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4-Hydroxy-2,2',3,4',5,5',6-Heptachlorobiphenyl (4-OH-CB187)-Mediated Decrease in Serum Thyroxine Level in Mice Occurs through Increase in Accumulation of Thyroxine in the Liver

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Running title: 4-OH-CB187-induced decrease in serum T_4 level

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Abbreviations: 4-OH-CB187, 4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl; PCB, polychlorinated biphenyl; T_4, thyroxine; TTR, transthyretin; TSH, thyroid-stimulating hormone; UDP-GT, UDP-glucuronosyltransferase.

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Abstract

4-Hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl (4-OH-CB187) was selected as a major hydroxylated polychlorinated biphenyl (PCB) metabolite detected from serum of the wildlife as well as humans and examined its effect on level of serum thyroid hormone in mice. At 4 days after treatments of C57BL/6 and DBA/2 mice with 4-OH-CB187 (1.0 mg/kg), the serum total thyroxine (T_4) and free T_4 levels were decreased in both strains of mice. On the other hand, no significant changes in the level and activity of the T_4-UDP-glucuronosyltransferases including UGT1a and UGT1a1 by the 4-OH-CB187-treatment were observed in either strain of mice. No 4-OH-CB187-mediated change in level of serum thyroid-stimulating hormone was observed in either strain of mice. Binding levels of [^{125}I]T_4 to serum proteins after administration of [^{125}I]T_4 were significantly changed in 4-OH-CB187-pretreated mice; decrease in the level of serum [^{125}I]T_4-transthyretin (TTR) complex and increase in the binding level of [^{125}I]T_4 to serum albumin and thyroxine binding protein in the both strains of mice. Clearance from serum of T_4 was promoted by 4-OH-CB187-pretreatment in both C57BL/6 and DBA/2 mice, and the levels of T_4 in several tissues, especially the liver, were increased. In addition, 4-OH-CB187-mediated decreases in serum total T_4 and free T_4 levels were observed in wild-type and TTR-heterozygous mice but not in TTR-deficient mice. The present findings demonstrate that 4-OH-CB187 shows a definite ability to decrease serum T_4 level and further indicate that the 4-OH-CB187-induced decrease would occur through increase in accumulation of T_4 in the liver.

Introduction

A large number of hydroxylated polychlorinated biphenyls (OH-PCBs) have been found in the blood of humans, birds, seals and polar bears (Hovander et al. 2002; Letcher et al. 2000; Sjödin et al. 2000). The concentration of OH-PCBs may exceed 10% of total amount of polychlorinated biphenyls (PCB) in human serum (Fängström et al. 2002; Hovander et al. 2002, 2006; Sandau et al, 2002). As the major OH-PCBs from human serum, 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl (4-OH-CB107), 3-hydroxy-2,2',4,4',5,5'-hexachlorobiphenyl (3-OH-CB153), 4-hydroxy-2,2',3,4',5,5'-hexachlorobiphenyl (4-OH-CB146), 3'-hydroxy-2,2',3,4,4',5'-hexachlorobiphenyl (3'-OH-CB138) and 4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl (4-OH-CB187) are identified. These OH-PCBs, especially 4-OH-CB187 (Fig. 1), show a high binding affinity for serum transthyretin (TTR) (Brouwer et al. 1998; Lans et al. 1993; Ucán-Marín et al. 2009). The binding affinity of 4-OH-CB187 is 5.3-fold-higher than that of endogenous ligand thyroxine (T_4) (Malmberg et al. 2004), strongly suggesting that 4-OH-CB187 would modify the metabolic fate and action of serum thyroid hormone.

Most PCB congeners are known to decrease the levels of serum thyroid hormone in rats (Craft et al. 2002; Van Birgelen et al 1995). As possible mechanisms for the PCB-mediated decrease, the induction of hepatic UDP-glucuronosyltransferases (UDP-GTs), especially UGT1As, responsible for thyroid hormone metabolism and the competitive inhibition on the thyroid hormone-TTR complex formation are considered (Brouwer et al 1998; Barter et al 1994). However, we have recently demonstrated that a consecutive treatment with Kanechlor-500 (KC500) resulted in significant decrease in

level of serum total T_4 not only in Wistar but also in Gunn rats (UGT1A-deficient Wistar rats) and further indicated that the KC500-induced decrease would occur through increase in accumulation (transportation from serum to tissues) of T_4 in several tissues, especially the liver, rather than increase in hepatic T_4-UDP-GT activity (Kato et al 2007).

In the present study, we selected 4-OH-CB187 as a major hydroxylated PCB detected from serum of the wildlife including humans and examined its effect on level of serum thyroid hormone in mice. The present results revealed that 4-OH-CB187 showed a definite ability to decrease serum T_4 level and strongly suggested that its decrease occurred mainly through increase in accumulation of T_4 in the liver.

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Materials and Methods

Chemicals. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan). The [^{125}I]T_4, radiolabelled at the 5'-position of the outer ring, was obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA). 4-OH-CB187 was synthesized by the Cadogan coupling reaction (Cadogan 1962) of 2,4,5-trichloroaniline with 2,3,5,6-tetrachloroanisole and subsequent demethylation of the resulting 4-methoxy-2,2',3,4',5,5',6-heptachlorobiphenyl using boron tribromide. The purity of the compound was >99% when analyzed by gas chromatography.

Animal Treatments. Male C57BL/6 mice (18-31 g) and the DBA/2 mice (18-28 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). TTR-deficient (TTR-/-) mice (15-24 g) were generated by using a homologous recombination method as described previously (Episkopou et al. 1993). Male TTR-heterozygous (TTR+/-) mice were backcrossed to C57BL/6 (wild-type, TTR+/+) female mice for 8 generations. The genotype of each pup was determined on the basis of the presence of the mutant TTR allele by PCR with genomic DNA taken from the tail. Male C57BL/6, DBA/2, TTR-heterozygous (TTR +/-) and TTR-/- mice 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℃, humidity, 55 ± 5%), and handled with animal care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received intraperitoneal injection of 4-OH-CB187 (1.0 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were

treated with vehicle alone (5 ml/kg).

A) *In Vivo* Study. Mice were killed by decapitation 4 days after the administration of 4-OH-CB187. The liver was removed, and hepatic microsomes were prepared according to the method of Kato et al. (1995) and stored at –85℃ 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℃ until use.

Analysis of serum hormones. Levels of total T_4, free T_4, and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using a Total T4 and Free T4 kit (Diagnostic Products Corporation; Los Angels, CA) and the rTSH [^{125}I] Biotrak assay system (GE Healthcare UK, Ltd., Little Chalfont, Buckinghamshine, UK), respectively.

Hepatic microsomal T_4-UDP-GT activity. The amount of hepatic microsomal protein was 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 (T_4-UDP-GT activity) was determined by the methods of Barter and Klaassen (1992).

