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A Possible Mechanism for the Decrease in Serum Thyroxine Level by a TCDD-Like PCB Congener, 3,3',4,4',5-Pentachlorobiphenyl in Mice

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Running title: CB126-induced decrease in serum T_4 level

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Abbreviations: CB126, 3,3',4,4',5-pentachlorobiphenyl; PCB, polychlorinated biphenyl; T_4, thyroxine; TTR, transthyretin; TSH, thyroid-stimulating hormone; UGT, UDP-glucuronosyltransferase.

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Abstract

Serum total thyroxine (T_4) and free T_4 levels were markedly decreased 7 days after treatment with 3,3',4,4',5-pentachlorobiphenyl (CB126) (2.5 mg/kg, intraperitoneal) in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive C57BL/6 mice but not in TCDD-resistant DBA/2 mice. At the same time, the level and activity of hepatic T_4-UDP-glucuronosyltransferase (T_4-UGT) were significantly increased in C57BL/6 mice but not in DBA/2 mice. Furthermore, the amounts of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide after injection of [^{125}I]T_4 were increased by CB126-pretretment in C57BL/6 mice, but not in DBA/2 mice. Clearance of [^{125}I]T_4 from serum was also promoted by CB126-pretreatment in C57BL/6 mice, but not in DBA/2 mice. On the other hand, no significant changes in the steady-state volumes of distribution of [^{125}I]T_4 and in the concentration ratio (Kp value) of the liver to serum by CB126-pretreatment were observed in either strain of mice. Because liver weight was increased by CB126-pretreatment in C57BL/6 mice, but not in DBA/2 mice, hepatic total [^{125}I]T_4 was increased only in C57BL/6 mice. The present findings indicate that CB126-mediated decrease in serum T_4 occurs through the increase in hepatic T_4-UGT and the enhanced accumulation of hepatic T_4 along with development of liver hypertrophy.

Introduction

Most polychlorinated biphenyl (PCB) congeners, such as 2,3',4,4',5-pentachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl (CB126), and 2,2',4,4',5,5'- and 2,3,3',4,4',5-hexachlorobiphenyls, are known to decrease the levels of serum thyroid hormone and to increase the activities of hepatic drug-metabolizing enzymes in rats and mice (Craft et al., 2002; Desaulniers et al., 1999; Ness et al., 1993; Van Birgelen et al., 1995). As possible mechanism for the PCB-mediated decrease in serum thyroid hormone, the induction of hepatic UDP-glucuronosyltransferases (UGTs), especially UGT1As, responsible for thyroid hormone metabolism and the competition of T_4 and the PCB for binding to transthyretin (TTR) have been proposed (Craft et al., 2002; Brouwer et al., 1998). In addition, hydroxylated PCB show a high binding affinity for serum TTR (Brouwer et al. 1998; Lans et al. 1993; Ucán-Marín et al. 2009). The decrease in serum thyroxine (T_4) by Aroclor 1254, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and TCDD-like PCB, CB126, in rats is reported to occur mainly through the induction of the UGT (T_4-UGT) responsible for T_4 glucuronidation through an aryl hydrocarbon (Ah) receptor-mediated mechanism (Barter and Klaassen, 1994; Schuur et al., 1997; Van Birgelen et al., 1995). However, we have demonstrated that a single and consecutive treatments with Kanechlor-500 (KC500), a commercial PCB mixture, resulted in a significant decrease in serum total T_4 not only in Wistar, but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007), and further indicated that the KC500-mediated decrease occurs through increased accumulation of T_4 in several tissues, especially the liver, rather than an increase in hepatic T_4-UGT activity (Kato et al., 2007).

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In the present study, therefore, to determine the mechanism for the decrease in serum thyroid hormone by a TCDD-like PCB, we selected CB126 (Fig. 1), because CB126 is a toxic coplanar PCB congener with a toxic equivalency factor (TEF) of 0.1 (Safe, 1994), and generally exists with mixtures of multiple PCB congeners in the environment, and also because it shows anti-estrogenic effects in a human breast cancer cell line (Krishnan and Safe, 1993; Gierthy et al., 1997). We also examined a relationship between the decrease in serum total T_4 and the increase in the hepatic T_4-UGT. It has been reported that TCDD-induced glucuronidation is observed in rats, but only marginally occurs in mice (Craft et al., 2002). In the present work, therefore, we examined the differences in the CB126-induced alteration of levels of thyroid hormones between TCDD-sensitive C57BL/6 mice and TCDD-insensitive DBA/2 mice. The results strongly suggest that the CB126-mediated decrease in serum total T_4 in mice occurs through the increase in hepatic T_4-UGT and the enhanced 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). CB126 were purchased from Cambridge Isotope Laboratories, Inc. (MA, USA). All the other chemicals used herein were obtained commercially in appropriate grades of purity.

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). Male C57BL/6 and DBA/2 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 CB126 (2.5 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 7 days after the administration of CB126. The thyroid gland and liver were removed and weighed. 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 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), respectivery.

Hepatic microsomal enzyme assays. The amount of hepatic microsomal protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Microsomal *O*-dealkylase activities of 7-benzyloxy-, 7-ethoxy-, and 7-pentoxy-resorufins were determined by the method of Burke *et al*. (1985). The activity of microsomal UGT toward T_4 (T_4-UGT activity) was determined by the method 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 detection kit, GE Healthcare UK, Ltd), and the level of each protein was determined densitometrically with LAS-1000 (Fuji Photo Film. Co., Ltd., Tokyo, Japan).

B) *Ex Vivo* Study. At 7 days after treatment with CB126, the mice were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml)

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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 intravenously given 0.1 ml of [^{125}I]T_4 (15 μCi /ml) dissolved in saline containing 10 mM NaOH and 1 % normal mouse serum. In addition, since bile was collected within 2.25 hr after pentobarbital administration, pentobarbital-mediated induction of the enzymes responsible for T_4 metabolism is little expected.

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. An aliquot (15 μl) of serum was used for measurement of [^{125}I]T_4 level by a γ-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences), and the assay was performed in duplicate.

