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A NOVEL MECHANISM FOR POLYCHLORINATED BIPHENYLS-INDUCED DECREASE IN SERUM THYROXINE LEVEL IN RATS

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Running title: PCB-induced decrease in serum T_4 level

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ABBREVIATIONS: PCB, polychlorinated biphenyls; T_3, triiodothyronine; T_4, thyroxine; TTR, transthyretin; TSH, thyroid-stimulating hormone; UDP-GT, UDP-glucuronosyltransferase.

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Abstract

We have previously suggested that the decrease in the levels of serum total thyroxine (T_4) and free T_4 by a single administration to rats of Kanechlor-500 (KC500) at a dose of 100 mg/kg is not necessarily dependent on the increase in hepatic T_4-UDP-glucuronosyltransferase (T_4-UDP-GT). In the present study, we determined whether or not a consecutive treatment with KC500 at a relatively low dose (10 mg/kg, i.p., once daily for 10 days) results in decrease in the level of serum total T_4 and further investigated an exact mechanism for the KC500-induced decrease in the T_4. At 4 days after final treatment with KC500, the serum total T_4 and free T_4 levels were markedly decreased in both Wistar and UGT1A-deficient Wistar (Gunn) rats, while significant increases in hepatic T_4-UDP-GT activity were observed in Wistar rats but not in Gunn rats. Level of serum thyroid-stimulating hormone was not significantly changed in either Wistar or Gunn rats. Clearance from serum of the [^{125}I]T_4 administered to the KC500-pretreated Wistar and Gunn rats was faster than that to the corresponding control (KC500-untreated) rats. The accumulated level of [^{125}I]T_4 was increased in several tissues, especially the liver, in the KC500-pretreated rats. The present findings demonstrated that a consecutive treatment with KC500 resulted in significant decrease in level of serum total T_4 in both Wistar and Gunn rats and further indicated that the KC500-induced decrease would occur through increase in accumulation of T_4 in several tissues, especially the liver, rather than increase in hepatic T_4-UDP-GT activity.

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Introduction

Most polychlorinated biphenyls (PCB) are known to decrease the level of serum thyroid hormone and to increase the activity of hepatic drug-metabolizing enzymes in rats (Craft et al., 2002; Van Birgelen et al., 1995). As possible mechanisms for the PCB-induced decrease in the level of serum thyroid hormone, enhancement of thyroid hormone metabolism by PCB, and displacement of the hormone from serum transport proteins, including transthyretin (TTR), by PCB and its ring-hydroxylated metabolites are considered (Barter and Klaassen, 1992a, 1994; Brouwer et al., 1998). Especially, the decrease in the level of serum thyroxine (T_4) by 3,3',4,4',5-pentachlorobiphenyl, Aroclor 1254, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats is believed to occur mainly through induction of the UDP-glucuronosyltransferases (T_4-UDP-GTs), especially UGT1A subfamily enzymes, responsible for glucuronidation of T_4 (Barter and Klaassen, 1994; Van Birgelen et al., 1995). However, magnitude of decrease in the level of serum total T_4 is not necessarily correlated with that of increase in T_4-UDP-GT activity (Craft et al., 2002; Hood et al., 2003). Furthermore, we have reported that in Kanechlor-500 (KC500)-treated mice, serum T_4 level decreased without increase in T_4-UDP-GT activity (Kato et al., 2003) and that the decrease in serum total T_4 level by a single administration of either Kanechlor-500 (KC500) or 2,2',4,5,5'-pentachlorobiphenyl occurred even in UGT1A-deficient Wistar (Gunn) rats (Kato et al., 2004). Thus, an exact mechanism for PCB-induced decrease in the level of serum thyroid hormone remains unclear. To date, most studies on biological effects of PCB have been performed using the experimental animals treated once at a high dose (more than 100 mg/kg body weight), and the effect of the consecutive treatment at a low

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dose has little been reported. Humans and wild animals are exposed to a wide variety of environmental chemicals, including PCB, at a low level over a long period of time. Therefore, a study on biological effects by the consecutive treatment with PCB at a low dose would be a very important.

In the present study, therefore, we examined whether or not a consecutive treatment with KC500 at a relatively low dose (10 mg/kg, i.p., once daily for 10 days) results in decrease in the level of serum total T_4 and further discussed a mechanism underlying the PCB-induced decrease in the T_4.