Western blot analysis. The polyclonal anti-peptide antibodies against the common region of rat UGT1A isoforms and specific antibodies against rat UGT1A1 and UGT2B1, which were established by Ikushiro et al. (1995, 1997), were used. Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita et al. (2001). The bands of mouse UGT1a1 and UGT2b1, which correspond to rat UGT1A1 and UGT2B1, respectively, were detected using chemical luminescence (ECL

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detection kit, GE Healthcare UK, Ltd), and the level of each protein band was determined densitometrically with LAS-1000 (Fuji Photo Film. Co., Ltd., Tokyo, Japan).

B) *Ex Vivo* Study. At 4 days after treatment with 4-OH-CB187, the mice 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, Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml), and then animal's body was warmed to 37℃. Fifteen minutes later, the mice were given i.v. 0.1 ml of [^{125}I]T_4 (15 μCi /ml) dissolved in the saline containing 10 mM NaOH and 1 % normal mouse serum.

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 (0.08 ml) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at -50℃ until use. Two aliquots (15 μl each) of each serum were used for determination of [^{125}I]T_4 level by a γ-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences).

Biliary excretion of [^{125}I]T_4 glucuronide. Amount of biliary [^{125}I]T_4 glucuronide were determined with HPLC as described of Vansell and Klaassen (2001). In brief, after the administration of [^{125}I]T_4, bile was collected on ice for 2 h at 30 min intervals.

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Bile volume was determined gravimetrically. Two aliquots (10 μl each) were taken from each bile sample for determination of [^{125}I]T_4 level by a γ-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences). A portion (10 μl) of bile was added to 2 volume methanol and kept at -20℃ for 1 h to precipitate protein. After the mixture was centrifuged at 12,000 g (4℃) 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 an Adsorbosphere C18 reverse-phase guard column (7.5×4.6 mm) (Alltech Associates, Inc., Deerfield, IL). 0.02 mM ammonium acetate (pH 4.0) containing 16~45% of acetonitrile solution was used for elution of [^{125}I]T_4 glucuronide; 16% of acetonitrile was used as a initial solution for 6 min, and then the 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 Radioisotope Detector 171 (Beckman Coulter, Inc. CA USA).

To identify [^{125}I]T_4 glucuronides, 100 μl of bile was incubated for 4 h at 37℃ with β-glucuronidase (250 units) in 100 mM phosphate buffer (100 μl, pH6.8), and the reaction was stopped by addition 50 μl of methanol and cooling on ice. After the reaction mixture was centrifuged at 12,000 g (4℃) for 10 min, the resultant supernatant was collected for HPLC analysis. [^{125}I]T_4 glucuronide in bile was confirmed by the disappearance of a peak responsible for [^{125}I]T_4 glucuronides by treatment with β-glucuronidase.

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Analysis of [^{125}I]T_4 bound to serum proteins. The levels of serum [^{125}I]T_4-thyroxine binding globulin (TBG), [^{125}I]T_4-albumin, and [^{125}I]T_4-TTR complexes were determined according to the method of Davis et al. (1970). In brief, serum was diluted with 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 - 20% gradient native polyacrylamide gels PAG Mid "Daiichi" 4/20 Daiichi Pure Chemicals Co., Ltd, Tokyo, Japan). The electrophoresis was performed at 4℃ for 11 h at 20 mA in the 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 a gel as templates. After the electrophoresis, a gel was dried and radioautographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd, Japan). 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 Bio Imaging Analyzer (BAS-2000II IP Reader, Fuji Photo Film Co., Ltd, Japan).

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 4-OH-CB187-pretreated mice, blood was sampled from abdominal aorta. Then, cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adreanal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, caecum, brown fat, skeletal muscle, bone marrow skin, spinal cord, and fat were removed and weighted. Radioactivities in serum and the tissues were determined by a γ-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical

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Sciences), and amounts of [^{125}I]T_4 in the tissues were shown as ratios to the amount in serum.

Statistics. The data obtained were statistically analyzed according to Student's *t* test or Dunnett's test after analysis of variance. In addition, clearance of [^{125}I]T_4 from serum, amount of biliary [^{125}I]T_4 glucuronide, and the binding level of [^{125}I]T_4 to serum proteins were statistically analyzed according to 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).

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Results

Serum Hormone Levels. The effect of 4-OH-CB187 on the level of serum thyroid hormone was examined in dioxin-sensitive C57BL/6 mice and dioxin-resistant DBA/2 mice (Fig. 2). In both C57BL/6 and DBA/2 mice, 4-OH-CB187-treatment resulted in significant decreases in the levels of serum total T_4 and free T_4. On the other hand, no significant change in TSH level was observed in either strain of mice.

To further clarify whether the 4-OH-CB187-mediated decrease in serum total T_4 level is dependent on the inhibition of a thyroid hormone-TTR complex formation, we examined effects of 4-OH-CB187 on the level of serum thyroid hormone in TTR-heterozygous and TTR-deficient mice (Fig. 3). 4-OH-CB187-mediated decreases in serum total T_4 and free T_4 levels were observed in the wild-type and TTR-heterozygous mice but not in TTR-deficient mice.

In addition, constitutive levels of serum total T_4 and free T_4 were 33-43% lower in TTR-heterozygous (TTR+/-) mice and 74-75% lower in TTR-deficient (TTR-/-) mice, as compared with that in wild-type (TTR+/+) mice.

Hepatic T_4-UDP-GT Enzymes. The effect of 4-OH-CB187 on hepatic microsomal activity of T_4-UDP-GTs was examined in C57BL/6 and DBA/2 mice. Treatment with 4-OH-CB187 resulted in no significant change in hepatic T_4-UDP-GT activity in either strain of mice (Fig. 4).

Levels of the proteins responsible for T_4-UDP-GT enzymes such as UGT1a, UGT1a1, and UGT2b1 were determined by Western blot analysis. No significant changes in the protein level of hepatic UGT1a, UGT1a1, and UGT2b1 after 4-OH-CB187-treatment

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were observed in either C57BL/6 or DBA/2 mice (Fig. 5).

Biliary Excretion of [^{125}I]T_4 Glucuronide. Effect of 4-OH-CB187 on the biliary excretion of [^{125}I]T_4-glucuronide was examined in C57BL/6 and DBA/2 mice. No significant change in the amount of biliary [^{125}I]T_4-glucuronide after 4-OH-CB187 pretreatment in either strain of mice (Fig. 6).

Serum Proteins Bound to [^{125}I]T_4. Effects of 4-OH-CB187 on the binding of [^{125}I]T_4 to serum proteins, such as TTR, albumin, and TBG, were examined in C57BL/6 and DBA/2 mice (Figs. 7 and 8). In both strains of mice, pretreatment with 4-OH-CB187 resulted in a significant decrease in the level of [^{125}I]T_4-TTR complex. On the contrary, the pretreatment led to significant increases in the levels of [^{125}I]T_4 bound to TBG and albumin.