Biliary excretion of total [^{125}I]T_4 and [^{125}I]T_4 glucuronide. After the administration of [^{125}I]T_4, bile was collected on ice for 2 h at 30 min intervals. Bile volume was determined gravimetrically. The amounts of total [^{125}I]T_4 and [^{125}I]T_4 glucuronide in bile were determined by the method of Vansell and Klaassen (2001). In brief, an aliquot (10 μl) of each bile sample was used for determining total [^{125}I]T_4 level by a γ-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences), and the assay was performed in duplicate. To measure the amount of

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[^{125}I]T_4 glucuronide in bile, a portion (10 μl) of each bile sample was added 2 volume methanol and stored at −20℃ for 1 h to precipitate protein. After the mixture was centrifuged at 12,000 g (4℃) for 10 min, and 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). A solution of 0.02 mM ammonium acetate (pH 4.0) containing 16-45% acetonitrile was used to elute [^{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 level of biliary [^{125}I]T_4 glucuronide was determined by Radioisotope Detector 171 (Beckman Coulter, Inc. CA USA).

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 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 4-20% 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, which were incubated with [^{125}I]T_4, were also

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applied on the 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 from 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 performed according to the modified method of Oppenheimer et al. (1968). In brief, at 5 min after administration of [^{125}I]T_4 to CB126-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 weighed. Radioactivities in serum and the tissues were determined by a γ-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical 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 bound 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).

Results

Serum Hormone Levels. We carried out the preliminary experiments of the dose-response (CB126: 0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg) and time-course (72, 120, and 168 hr). On the basis of the results, we determined the suitable dose and time (CB126: 2.5 mg/kg, 7 days after the dosing). The effects of CB126 on the levels of serum thyroid hormones were examined in C57BL/6 and DBA/2 mice (Figure 2). Serum total T_4 and free T_4 levels 7 days after the treatment with CB126 were markedly decreased in C57BL/6 mice, but not in DBA/2 mice. On the other hand, no significant increase in the level of serum TSH by CB126-pretreatment was observed in either strain of mice.

Hepatic Drug-Metabolizing Enzymes. Effects of CB126 on hepatic microsomal activities of ethoxyresorufin *O*-dealkylase (Cyp1a1/2), benzyloxyresorufin *O*-dealkylase (Cyp2b1/2 and Cyp3a1/2), and pentoxyresorufin *O*-dealkylase (Cyp2b1/2) were examined in C57BL/6 and DBA/2 mice. Treatment of C57BL/6 mice with CB126 resulted in remarkable increases in hepatic microsomal enzyme activities; 73-fold for ethoxyresorufin *O*-dealkylase activity, 7-fold for pentoxyresorufin *O*-dealkylase activity, and 3-fold for benzyloxyresorufin *O*-dealkylase activity. On the other hand, no such CB126-mediated increase was observed in DBA/2 mice (Table 1).

Hepatic T_4-UGT. T_4 glucuronidation is reported to be primarily mediated by hepatic T_4-UGT, including UGT1A1 and UGT1A6, in the rat liver (Visser, 1996). Therefore, we examined the effects of CB126 on hepatic microsomal T_4-UGT activity in C57BL/6

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and DBA/2 mice. The activity of hepatic T_4-UGT was significantly increased by CB126 in C57BL/6 mice, but not in DBA/2 mice (Figure 3).

The amounts of the proteins responsible for T_4-UGTs, including total Ugt1a, Ugt1a1, and Ugt2b1, in mice were determined by Western blot analysis. The amounts of the proteins responsible for the Ugt1a enzymes in the liver were significantly increased by CB126 in C57BL/6 mice but not in DBA/2 mice. The level of hepatic Ugt2b1 was decreased in C57BL/6 mice, whereas the level was not significantly changed in DBA/2 mice by CB126 treatment (Figure 4).

Biliary Excretion of [^{125}I]T_4 and [^{125}I]T_4 Glucuronide. We examined effects of CB126 on the levels of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide in C57BL/6 and DBA/2 mice. The amounts of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide after intravenous injection of [^{125}I]T_4 were increased by CB126-pretreatment in C57BL/6 mice, but not in DBA/2 mice (Figure 5).

Serum Proteins Bound to [^{125}I]T_4. The effects of CB126 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 (Figures 6 and 7). In CB126-pretreated C57BL/6 mice, the level of serum [^{125}I]T_4-TTR complex slightly increased, while the binding levels of [^{125}I]T_4 to serum albumin and TBG slightly decreased. In DBA/2 mice, no such effects of CB126-pretreatment were observed.

Clearance of [^{125}I]T_4 from Serum After an intravenous administration of [^{125}I]T_4 to the CB126-pretreated C57BL/6 and DBA/2 mice, concentrations of [^{125}I]T_4 in the

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serum were measured at the indicated times (Figure 8). CB126-pretreatment resulted in a clear enhancement of the clearance of [^{125}I]T_4 from the serum in C57BL/6 mice, but not in DBA/2 mice. The serum [^{125}I]T_4 level was decreased by about 35% of control level within 5 min, and the decrease remained up to 120 min later. The serum pharmacokinetic parameters of the [^{125}I]T_4 estimated from these data (Figure 8) were summarized in Table 2. The mean total body clearance (Cl_{tb}) of [^{125}I]T_4 in the CB126-pretreated C57BL/6 mice increased 1.7-fold, as compared with the control mice. On the other hand, no significant change in the steady-state volumes of distribution of [^{125}I]T_4 by CB126-pretreatment was observed in either strain of mice (Table 2).

Tissue Distribution of [^{125}I]T_4. Effects of CB126-pretretment on the tissue-to-serum concentration ratio (Kp value) and tissue distribution level of [^{125}I]T_4 were examined in C57BL/6 and DBA/2 mice. The Kp values in the thyroid gland, liver, and kidney were the greatest in either strain of control mice (Figure 9). Kp value in the thyroid gland was reduced by CB126-pretreatment in C57BL/6 mice, but not in DBA/2, whereas no such significant changes in the Kp values of the liver and kidney were observed in either strain of mice (Figure 9).

In the control C57BL/6 and DBA/2 mice, accumulation of [^{125}I]T_4 was the highest in the liver among the tissues examined (Figure 10). In C57BL/6 mice, pretreatment with CB126 resulted in increase in hepatic total [^{125}I]T_4, and the accumulated level in the liver was to more than 49% of the [^{125}I]T_4 dosed (Figure 10), whereas no significant change in liver accumulation (per g liver) of [^{125}I]T_4 by CB126-pretreatment was observed (Table 3). In DBA/2 mice, no significant change in accumulation of [^{125}I]T_4 in the liver by CB126 occurred. In addition, treatment of C57BL/6 mice with CB126

resulted in significant increases in weights of liver and thyroid gland and in significant decreases in weights of thymus and caecum. In DBA/2 mice, the CB126 treatment resulted in slight increases in weights of liver, kidney, seminal vesicle, stomach, jejunum, ileum and caecum and in a significant decrease in weight of thymus (Table 4).