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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 (Boston, MA). The KC500 used in the present experiments contains 2,2',5,5'-tetrachlorobiphenyl (5.6% of total PCBs), 2,2',3,5',6-pentachlorobiphenyl (6.5%), 2,2',4,5,5'-pentachlorobiphenyl (10%), 2,3,3',4',6-pentachlorobiphenyl (7.4%), 2,3',4,4',5-pentachlorobiphenyl (7.7%), 2,2',3,4,4',5'-hexachlorobiphenyl (5.6%) and 2,2',4,4',5,5'-hexachlorobiphenyl (5.4%) as major PCB congeners (Haraguchi et al, 2005). All the other chemicals used herein were obtained commercially in appropriate grades of purity.

Animal Treatments. Male Wistar rats (160-200 g) and UGT1A-deficient Wistar rats (Gunn rats, 190-260 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male Wistar and Gunn rats were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM-8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1℃; humidity, 55 ± 5%), and handled with human care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Rats received consecutive intraperitoneal injections of KC500 (10 mg/kg) dissolved in Panacete 810 (5 ml/kg) at 24 h-intervals for 10 days. Control animals were treated with a vehicle alone (5 mg/kg).

A) *In Vivo* Study. Rats were killed by decapitation 4 days after the final

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administration of KC500. 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, total T_3 and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using Total T4 and Free T4 kit (Diagnostic Products Corporation; Los Angels, CA), the Triiodothyronine kit GammaCoat^{TM} T_3 II (DiaSorin Inc; Stillwater, MN), and the rTSH [^{125}I] Biotrak assay system (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshine, UK), respectivery.

Hepatic microsomal enzyme assays. Hepatic microsomal fraction was prepared according to the method as described previously (Kato *et al*., 1995), and 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).

Hepatic T_4-Metabolizing Enzyme Aassay. The activity of microsomal UDP-GT toward T_4 (T_4-UGT activity) was determined by the methods of Barter and Klaassen (1992b).

Western Blot Analysis. The polyclonal anti-peptide antibodies against the

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common region of UGT1A isoforms and specific antibodies against UGT1A1, UGT1A6, 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 corresponding to UGT1A1, UGT1A6, and UGT2B1 on a sheet were detected using chemical luminescence (ECL detection kit, Amersham Biosciences Inc., Piscataway, NJ), and the level of each protein was determined densitometrically with LAS-1000 (Fuji Photo Film. Co., Ltd., Tokyo, Japan).

B) *Ex Vivo* Study. At 4 days after a consecutive 10-day treatment with KC500, the rats were anesthetized with a saline (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (Polyethylene tube SP31, 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 rats were given i.v. 1 ml of [^{125}I]T_4 (15 μCi /ml) dissolved in the saline containing 10 mM NaOH and 1 % normal rat serum.

Clearance of [^{125}I]T_4 from Serum. The study on the clearance of [^{125}I]T_4 from serum was performed according to the method of Oppenheimer et al. (1968). Briefly, after the administration of [^{125}I]T_4, a portion (0.3 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) were taken from each serum sample for determining [^{125}I]T_4 level by a γ-counter (COBRA^{TM} II AUTO-GAMMA$^{®}$5002, Packard Co., Meriden,

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USA).

Analysis of [^{125}I]T_4 Bound to Serum Proteins. The levels of serum [^{125}I]T_4-albumin and [^{125}I]T_4-TTR complexes were determined according to the method of Davis et al. (1970). Briefly, serum was diluted in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels PAG Mid "Daiichi" 4/20 (Daiichi Pure Chemicals Co., Ltd, 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 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-albumin and [^{125}I]T_4-TTR in serum were determined by counting the gel fractions identified from Bio Imaging Analyzer (BAS-2000II IP Reader, Fuji Photo Film Co., Ltd, Japan).

Tissue Distribution of [^{125}I]T_4. The study on the tissue distribution of [^{125}I]T_4 was performed according to the modified method of Oppenheimer et al. (1968). Briefly, at 60 min after administration of [^{125}I]T_4 to KC500-pretreated rats, 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, lleum, caecum, brown fat, skeletal muscle, bone marrow skin, spinal cord, fat, were removed and weighted. Radioactivities in serum and the tissues were determined

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by a γ-counter (COBRA^{TM} II AUTO-GAMMA$^{®}$5002, Packard Co., Meriden, USA), and amounts of [^{125}I]T_4 in various tissues were shown as ratios of tissue-to-serum.

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

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Results

Serum hormone levels. Effects of KC500 on levels of serum thyroid hormones were examined in Wistar and Gunn rats (Fig. 1). In both Wistar and Gunn rats, KC500 treatment resulted in decreases of the serum total T_4 and free T_4, and the magnitude of the decrease in each serum thyroid hormone was almost the same in the both strains of rats. On the other hand, significant decrease in the level of serum total T_3 was observed in Gunn rats but not in Wistar rats. In addition, no significant change in TSH level was observed in either Wistar or Gunn rats.