Clearance of [^{125}I]T_4 from Serum After an i.v. administration of [^{125}I]T_4 to the 4-OH-CB187-pretreated C57BL/6 and DBA/2 mice, concentrations of [^{125}I]T_4 in the serum were measured at the indicated times (Fig. 9). In both C57BL/6 and DBA/2 mice, pretreatment with 4-OH-CB187 promoted the clearance of [^{125}I]T_4 from serum. Their serum [^{125}I]T_4 levels were decreased by about 35% of the corresponding control levels within 5 min, and the decreases remained up to 120 min later. The serum pharmacokinetic parameters of the [^{125}I]T_4 estimated from these data (Fig. 9) were summarized in Table 1. The mean total body clearances (Cl_{tb}) of [^{125}I]T_4 in the 4-OH-CB187-pretreated C57BL/6 and DBA/2 mice increased to about 1.5-times, as compared with the corresponding control mice. The steady-state volumes of

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distribution (Vd_{ss}) in the 4-OH-CB187-pretreated mice also increased to 1.5-times, as compared with the corresponding control mice.

Tissue Distribution of [^{125}I]T_4. Effects of 4-OH-CB187-pretretment on the tissue-to-serum concentration ratio (Kp value) and distribution level of [^{125}I]T_4 in a tissue after the administration of [^{125}I]T_4 were examined using C57BL/6 and DBA/2 mice. The Kp values in the thyroid gland and liver were the greatest in either strain of control (4-OH-CB187-untreated) mice (Fig. 10). Pretreatment with 4-OH-CB187 resulted in significant increase in the Kp values in the tissues including thyroid gland and liver in either strain of mice (Fig. 10) .

Accumulation level of [^{125}I]T_4 was the highest in the liver among the tissues examined in either strain of mice (Fig. 11). In both strains of mice, pretreatment with 4-OH-CB187 resulted in significant increase in the accumulation in tissues, especially the liver, and the level in the liver achieved to more than 40% of the [^{125}I]T_4 dosed (Fig. 11). In addition, the accumulation level per g liver was also increased in 4-OH-CB187-pretreated mice (Table 2). On the other hand, no significant change in the liver weight after 4-OH-CB187-pretreatment was observed in either strain of mice (Table 3).

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Discussion

In the present study, we first demonstrate that the treatment with 4-OH-CB187 (a single i.p. administration at a dose of 1.0 mg/kg) promoted accumulation of T_4 in several tissues, especially the liver, and resulted in a drastic decrease in the levels of serum total T_4 and free T_4 in both C57BL/6 and DBA/2 mice.

As a possible explanation for a chemical-induced decrease in serum thyroid hormones, a hepatic T_4-UDP-GT-dependent mechanism is generally considered, because T_4-UDP-GT inducers, including PCB, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), phenobarbital, 3-methylcholanthrene, pregnenolone-16α-carbonitrile and clobazam, show strong activities for decreasing level of serum total thyroid hormones, including T_4 and T_3 (Barter and Klaassen, 1994; Miyawaki et al., 2003; Van Birgelen et al., 1995). However, among experimental animals treated with T_4-UDP-GT inducers, difference in magnitude of decrease in the level of serum total T_4 is not necessarily correlated with that of increase in hepatic T_4-UDP-GT activity (Craft et al., 2002, Hood et al., 2003, Kato et al., 2003). Furthermore, our previous studies (Kato et al., 2004; 2005, 2007) using Wistar and Gunn rats supported a hypothesis that decreases in the level of serum total thyroid hormones by PCB and phenobarbital occur primarily in a hepatic T_4-UDP-GT-independent pathway.

In the present study, we demonstrated that the level and activity of hepatic T_4-UDP-GTs, especially the UGT1a and UGT1a1 responsible for glucuronidation of T_4, were little changed by 4-OH-CB187-treatment in both C57BL/6 and DBA/2 mice, although serum total T_4 level was markedly decreased in both strains of mice by the treatment. This indicates that 4-OH-CB187-induced decrease in serum T_4 level occurs in a

T_4-UDP-GT-independent manner.

Furthermore, 4-OH-CB187-treatment led to no significant change in the level of serum TSH in either strain of mice, although serum TSH is considered as one of the factors regulating the level of serum total T_4. No significant change in the level of serum TSH in PCB-treated rats has also been reported (Hallgren et al., 2001; Hood et al., 1999; Liu et al., 1995, Kato et al., 2004).

As another possible mechanism for the 4-OH-CB187-induced decrease in level of serum total T_4, TTR-associated pathway might be considered, because PCB and its hydroxylated metabolites act as T_4 antagonists to TTR (Brouwer et al., 1998; Lans et al., 1993; Meerts et al., 2002; Kato et al., 2004) and because serum TTR level is closely correlated with serum thyroid hormone level (Episkopou *et al.,* 1993). Accordingly, competitive inhibition of a T_4-TTR complex formation by 4-OH-CB187 is considered to promote a decrease in the level of serum total T_4. In the present study, both the decrease in the level of [^{125}I]T_4 bound to serum TTR and the increase in the level of [^{125}I]T_4 bound to serum albumin and TBG were confirmed in 4-OH-CB187-pretreated mice. Furthermore, 4-OH-CB187-mediated decrease in serum total T_4 and free T_4 levels occurred in wild-type and TTR-heterozygous mice but not in TTR-deficient mice. These findings indicate that 4-OH-CB187 inhibits formation of serum T_4-TTR complex and further suggest that 4-OH-CB187-induced inhibition of the T_4-TTR complex formation might lead to change in tissue distribution of T_4.

Since distribution levels of [^{125}I]T_4 to plasma and tissues after a [^{125}I]T_4-treatment is not significantly changed up to 48 hr later (Oppenheimer et al., 1968), distribution levels of [^{125}I]T_4 in several tissues were examined at 5 min after the [^{125}I]T_4-administration to 4-OH-CB187-pretreated mice. The results indicated that the mean total body

clearance of [^{125}I]T_4 and the distribution volume of [^{125}I]T_4 to tissues were increased by 4-OH-CB187-pretreatment in both C57BL/6 and DBA/2 mice. A tissue-to-serum concentration ratio (Kp value) also increased in several tissues, especially the liver, in the 4-OH-CB187-pretreated C57BL/6 and DBA/2 mice, as compared with the corresponding control mice. In addition, more than 40% of the [^{125}I]T_4 dosed was accumulated in the liver in 4-OH-CB187-pretreated mice.

In conclusion, the present findings demonstrate that 4-OH-CB187 possesses the ability to reduce serum thyroid hormone level in mice and further indicate that the 4-OH-CB187-mediated decrease occurs mainly through increase in accumulation (transportation from serum to liver) of T_4 in the liver. Furthermore, the present findings strongly suggest that the increased accumulation in the liver would be attributed to the 4-OH-CB187-mediated inhibition of a T_4-TTR complex formation in serum.

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Footnotes

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Legends for figures

Fig. 1. Chemical structure of 4-OH-CB187

Fig. 2. Effects of 4-OH-CB187 on levels of serum total T_4, free T_4, and TSH in mice. Animals were killed 4 days after the administration of 4-OH-CB187 (1.0 mg/kg), and levels of serum thyroid hormones were measured, as described in *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for four to six animals. **P*<0.01, significantly different from each control.