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Discussion

In the present study, treatment with CB126 (a single intraperitoneal administration at a dose of 2.5 mg/kg) resulted in a significant decrease in serum total T_4 and free T_4 in C57BL/6 mice, but not in DBA/2 mice. The strain-difference in CB126-mediated decrease in the serum T_4 level was closely correlated with those in the increases in the activities of hepatic drug metabolizing enzymes, including T_4-UGT and in accumulation of T_4 in the liver.

As a possible explanation for the TCDD-like PCB-induced decrease in serum thyroid hormones, hepatic T_4-UGT-dependent mechanism was considered, because T_4-UGT inducers, such as TCDD and CB126, have strong activities for decreasing serum total thyroid hormones in rats (Schuur et al., 1997; Van Birgelen et al., 1995). Furthermore, we confirmed that the activity of hepatic T_4-UGT (Ugt1a and Ugt1a1) was significantly increased by CB126-treatment in C57BL/6 mice, but not in DBA/2 mice, and further found that the amounts of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide after intravenous injection of [^{125}I]T_4 were increased by CB126-pretreatment in C57BL/6 mice, but not in DBA/2 mice, suggesting that the induction of hepatic T_4 removal occurs more likely at the level of basolateral transport of T_4. The previous reports and the present findings strongly suggest that induction of hepatic T_4-UGT is one of factors that mediate the decrease in serum T_4 level by CB126. In addition, our present result of the CB126-mediated decrease in the level of Ugt2b1 in C57BL/6 mice was identified with the previous results (Buckley and Klaassen, 2009).

However, among the rats and mice treated with a TCDD-like PCB, the difference in magnitude of decrease in serum total T_4 dose not correlate with that of hepatic T_4-UGT

activity (Craft et al., 2002, Hood et al., 2003). Recently, we have demonstrated a hepatic T_4-UGT-independent pathway for PCB-mediated decrease in serum total thyroid hormones using Wistar and Gunn (T_4-UGT-deficient) rats and further indicated that an increase in the accumulation of T_4 in livers of PCB-treated rats is partially responsible for the decrease in serum total thyroid hormones including T_4 (Kato et al., 2007). In addition, serum TSH and hepatic type-I iodothyronine deiodinase, which are important the factors regulating serum thyroid hormones, are not induced by PCB in rats (Hallgren et al., 2001; Hood et al., 1999; Liu et al., 1995, Kato et al., 2004). Likewise, CB126-treatment did not increase serum TSH in both C57BL/6 and DBA/2 mice. Furthermore, enhanced accumulation of T_4 in the tissues, especially liver, was observed in CB126-pretreated C57BL/6 mice, but not in CB126-pretreated DBA/2 mice, strongly suggesting that the enhancement of overall amount of T_4 in liver is one of the factors which mediate PCB-induced decrease in serum total thyroid hormones in mice.

As a possible mechanism for CB126-mediated enhancement of T_4 accumulation in liver, a TTR-associated pathway might be considered, because PCB and its hydroxylated metabolites act as competitors for T_4 for binding to TTR, and because a decrease in T_4-TTR complex formation results in an increase in serum free T_4 and then in uptake of T_4 into the liver (Brouwer et al., 1998; Lans et al., 1993; Meerts et al., 2002; Kato et al., 2004). However, the present study concerning the fate of serum T_4 using [^{125}I]T_4 indicates that only a slight increase in serum [^{125}I]T_4-TTR complex and only a slight decrease in binding of level of [^{125}I]T_4 to serum albumin and TBG in CB126-pretreated C57BL/6 mice. In addition, although some hydroxylated metabolites of PCBs are known to show higher capacity for binding to TTR (Brouwer et al., 1998; Lans et al., 1993; Ucán-Marín et al., 2009), the hydroxylated metabolites of

CB126 were minimally detected in the serum and liver in CB126-pretreated C57BL/6 mice (data not shown), as described previously by Koga et al (1990). The previous reports and the present findings indicate that a TTR-associated pathway is not considered a primarily mechanism for CB126-mediated decrease in serum total T_4. However, it was found that CB126-induced liver hypertrophy occurred in C57BL/6 mice but not in DBA/2 mice, and only a small change in the concentration of T_4 (per g tissue) livers of both C57BL/6 and DBA/2 mice. Therefore, the CB126-induced increase in accumulation of hepatic T_4 in C57BL/6 mice is thought to occur mainly through development of liver hypertrophy.

However, the increases in tissue weights were not necessarily correlated with those in accumulation of [^{125}I]T_4 in the tissues of the CB126-pretreated mice. For example, no CB126-induced accumulation of [^{125}I]T_4 was observed in the thyroid gland in C57BL/6 mice. Although an exact mechanism for a liver-selective accumulation of [^{125}I]T_4 by CB126 remains unclear, the liver-selective apparatus for T_4-transportation might exist. In addition, CB126-induced development of liver hypertrophy was herein found to occur in C57BL/6 mice, but not DBA/2 mice, confirming that the development of liver hypertrophy occurs in an Ah receptor-dependent pathway (Yoshizawa et al., 2007). Although it has been reported that CB126-induced development of liver hypertrophy occurs in the rat liver (Yoshimura et al., 1979), there is no report, exception with our present paper, concerning the CB126-induced change in the level of T_4 in the liver. Therefore, it is unclear whether CB126-enhanced accumulation of [^{125}I]T_4 in the liver occurs specifically in mice. Furthermore, in DBA/2 mice, CB126-pretreatment resulted in increases in Kp values of [^{125}I]T_4 in the thymus and fat, while it did in decrease in the value in the heart. A mechanism for the tissue-difference in the CB126-mediated

changes in the Kp value remains unclear.

In conclusion, we demonstrate that CB126-mediated decrease serum T_4 in mice occurs not only through increases in hepatic drug-metabolizing enzymes, especially T_4-UGT, but also through development of liver hypertrophy.

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Footnotes

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Legends for figures

Fig. 1. Chemical structure of 3,3',4,4',5-pentachlorobiphenyl.

Fig. 2. Effects of CB126 on the levels of serum total T_4, free T_4, and TSH. Animals were killed 7 days after the administration of CB126 (2.5 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 seven animals. **P*<0.05, significantly different from each control.

Fig. 3. Effect of CB126 on the activity of hepatic microsomal T_4-UDPGT. Hepatic microsomes from individual animals were used for T_4-UDPGT 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. 4. Representive Western blot patterns for hepatic microsomal Ugt isoforms in CB126-treated mice. Hepatic microsomes from individual animals were used for Western blot analysis, as described in *Materials and Methods*.