Hepatic drug-metabolizing enzymes. Effects of KC500 on hepatic microsomal activities of benzyloxyresorufin *O*-dealkylase (CYP2B1/2 and CYP3A1/2), pentoxyresorufin *O*-dealkylase (CYP2B1/2), and ethoxyresorufin *O*-dealkylase (CYP1A1/2) were examined in Wistar and Gunn rats. In both Wistar and Gunn rats, these enzyme activities were significantly increased by KC500 (Table 1), and the increase in each enzyme activity was much greater in Wistar rats than in Gunn rats.

Hepatic T_4-metabolizing enzyme activities. T_4 glucuronidation is primarily mediated by hepatic T_4-UDP-GT, such as UGT1A1 and UGT1A6, in the rat liver (Visser, 1996), and a chemical-mediated induction of the enzymes is considered to contribute to the decrease in the level of serum total T_4. Therefore, we examined effects of KC500 on hepatic microsomal T_4-UDP-GT activity in Wistar and Gunn rats. Constitutive activity of T_4-UDP-GT was about 2.2-fold higher in Wistar rats than in Gunn rats. Treatment with KC500 resulted in significant increase of T_4-UDP-GT activity in Wistar

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rats but not in Gunn rats (Fig. 2).

Western blot analysis for UGT1As. Levels of the proteins responsible for UGT1A enzymes, UGT1A1 and UGT1A6, were increased by KC500 treatment in Wistar rats but not in Gunn rats (Figs. 3 and 4). In addition, no expression of the UGT1A enzymes was confirmed in Gunn rats. On the other hand, the level of UGT2B1 was significantly increased by KC500 in both Wistar and Gunn rats, and magnitudes of the increase in the both strains of rats were almost the same (Figs. 3 and 4).

Serum proteins bound to [^{125}I]T_4. The effects of KC500 on the binding of [^{125}I]T_4 to serum proteins, TTR and albumin, were examined in Wistar and Gunn rats (Figs. 5 and 6). In both Wistar and Gunn rats, pretreatment with KC500 resulted in a significant decrease in the level of [^{125}I]T_4-TTR complex, whereas it did in a significant increase in the level of [^{125}I]T_4 bound to albumin (Figs. 5 and 6).

Clearance of [^{125}I]T_4 from serum After an iv administration of [^{125}I]T_4 to the KC500-pretreated Wistar and Gunn rats, concentrations of [^{125}I]T_4 in sera were measured at the indicated times (Fig. 7). In both Wistar and Gunn rats, pretreatment with KC500 promoted the clearance of [^{125}I]T_4 from serum, and their serum [^{125}I]T_4 levels were decreased to about 40 % of the initial level within 5 min. In the KC500-untreated Wistar and Gunn rats, serum [^{125}I]T_4 levels were gradually decreased to about 40% of the initial level at 120 min later. The serum pharmacokinetic parameters of the [^{125}I]T_4 estimated from these data (Fig. 7) were summarized in Table 2. The mean total body clearances (Cl_{tb}) of [^{125}I]T_4 in the KC500-pretreated rats were

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2.4- and 2.9-times, respectively, greater than those in the corresponding control rats. The steady state volumes of distribution (Vd_{ss}) in the KC-500-pretreated rats were 1.6- and 2.4-times, respectively, larger than those in the corresponding control rats.

Tissue distribution of [^{125}I]T_4. 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 to the KC500-pretreated Wistar and Gunn rats are shown in Figures 8 and 9, respectively. Kp values of the thyroid gland and liver were the greatest among those of the tissues examined in either Wistar or Gunn rats (Fig. 8). In addition, Kp values in the all tissues examined, with exception of the testis and ileum, were greater in KC500-pretreated Wistar rats than those in the corresponding control (KC500-untreated) rats. Kp values in the thyroid gland, liver, and jejunum in the KC500-pretreated Wistar and Gunn rats were 1.6-1.8, 3.3-3.8 and 4.7-11.5 times, respectively, higher than those in corresponding control rats (Fig. 8).

In the control Wistar and Gunn rats, accumulation level of [^{125}I]T_4 was the highest in the liver among the tissues examined (Fig. 9). In both Wistar and Gunn rats, pretreatment with KC500 resulted in increase in the accumulation level in the liver, and the levels achieved to more than 40% of the [^{125}I]T_4 dosed (Fig. 9). Likewise, significant increase in accumulation of [^{125}I]T_4 was observed in the jejunum (Fig. 9). In addition, significant increases in the liver weight and accumulation level (per g liver) of [^{125}I]T_4 occurred in KC500-pretreated Wistar rats, but not in Gunn rats (Tables 3 and 4).