Fig. 3. Effects of 4-OH-CB187 on levels of serum total T_4, free T_4, and TSH in TTR-heterozygous and TTR-deficient mice. Animals were killed 4 days after the administration of 4-OH-CB187 (1.0 mg/kg), and levels of serum thyroid hormones were measured as described in *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for three to six animals. **P*<0.01, significantly different from corresponding control. †*P*<0.05, significantly different from control of TTR+/+ mice. ‡*P*<0.05, significantly different from control of TTR+/- mice.

Fig. 4. Effect of 4-OH-CB187 on the activity of hepatic microsomal T_4-UDP-GT in mice. Hepatic microsomes from individual animals were used for T_4-UDP-GT enzyme assay, as described in *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for four to seven animals. **P*<0.05, significantly different from each control.

Fig. 5. Representive Western blot profiles for hepatic microsomal UGT isoforms in the 4-OH-CB187-treated mice. Hepatic microsomes from individual animals were used for Western blot analysis, as described in *Materials and Methods*.

Fig. 6. Effect of 4-OH-CB187 on amount of the biliary [^{125}I]T_4-glucuronide in mice. The level of [^{125}I]T_4-glucuronide excreted was measured in bile collected at 30 min intervals after the i.v. administration of [^{125}I]T_4. Each point represents the mean ± S.E. (vertical bars) for five to eight mice. **P*<0.01, significantly different from each control. —○— , control; —●— , 4-OH-CB187.

Fig. 7. Effect of 4-OH-CB187 on the binding of [^{125}I]T_4 to serum proteins in C57BL/6 mice. The amounts of [^{125}I]T_4 bound to the serum proteins 5 min after [^{125}I]T_4-administration were assessed by the method as described in *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for four to five animals. **P*<0.001, significantly different from each control.

Fig. 8. Effect of 4-OH-CB187 on the binding of [^{125}I]T_4 to serum proteins in DBA/2 mice. Experimental protocols were the same as those described in the legend of Fig. 7. Each column represents the mean ± S.E. (vertical bars) for five to seven animals. **P*<0.001, significantly different from each control.

Fig. 9. Effects of 4-OH-CB187 on the clearance of [^{125}I]T_4 from serum in mice. The amount of serum [^{125}I]T_4 was measured at the indicated times after the i.v. administration of [^{125}I]T_4. Each point represents the mean ± S.E. (vertical bars) for

five to eight mice. *P<0.05, significantly different from each control. —○— , control; —●— , 4-OH-CB187.

Fig. 10. Tissue-to-serum concentration ratio (Kp value) of [^{125}I]T_4 in various tissues after administration of [^{125}I]T_4 to 4-OH-CB187-pretreated mice. 4-OH-CB187 (1.0 mg/kg) was given to mice, and 96 hr after the 4-OH-CB187-treatment, [^{125}I]T_4 were further administered to the mice. At 5 min after the [^{125}I]T_4-administration, the radioactivity in each tissue was measured, as described in *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for five to seven animals. *P<0.05, significantly different from each control. ▭, control; ▨, 4-OH-CB187.

Fig. 11. Tissue distribution of [^{125}I]T_4 after administration of [^{125}I]T_4 to 4-OH-CB187-pretreated mice. Experimental protocols were the same as those described in the legend of Fig. 10. Each column represents the mean ± S.E. (vertical bars) for five to seven animals. *P<0.05, significantly different from each control. ▭, control; ▨, 4-OH-CB187.

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Table 1. *Pharmacokinetic parameters for [^{125}I]T_4 after the administration of [^{125}I]T_4 to the 4-OH-CB187-pretreated mice*

Animal	Pretreatment	Mean total body clearance × 100 (ml/min)	Distribution volume (ml)
C57BL/6	None (control)	1.48 ± 0.08	2.90 ± 0.17
	4-OH-CB187	2.23 ± 0.15*	4.48 ± 0.24*
DBA/2	None (control)	1.24 ± 0.02	2.47 ± 0.06
	4-OH-CB187	2.01 ± 0.12*	3.76 ± 0.24*

The data shown was calculated from the data in Fig. 9. The values shown are expressed as the mean ± S.E. for five to eight mice. *P<0.05, significantly different from each control.

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Table 2. *Accumulation of [^{125}I]T_4 in the 4-OH-CB187-pretreated mice livers*

	[^{125}I]T_4 (% of dose/g liver)	
Animal	Control	4-OH-CB187
C57BL/6	27.13 ± 1.06	35.14 ± 1.19*
DBA/2	25.25 ± 1.02	31.29 ± 1.25*

The radioactivity in the liver was measured at 5 min after the [^{125}I]T_4-administration. The values shown are expressed as the mean ± S.E. for five to seven mice. *P<0.01, significantly different from each control.

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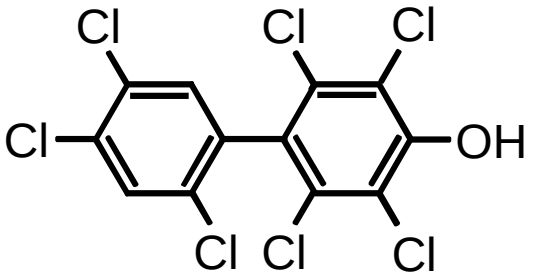
Table 3. *Liver weights after the administration of 4-OH-CB187 to mice*

	Liver weight (% of body weight)	
Animal	Control	4-OH-CB187
C57BL/6	4.63 ± 0.29	4.71 ± 0.09
DBA/2	4.90 ± 0.17	4.92 ± 0.12

Animals were killed 4 days after the administration of 4-OH-CB187 (1.0 mg/kg), and the liver weight was measured. The values shown are expressed as the mean ± S.E. for five to eight animals. **P*<0.001, significantly different from each control.