Fig. 5. Effect of CB126 on amounts of the biliary total [^{125}I]T_4 and [^{125}I]T_4-glucuronide. The levels of total [^{125}I]T_4 and [^{125}I]T_4-glucuronide excreted were measured in the bile collected at 30 min intervals after the i.v. administration of [^{125}I]T_4.

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Each point represents the mean ± S.E. (vertical bars) for five to eight mice. *P<0.05, significantly different from each control. —○—, control; —■— , CB126.

Fig. 6. Effects of CB126 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 five to six animals. *P<0.05, significantly different from each control.

Fig. 7. Effects of CB126 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. 6. Each column represents the mean ± S.E. (vertical bars) for four to five animals.

Fig. 8. Effects of CB126 on the clearance of [^{125}I]T_4 from serum. 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; —■— , CB126.

Fig. 9. Tissue-to-serum concentration ratio (Kp value) of [^{125}I]T_4 in various tissues after administration of [^{125}I]T_4 to CB126-pretreated mice. CB126 (2.5 mg/kg) was given to mice, and 168 hr after the CB126-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; ▨, CB126.

Fig. 10. Tissue distribution of [^{125}I]T_4 after the administration of [^{125}I]T_4 to CB126-pretreated mice. Experimental protocols were the same as those described in the legend of Fig. 9. Each column represents the mean ± S.E. (vertical bars) for five to seven animals. *P<0.05, significantly different from each control. ▭, control; ▨, CB126.

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Table 1. Effects of CB126 on the activity of hepatic microsomal alkoxyresorufin *O*-dealkylases in C57BL/6 and DBA/2 mice

O-Dealkylase of alkoxyresolfin	C57BL/6		DBA/2	
	Control	CB126	Control	CB126
7-Ethoxy-	0.20 ± 0.02	14.29 ± 0.56*	0.18 ± 0.01	0.21 ± 0.01*
7-Benzyloxy-	0.12 ± 0.02	0.38 ± 0.01*	0.07 ± 0.01	0.10 ± 0.01
7-Pentoxy-	0.02 ± 0.002	0.15 ± 0.01*	0.02 ± 0.001	0.03 ± 0.003*

Animals were killed 7 days after the administration of CB126 (2.5 mg/kg). The activities of alkoxyresolfin *O*-dealkylase are represented as nmol of the resolfin formed /mg protein/min. Data represent the mean ± S.E. for four to five mice. *$P<0.05$, significantly different from each control.

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Table 2. Pharmacokinetic parameters for [^{125}I]T_4 after the administration of [^{125}I]T_4 to the CB126-pretreated mice

	C57BL/6		DBA/2	
	Non (control)	CB126	Non (control)	CB126
Mean total body clearance (ml/min)	0.015 ± 0.001	0.025 ± 0.002*	0.012 ± 0.0003	0.012 ± 0.001
Distribution volume (ml)	2.90 ± 0.17	3.06 ± 0.12	2.50 ± 0.08	2.72 ± 0.06

The data shown was calculated from the data in Fig. 8. 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 3. Accumulation of [^{125}I]T_4 in the CB126-pretreated mouse livers

	C57BL/6		DBA/2	
	Control	CB126	Control	CB126
[^{125}I]T_4	27.13 ± 1.06	24.16 ± 0.67	25.25 ± 1.02	23.59 ± 0.62

[^{125}I]T_4 level is represented as % dose per g liver. The radioactivity in the liver was measured at 5 min after the [^{125}I]T_4-administration. Data represent the mean ± S.E. for five to seven mice.

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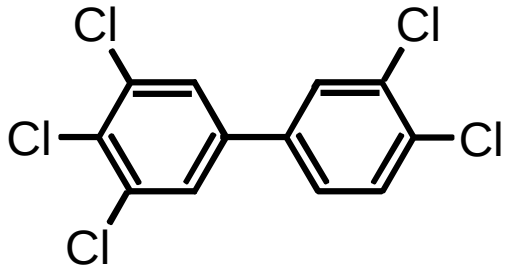
Table 4. Changes in the body and tissue weights after the administration of CB126 to C57BL/6 and DBA/2 mice

Body and tissues	C57BL/6		DBA/2	
	Control	CB126	Control	CB126
Body	25.9 ± 0.21	25.4 ± 0.20	25.2 ± 0.30	25.8 ± 0.53
Cerebrum	0.322 ± 0.004	0.311 ± 0.004	0.275 ± 0.005	0.268 ± 0.002
Cerebellum	0.094 ± 0.008	0.107 ± 0.0003	0.114 ± 0.002	0.118 ± 0.004
Pituitary gland	0.0011 ± 0.0001	0.0013 ± 0.0001	0.0013 ± 0.0002	0.0014 ± 0.0001
Thyroid gland	0.0019 ± 0.0001	0.0023 ± 0.0001*	0.0022 ± 0.0001	0.0023 ± 0.0002
Sublingual gland	0.048 ± 0.003	0.047 ± 0.002	0.054 ± 0.002	0.058 ± 0.003
Submandibular gland	0.092 ± 0.006	0.091 ± 0.003	0.094 ± 0.002	0.098 ± 0.002
Thymus	0.020 ± 0.002	0.008 ± 0.0007*	0.033 ± 0.001	0.029 ± 0.001*
Heart	0.112 ± 0.003	0.107 ± 0.002	0.117 ± 0.002	0.119 ± 0.003
Lung	0.129 ± 0.003	0.132 ± 0.002	0.121 ± 0.0030	0.123 ± 0.0019
Liver	1.149 ± 0.073	2.029 ± 0.030*	1.234 ± 0.034	1.366 ± 0.041*
Kidney	0.319 ± 0.008	0.330 ± 0.004	0.361 ± 0.011	0.407 ± 0.014*
Adrenal gland	0.0028 ± 0.0002	0.0027 ± 0.0003	0.0030 ± 0.0001	0.0030 ± 0.0002
Spleen	0.071 ± 0.005	0.063 ± 0.002	0.082 ± 0.001	0.090 ± 0.006
Testis	0.194 ± 0.004	0.189 ± 0.003	0.224 ± 0.007	0.233 ± 0.005
Prostate gland	0.040 ± 0.002	0.044 ± 0.003	0.046 ± 0.003	0.057 ± 0.004
Seminal vesicle	0.165 ± 0.010	0.171 ± 0.013	0.160 ± 0.010	0.194 ± 0.004*
Stomach	0.257 ± 0.026	0.262 ± 0.020	0.299 ± 0.023	0.438 ± 0.039*
Duodenum	0.186 ± 0.006	0.180 ± 0.007	0.147 ± 0.001	0.154 ± 0.009
Jejunum	0.390 ± 0.006	0.399 ± 0.023	0.401 ± 0.018	0.453 ± 0.009*
Ileum	0.511 ± 0.047	0.457 ± 0.024	0.488 ± 0.019	0.641 ± 0.050*
Caecum	0.477 ± 0.042	0.294 ± 0.009*	0.290 ± 0.004	0.390 ± 0.027*

Animals were killed 7 days after the administration of CB126 (2.5 mg/kg). Data represent the mean ± S.E. for five to seven mice. *P<0.05, significantly different from each control.

