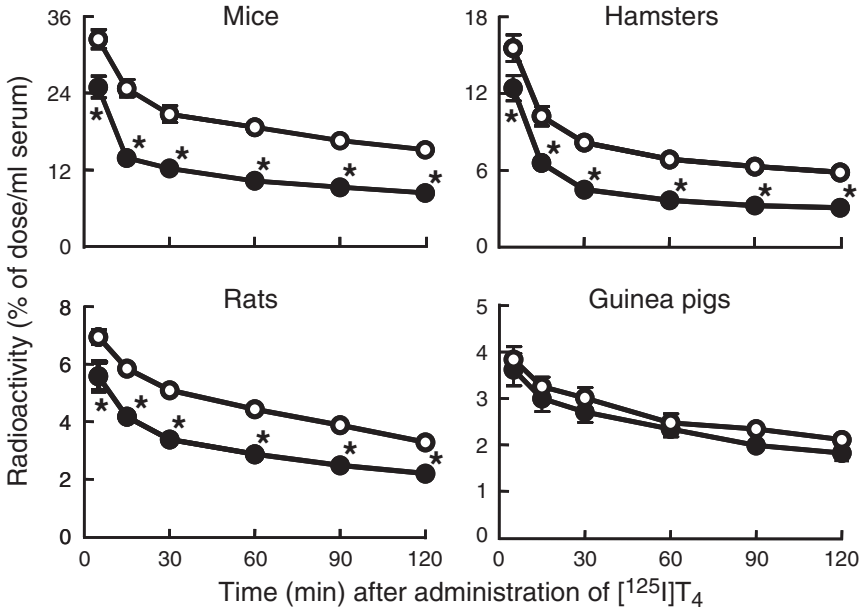

Fig. 9. Effect of PB pretreatment on the clearance of [^{125}I]T_4 from serum. A portion of [^{125}I]T_4 (15 μCi/ml) was intravenously administered to the animal pretreated with PB or vehicle alone (control). The amount of serum [^{125}I]T_4 was measured at the indicated times after the intravenous administration of [^{125}I]T_4. Each point represents the mean ± SE (vertical bars) for three to four animals. *P<0.05, significantly different from each control. —○—, Control; —●—, PB.

Table 1
Pharmacokinetic parameters for [^{125}I]T_4 after the administration of [^{125}I]T_4 to the PB-pretreated animals.

Animal	Pretreatment	Mean total body clearance × 100 (ml/min)	Distribution volume (ml)
Mice	Control	1.35 ± 0.16	4.38 ± 0.38
	PB	2.84 ± 0.19*	6.87 ± 0.66*
Hamsters	Control	3.62 ± 0.59	11.03 ± 0.57
	PB	7.04 ± 0.98*	19.15 ± 1.58*
Rats	Control	7.83 ± 0.61	16.56 ± 0.39
	PB	10.93 ± 0.60*	25.83 ± 1.91*
Guinea pigs	Control	9.71 ± 0.79	32.35 ± 3.45
	PB	13.84 ± 1.87	32.86 ± 1.83

The pharmacokinetic parameters of [^{125}I]T_4 were calculated from the data in Fig. 9 with non-compartmental methods as described previously (Tabata et al., 1999). The values shown expressed as the mean ± SE for three to four animals.
* $P<0.05$, significantly different from each control.

that the PB-mediated increase in the excretion level of biliary T_4-glucuronide was observed only in rats, despite that PB-mediated increases in the level and activity of hepatic T_4-UGT (UGT1As) were observed in mice, hamsters, and rats. Therefore, the PB-induced decrease in the serum T_4 level in mice, hamsters, and rats should be thought to primarily occur in a T_4-UGT-independent manner.

PB treatment led to decrease in the level of serum TSH only in hamsters, but not in any species of animals examined, suggesting that decrease in the TSH level is not a primary incidence leading to the PB-induced decrease in serum T_4 level, although serum TSH is considered as one of the factors regulating the level of serum total T_4 (Capen, 2008). In addition, the previous reports on PB-mediated alteration of serum TSH level have been often conflicting. Some reports (Hood et al., 1999; O'Connor et al., 1999) indicate a significant increase in the serum T_4 level by PB, while in other reports (Klaassen and Hood, 2001; McClain et al., 1989; Kato et al., 2005), no such PB-mediated increase.