Figure 1



4-OH-CB187

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Figure 2

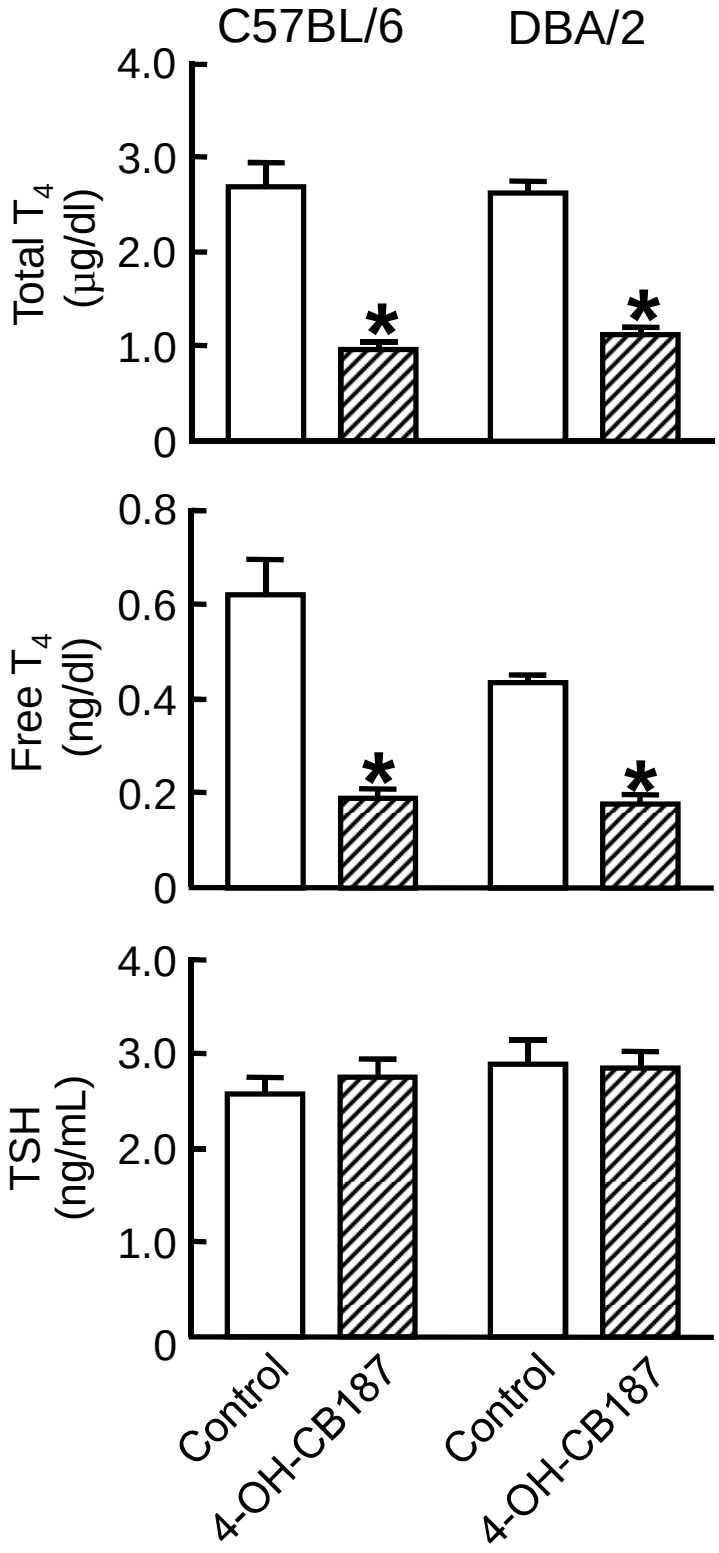

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Figure 3