Fig. 1



3,3',4,4',5-pentachlorobiphenyl
(CB126)

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Fig. 2

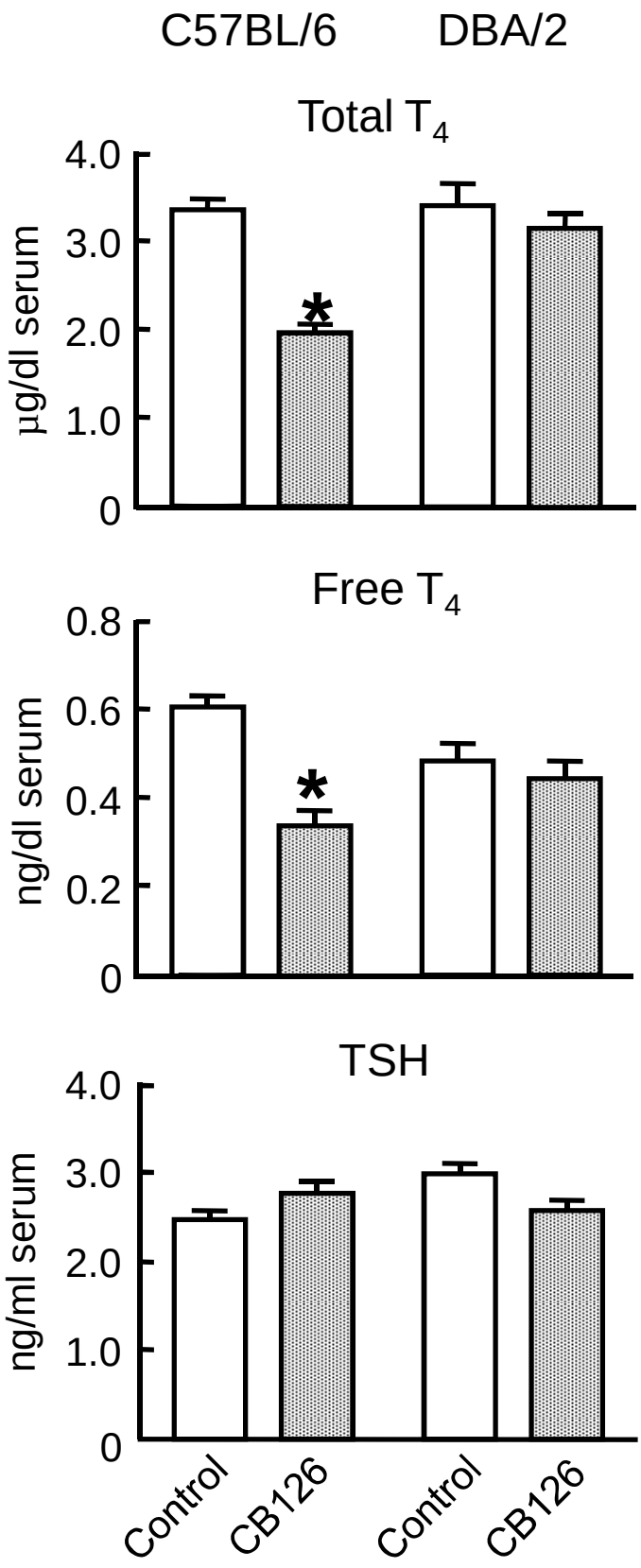