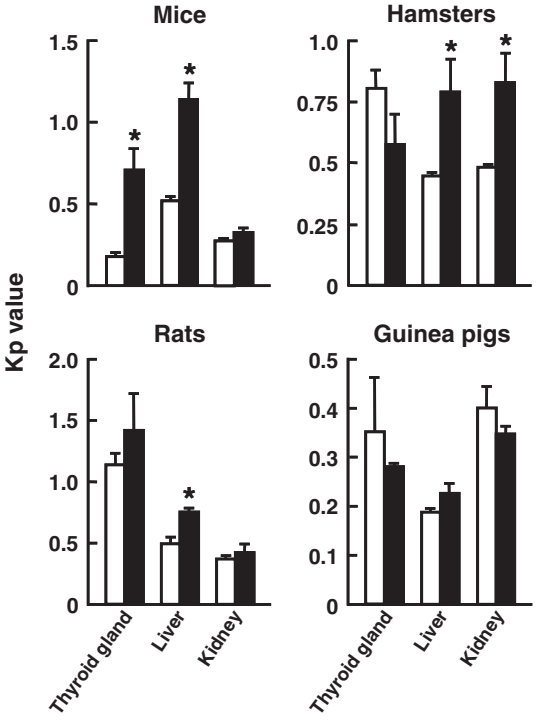

Fig. 10. Effects of PB pretreatment on the tissue-to-serum concentration ratios (Kp values) of [^{125}I]T_4 in various tissues. A portion of [^{125}I]T_4 (15 μCi/ml) was intravenously administered to the animal pretreated with PB or vehicle alone (control), and at 5 min after the [^{125}I]T_4 administration, the radioactivity in each tissue was measured. Each column represents the mean ± SE (vertical bars) for three to five animals. *$P<0.05$, significantly different from each control. □, Control; ■, PB.

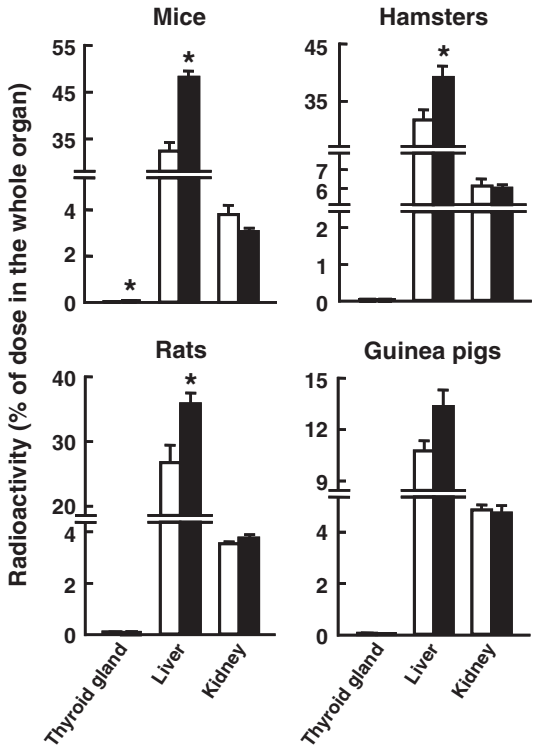

Fig. 11. Effect of PB pretreatment on tissue distribution of [^{125}I]T_4. Experimental protocols were the same as those described in the legend of Fig. 10. Each column represents the mean ± SE (vertical bars) for four to five animals. *$P<0.05$, significantly different from each control. □, Control; ■, PB.

Thus, difference might be attributed to the differences in the dose of PB and its treatment protocol.

Hepatic type-I iodothyronine deiodinase and sulfotransferase are also considered as the factors, which regulate the level of serum total T_4. Significant decrease in hepatic type-I iodothyronine deiodinase activity by PB was observed in hamsters and rats, whereas no significant change occurred in mice and guinea pigs. As for hepatic sulfotransferase activity, no significant change by PB was observed in any species of the animals examined. Therefore, PB-mediated decrease in the serum T_4 level seems to occur in the type-I iodothyronine deiodinase- and T_4-sulfotransferase-independent pathways.