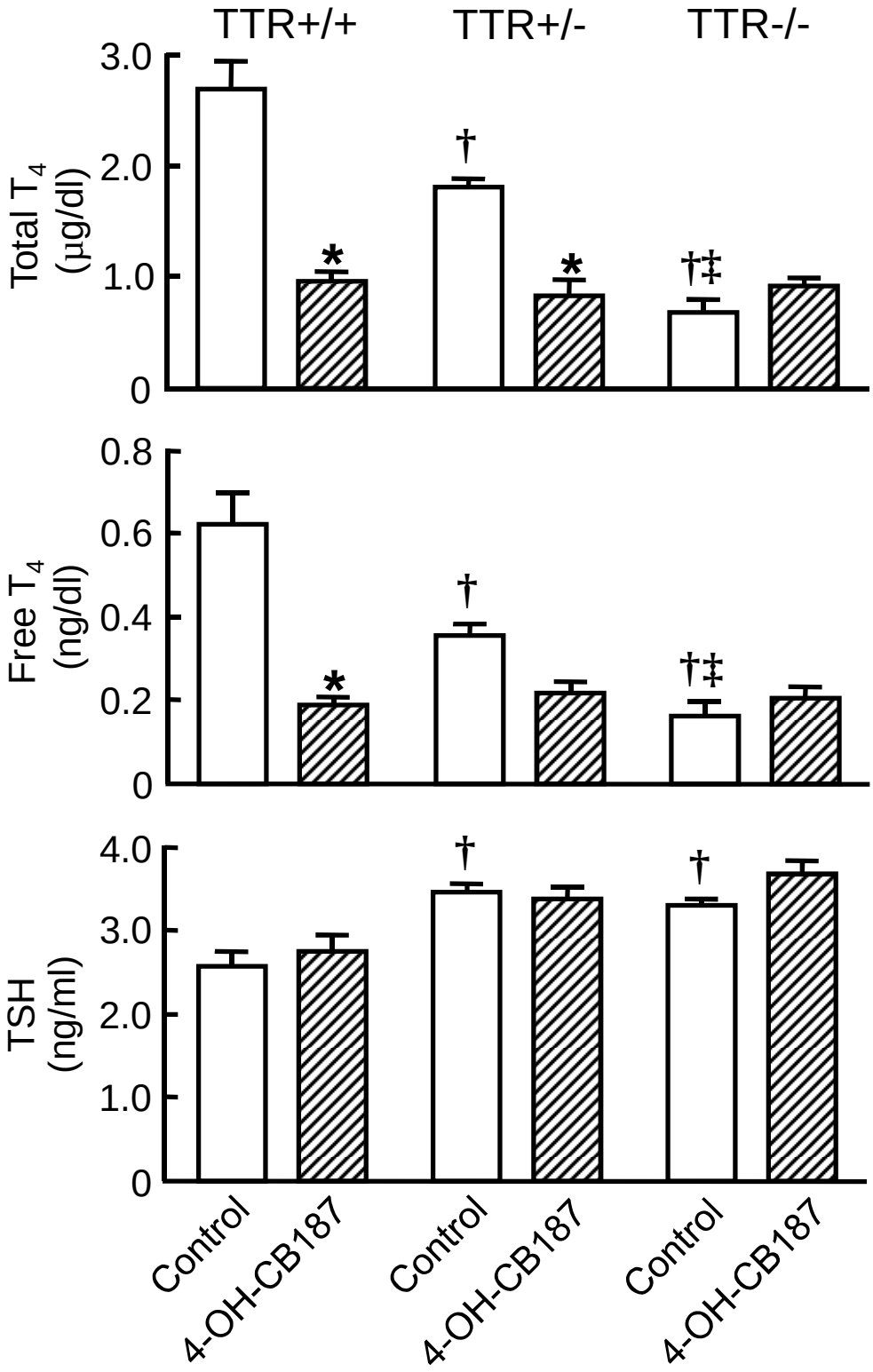

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Figure 4

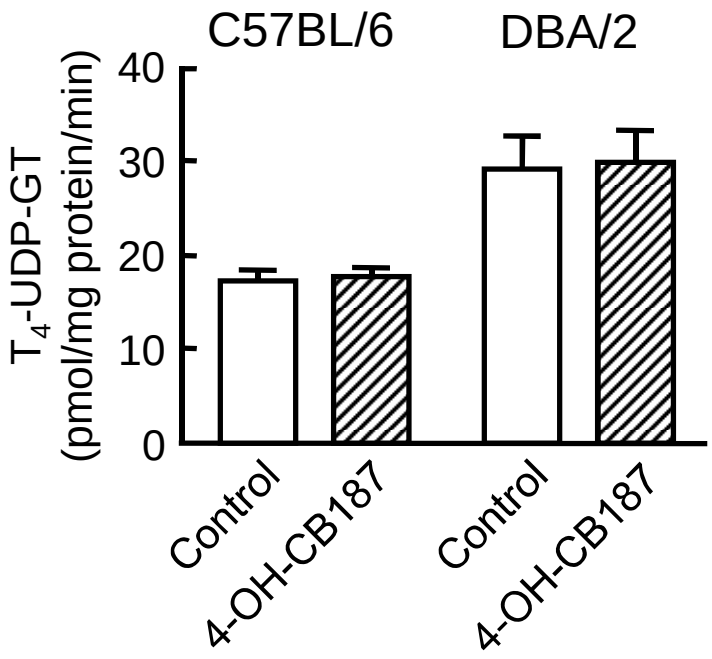

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Figure 5