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Fig. 3

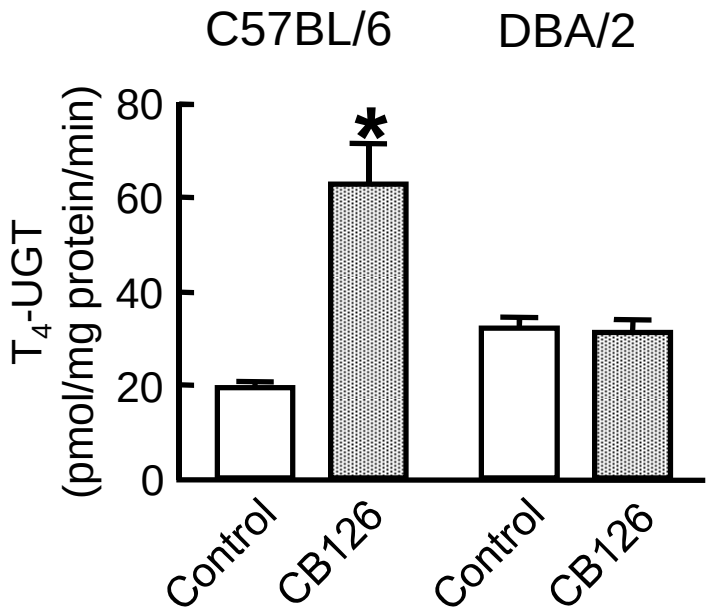

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Fig. 4

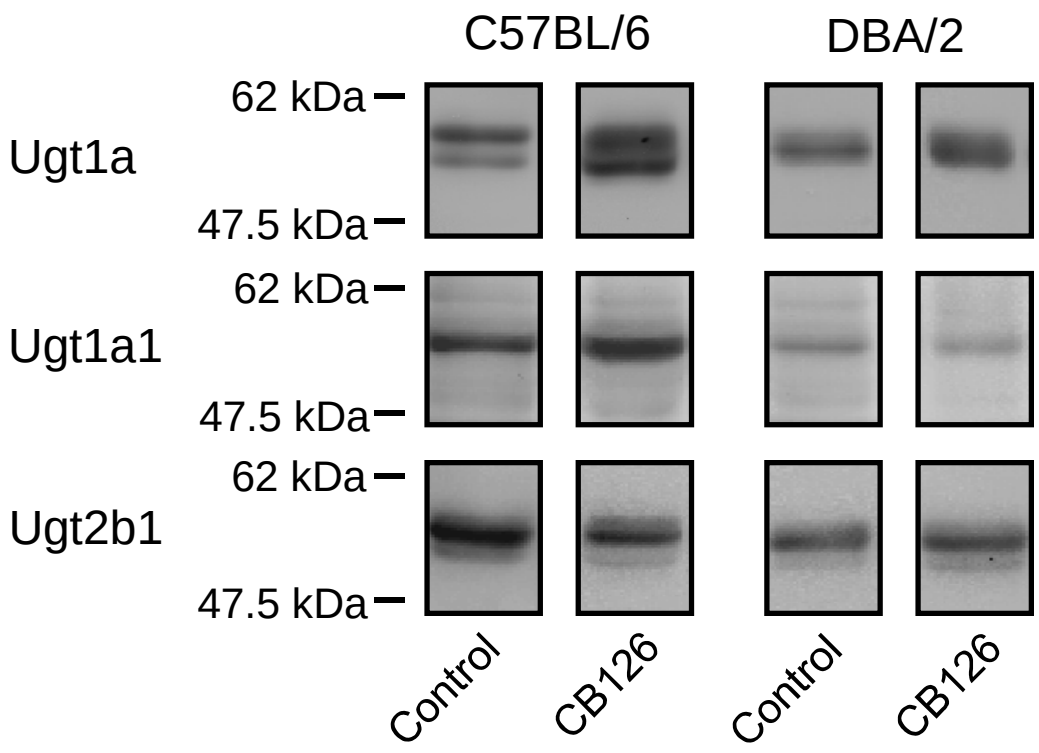