Although it has been reported that the bindings of the PCB and its hydroxylated metabolites to TTR, a major T_4 transporting protein, might be attributed to decrease of the serum T_4 level in PCB-treated rats (Lans et al., 1993; Brouwer et al., 1998; Meerts et al., 2002; Kato et al., 2004), PB-mediated displacement of T_4 from serum TTR did not occur in all the animals examined, with the exception of the slight displacement 120 min after PB treatment in rats. Accordingly, PB-mediated decreases of the serum T_4 level in mice, hamsters, and rats are thought to occur in a TTR-independent pathway.

Table 2
Accumulation of [^{125}I]T_4 in the PB-pretreated mice, hamsters, rats, and guinea pigs livers.

	[^{125}I]T_4 (percentage of dose/g liver)	
Animal	Control	PB
Mice	15.86 ± 1.10	21.00 ± 1.14*
Hamsters	6.59 ± 0.70	6.56 ± 0.80
Rats	3.05 ± 0.27	3.47 ± 0.30
Guinea pigs	0.50 ± 0.04	0.61 ± 0.04

The radioactivity in the liver was measured at 5 min after the [^{125}I]T_4 administration, as described in Materials and methods. The values shown are expressed as the mean ± SE for four to five animals.
* $P<0.05$, significantly different from each control.

Table 3
Liver weights after administration of PB to animals.

Animal	Relative liver weight (percentage of body weight)	
	Control	PB
Mice	5.37 ± 0.31	6.04 ± 0.14
Hamsters	4.18 ± 0.13	5.28 ± 0.17*
Rats	4.32 ± 0.13	4.90 ± 0.19*
Guinea pigs	3.74 ± 0.21	3.80 ± 0.19

Animals were killed 1 day after the administration of PB (80 mg/kg, intraperitoneal, once daily for 4 days), and the liver weight was measured. The values shown are expressed as the mean ± SE for three to five animals.
* $P<0.05$, significantly different from each control.

To clarify the PB-mediated decrease in the serum total T_4 level, we administered [^{125}I]T_4 to the PB-pretreated animals and measured the levels of [^{125}I]T_4 in their various tissues. Marked increases in the mean total body clearance of [^{125}I]T_4 and in the steady-state distribution volume of [^{125}I]T_4 were observed in the PB-pretreated mice, hamsters, and rats. The liver-to-serum concentration ratio (Kp value) was greater in PB-pretreated animals than in the corresponding control animals. In addition, more than 48%, 39%, and 36% of the [^{125}I]T_4 dosed were accumulated in the liver of the PB-pretreated mice, hamsters, and rats, respectively. Furthermore, PB-induced liver hypertrophy was observed in hamsters and rats, but not in mice. On the other hand, a significant increase in the concentration of T_4 (per gram of tissue) in livers by PB pretreatment was observed only in mice, but not in hamsters and rats, indicating species-specific differences in the PB-mediated development of liver hypertrophy and stimulation of the T_4 influx into the liver. Although the species-specific mechanisms for the PB-mediated biological changes remain unclear, the present findings suggest that PB-induced increases in accumulation of hepatic T_4 in hamsters and rats occur mainly through development of liver hypertrophy without a stimulated influx of T_4 into the liver, while the increase in mice occurs through stimulation of the influx.

In conclusion, we demonstrate for the first time that PB-mediated decrease in serum T_4 level occurs in mice, rats, and hamsters, but not in guinea pigs. We further suggest that PB-induced decrease in serum T_4 level occurs through an increase in accumulation (transportation from serum to liver) of T_4 in the liver rather than through the induction of hepatic T_4-UGT and that the PB-induced increase in accumulation of hepatic T_4 occurs mainly through either development of liver hypertrophy (in rats and hamsters) or increase in the influx of T_4 into the liver (in mice). Furthermore, we indicated herein that in contrast to mice, rats, and hamsters, guinea pigs showed no response to PB-mediated biological changes such as decrease in serum T_4 level, development of liver hypertrophy, and increase in accumulation level