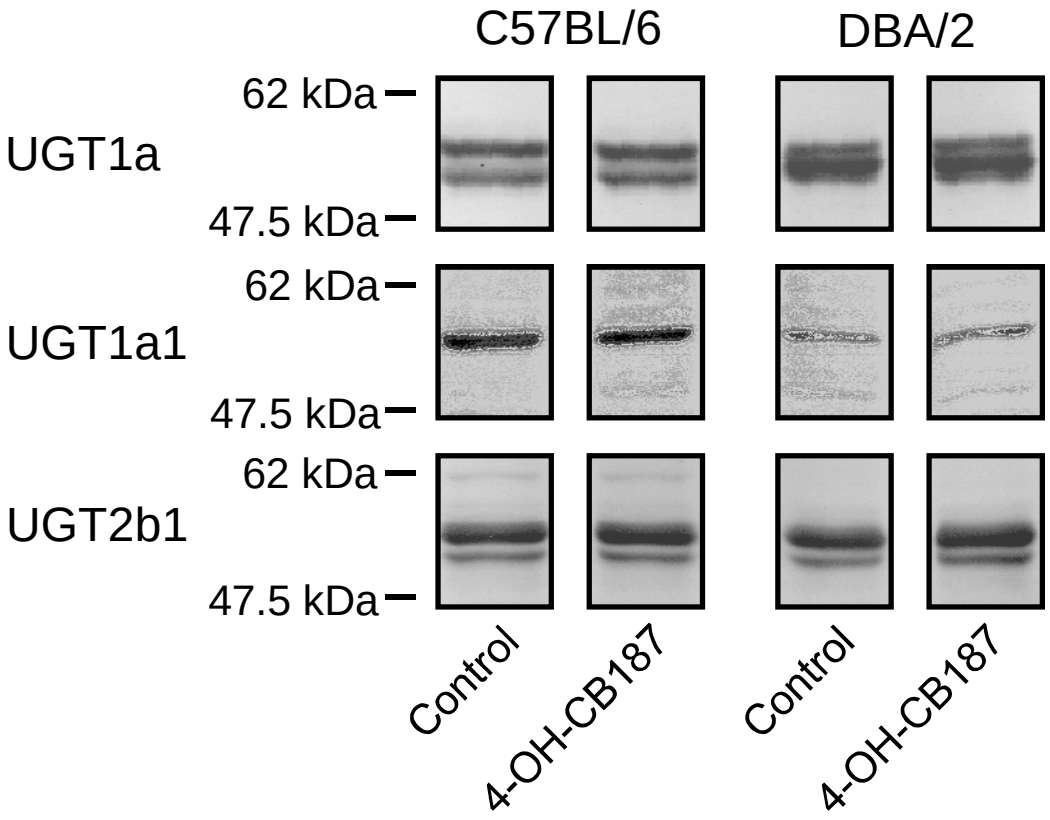

Figure 6

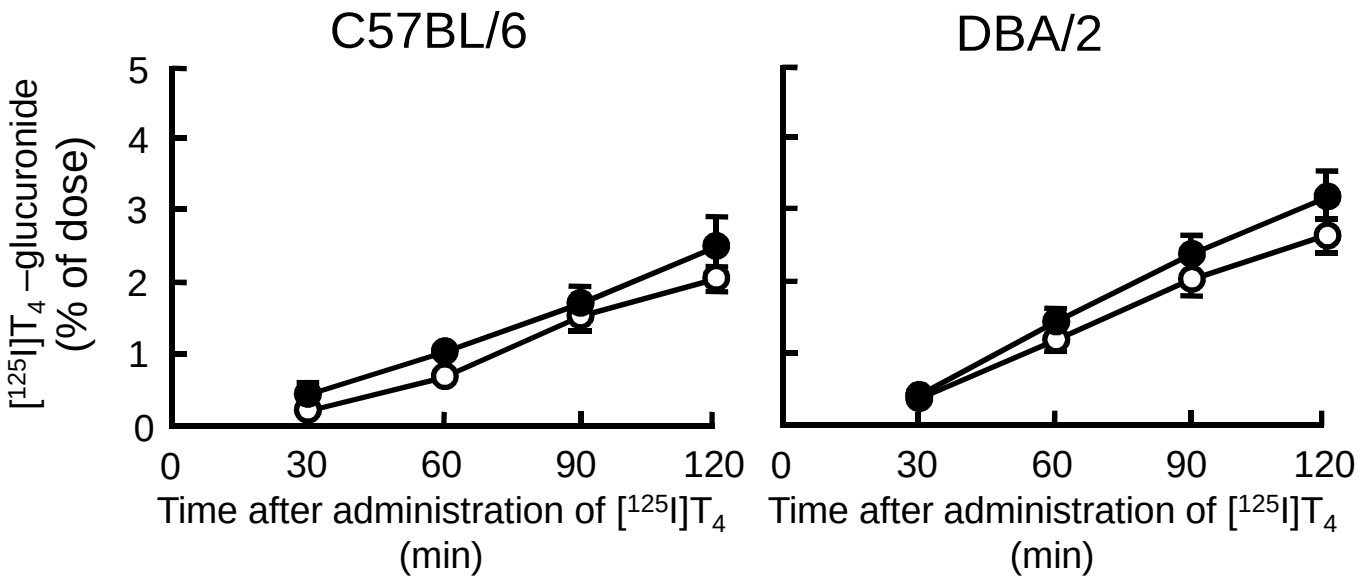

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Figure 7

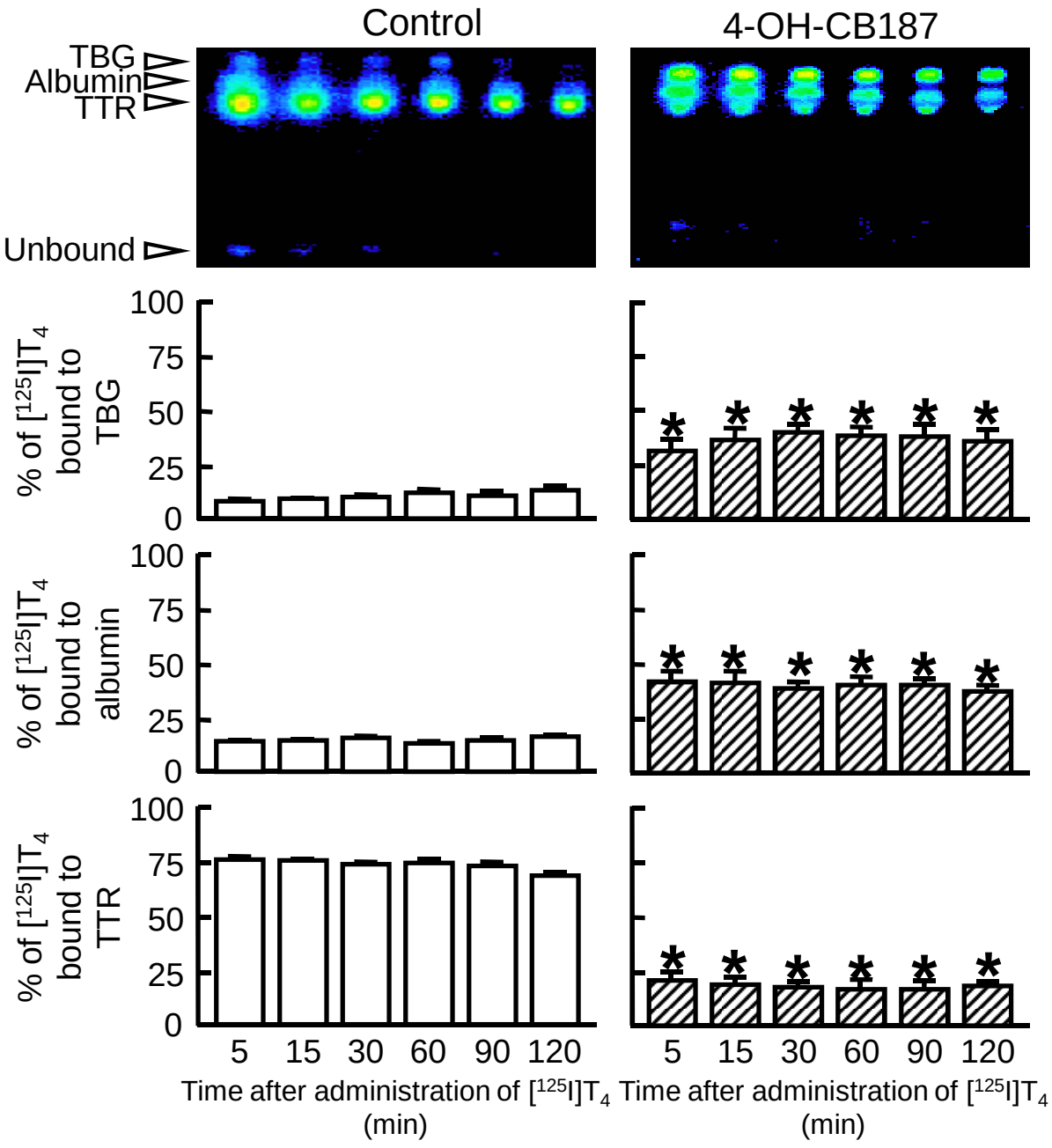