Fig. 5

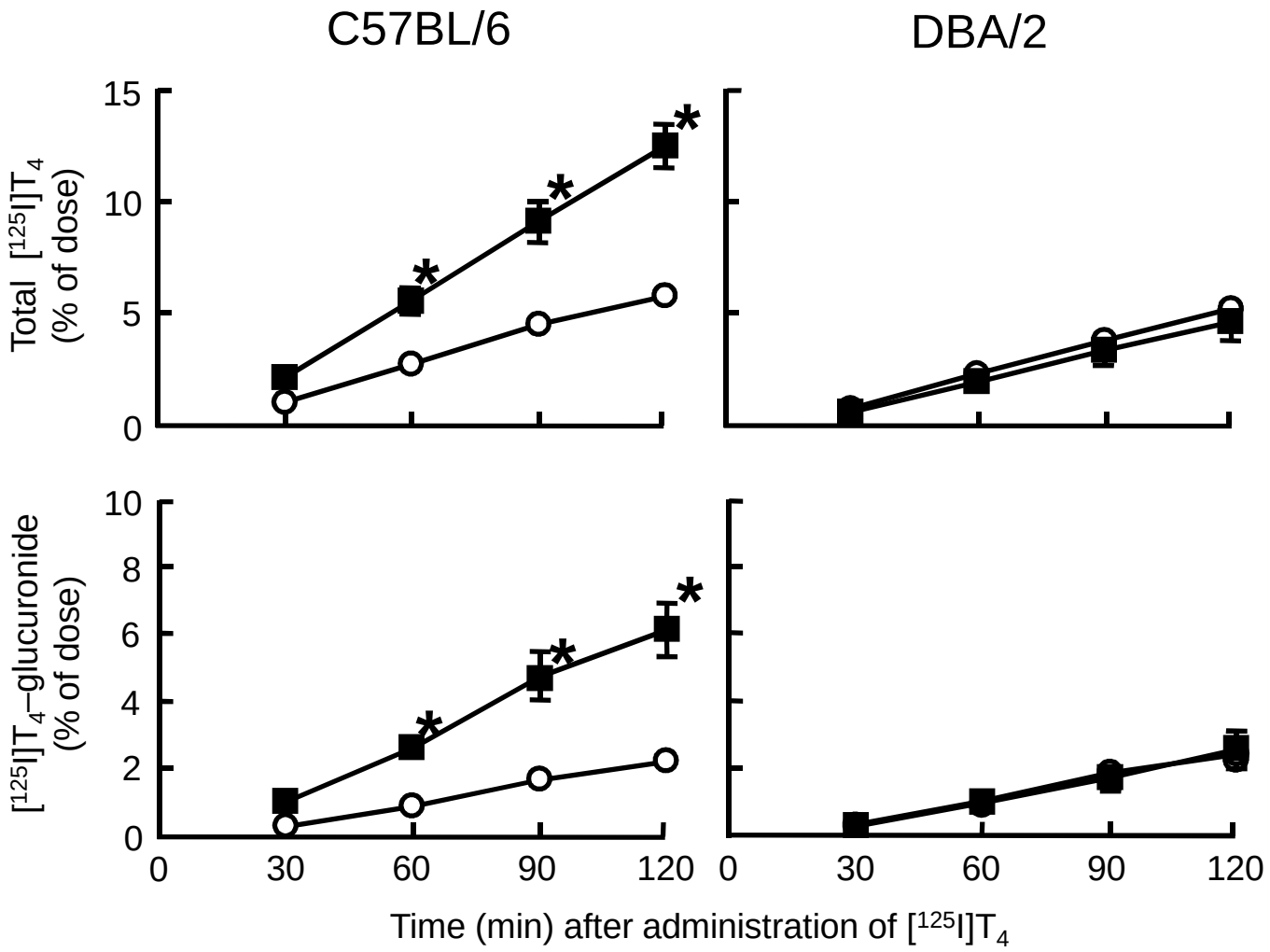

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Fig. 6

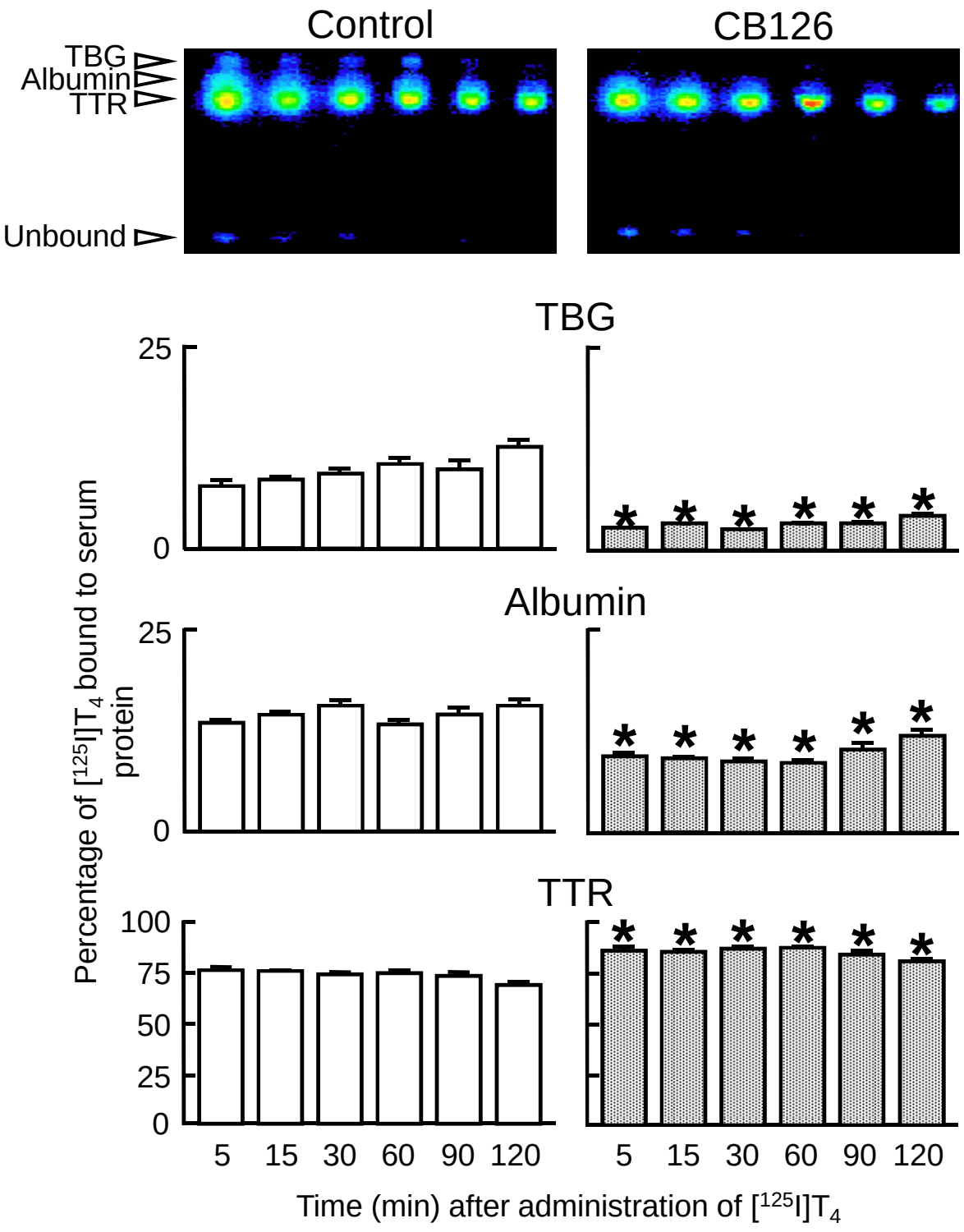

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