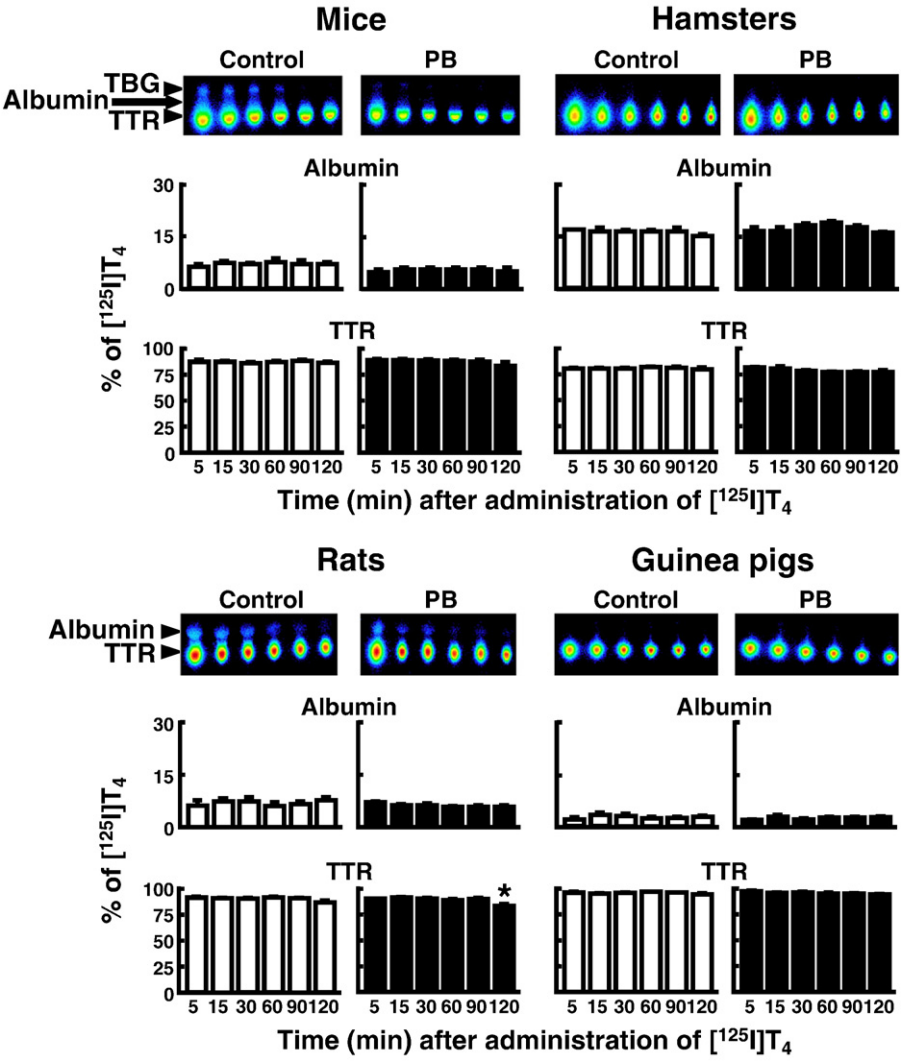

Fig. 12. Effect of PB pretreatment on the binding of [^{125}I]T_4 to serum proteins. A portion of [^{125}I]T_4 (15 μCi/ml) was intravenously administered to the animal pretreated with PB or vehicle alone (control), and the amounts of [^{125}I]T_4 bound to the serum proteins 5 min after [^{125}I]T_4 administration were assessed. Each column represents the mean ± SE (vertical bars) for four to five animals. *$P<0.05$, significantly different from each control.

of T_4 in the liver. The reason why guinea pigs show no response to the PB-mediated biological effects remains unclear, although it was previously reported that constitutive level of the serum free T_4 in guinea pigs is similar to those in rats and humans, while the level of serum total T_4 in guinea pigs is similar to that in rats and lower than that in humans (Castro et al., 1986). Further studies on such species differences are needed.

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Conflict of interest statement

The authors do not have any conflict of interest.

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