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Figure 8

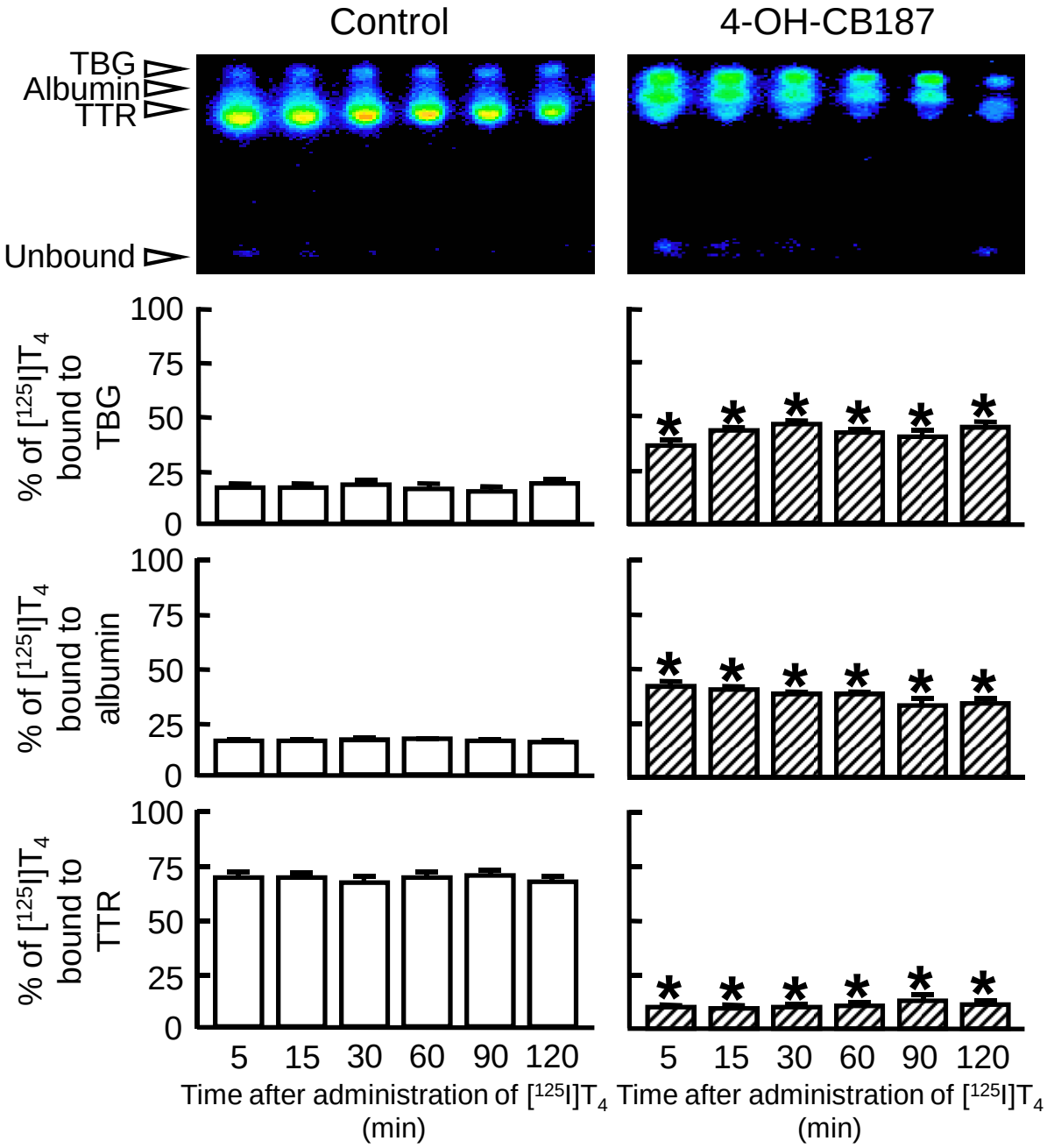

Figure 9

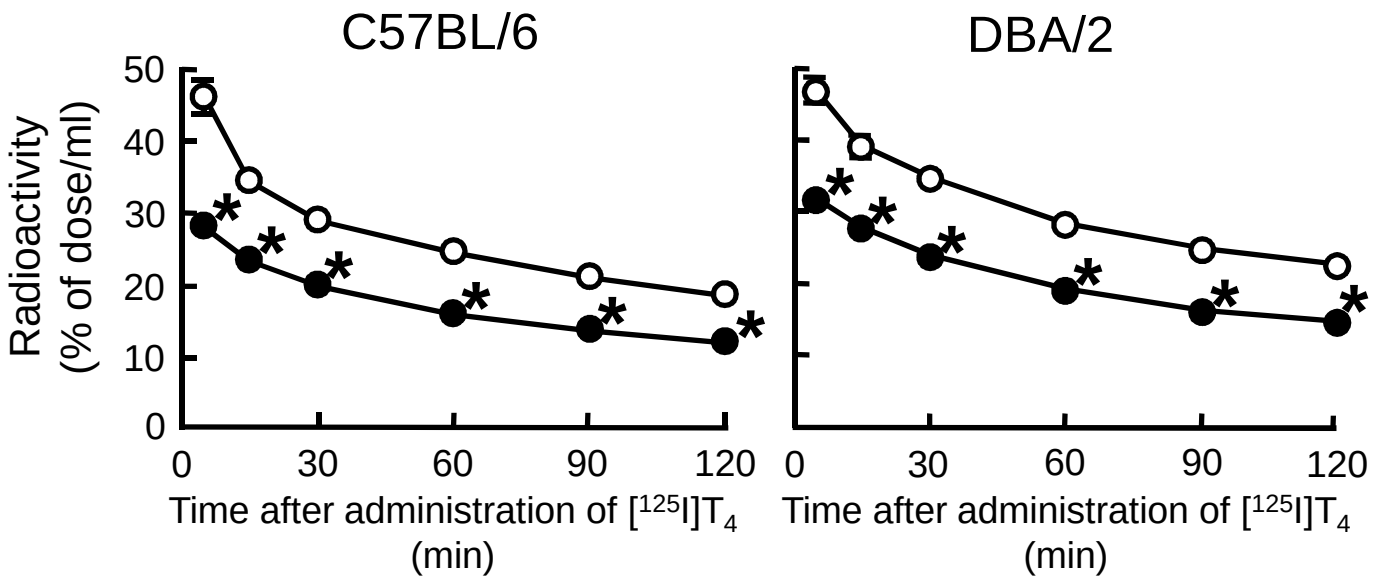

Figure 10

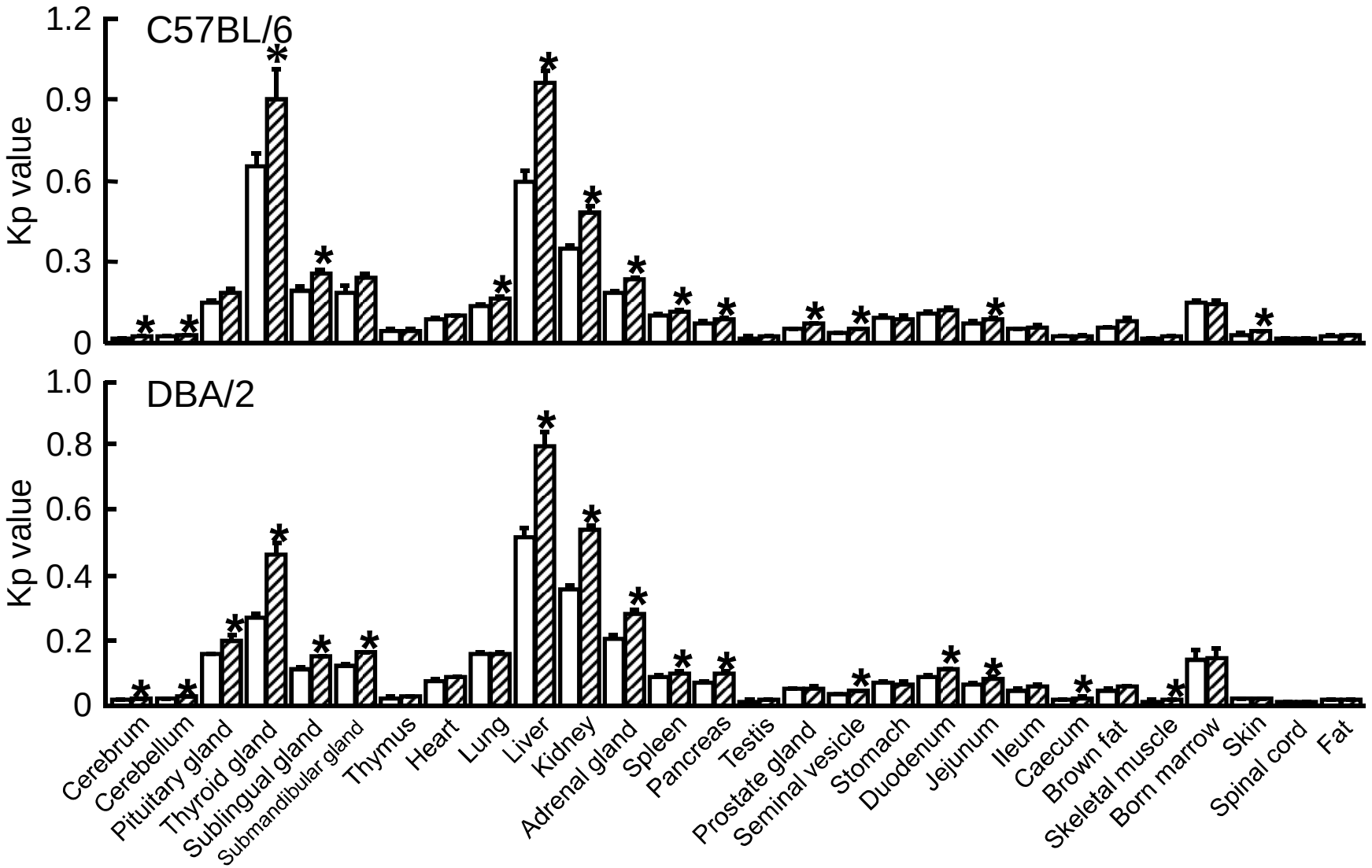

Figure 11