Fig. 7

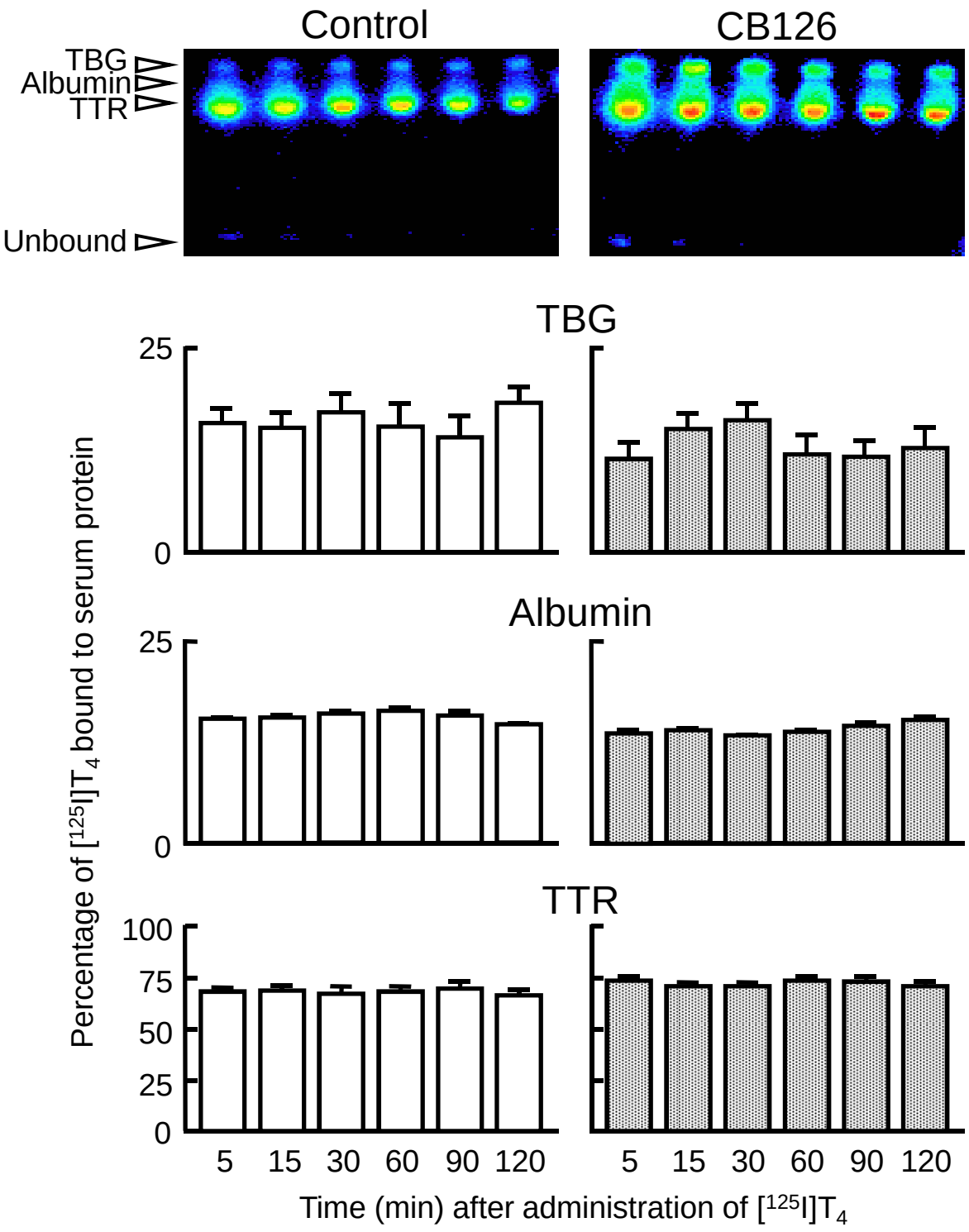

Fig. 8

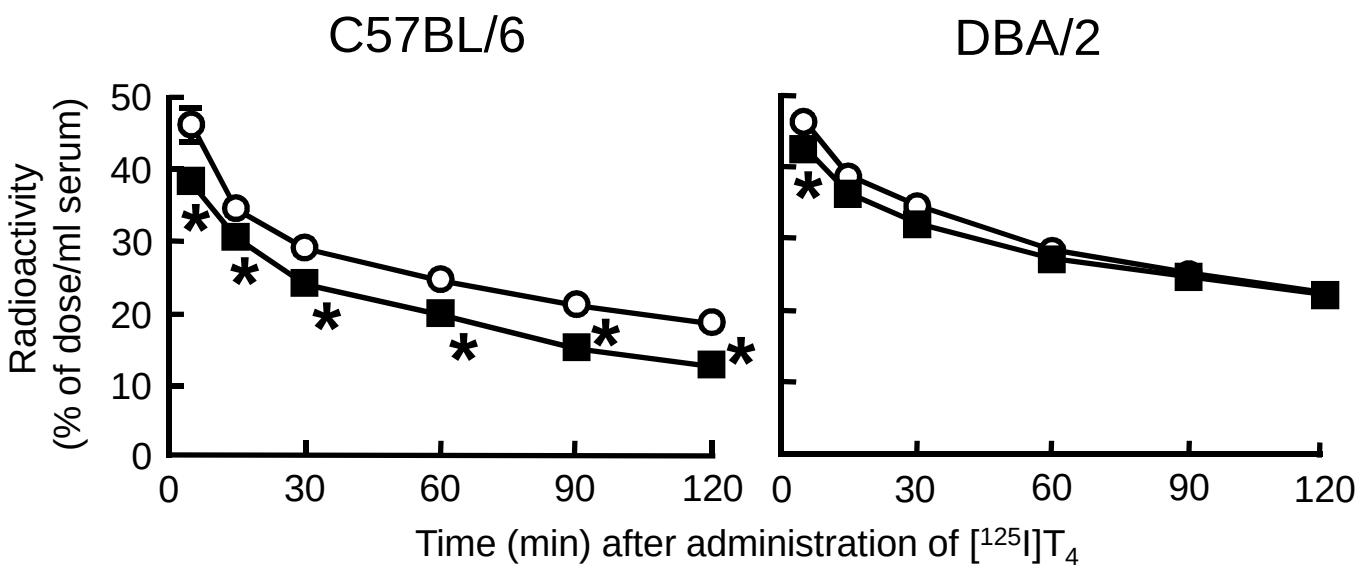

Fig. 9

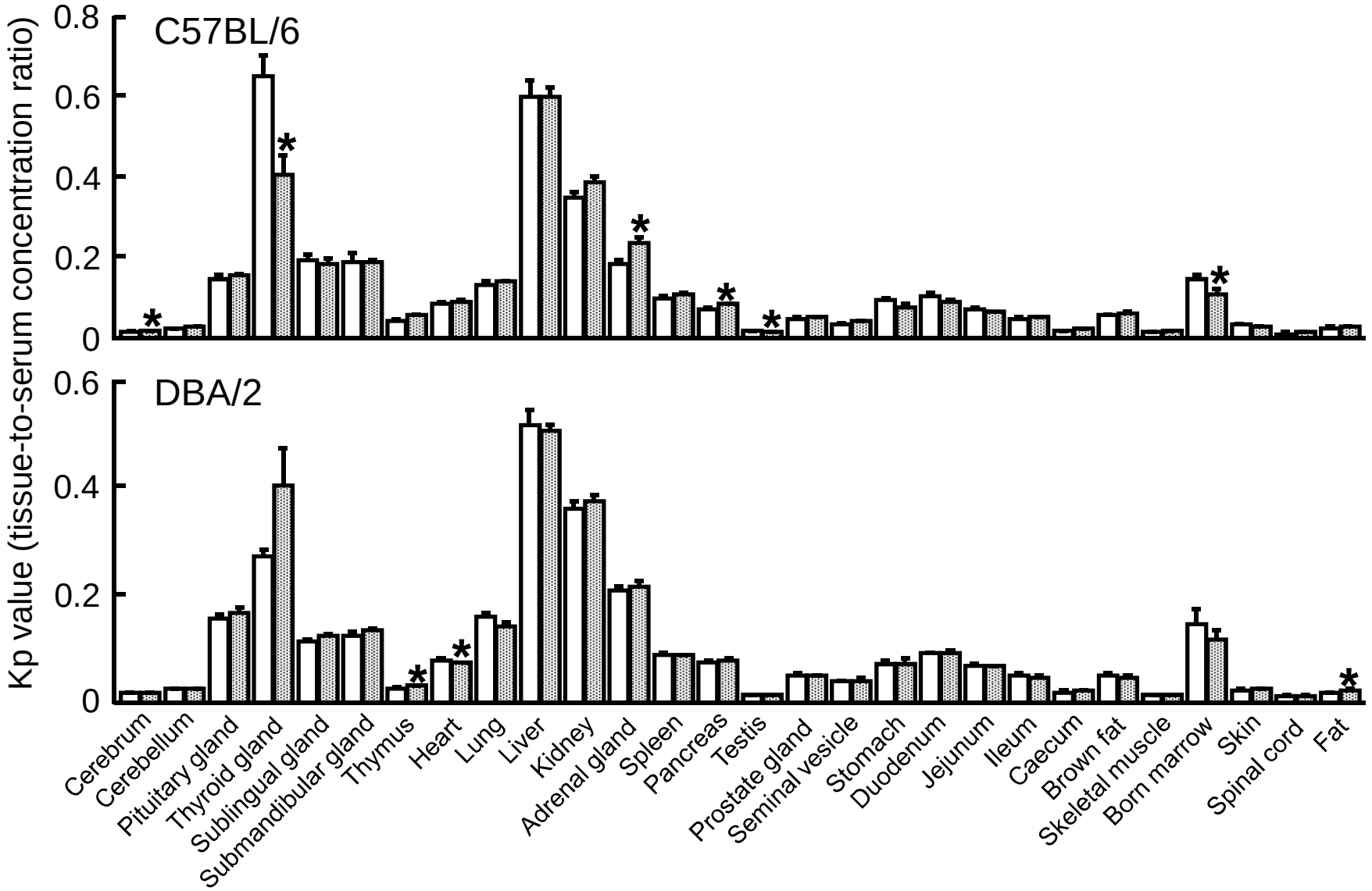

Fig. 10