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Discussion

In the present study, we found that consecutive treatment with KC500 (10 mg/kg, i.p., once daily for 10 days; total dose, 100mg/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 Wistar and Gunn (UGT1A-deficient) rats. Thus decrease in level of serum total T_4 is also observed in the Wistar and Gunn rats treated with KC500 (a single i.p. administration at a dose of 100 mg/kg) (Kato et al., 2004). In addition, constitutive levels of serum total T_4 and T_3 were higher in Gunn rats than in Wistar rats, and the results were identified with those as previously described by Benathan et al. (1983). The difference in constitutive level of serum thyroid hormone between Wistar and Gunn rats seems to be dependent on differences in the level and/or activity of T_4/T_3-UDP-GTs.

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, 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 the experimental animals treated with a T_4-UDP-GT inducer, difference in magnitude of decrease in the level of serum total T_4 is not necessarily correlated with that of hepatic T_4-UDP-GT activity (Craft et al., 2002, Hood et al., 2003, Kato et al., 2003). Our present and previous results (Kato et al., 2004; 2005) using Wistar and Gunn rats support a hypothesis that significant decrease in the level of serum total thyroid hormones by

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either PCB or phenobarbital occurs primarily in a hepatic T_4-UDP-GT-independent pathway.

As a possible mechanism for the PCB-induced decrease in serum T_4 level, increase in hepatic drug-metabolizing enzymes might be considered. However, these are induced greater in the Wistar rats than in the Gunn rats, while magnitudes of decrease in serum T_4 level in Wistar and Gunn rats were almost the same. Accordingly, the KC500-induced decrease in serum T_4 level is thought to be independent on the KC500-induced drug-metabolizing enzymes, including UDT-GTs and CYPs.

As the factors regulating the level of serum total T_4, serum TSH, hepatic type-I iodothyronine deiodinase and T_4 transporting protein (TTR) are known. However, no significant change in the level of serum TSH occurs in the PCB-treated rats (Hallgren et al., 2001; Hood et al., 1999; Liu et al., 1995, Kato et al., 2004). Hepatic type-I iodothyronine deiodinase activity was significantly decreased in Wistar and Gunn rats by KC500 (Kato et al., 2004). On the other hand, TTR-associated pathway might be considered for explanation on PCB-induced decrease in level of serum total T_4, because PCB and its ring-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). Thus competitive inhibition by PCB and/or its metabloites would promote a decrease in the level of serum total T_4. In the present study, significant decrease in level of [^{125}I]T_4 bound to serum TTR and increase in level of [^{125}I]T_4 bound to serum albumin occurred in both KC500-pretreated Wistar and Gunn rats, suggesting that PCB and/or its metabolite(s) inhibit a formation of serum T_4-TTR complex.

Thus inhibition of the T_4-TTR formation might lead to change in tissue distribution of T_4. Therefore, to clarify this, we administered [^{125}I]T_4 to KC500-pretreated Wistar and

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Gunn rats and thereafter, determined the levels of [^{125}I]T_4 in their tissues. In addition, since [^{125}I]T_4 in either plasma or tissues is known to be stable during the 48 hr (Oppenheimer et al., 1968), the radioactivity detected in the serum and tissues would be attributed to [^{125}I]T_4 in each tissue. 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 KC500-pretreated rats. A tissue-to-serum concentration ratio (Kp value) was greater in several tissues, especially the liver, of the KC500-pretreated Wistar and Gunn rats than in the corresponding control (KC-untreated) rat tissues. In addition, in both KC500-pretreated Wistar and Gunn rats, more than 40% of the [^{125}I]T_4 dosed was accumulated in the liver.

In conclusion, the present findings confirmed that PCB-induced decrease in serum T_4 occurs not only in Wistar rats but also in Gunn (UGT1A-deficient) rats and further led to a hypothesis that the PCB-induced decrease occurs through increase in accumulation (transportation from serum to liver) of T_4 in the liver rather than through induction of hepatic T_4-UDP-GT. In addition, the increased accumulation in the liver might be attributed to the PCB- and its metabolite(s)-mediated inhibition of formation of serum T_4-TTR complex.

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Footnotes

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Legends for figures

FIG. 1. Effects of KC500 on levels of serum total thyroxine (T_4), free T_4, total triiodothyronine (T_3) and thyroid-stimulating hormone (TSH) in Wistar and Gunn rats. Animals were killed 4 days after the final administration of KC500 (10 mg/kg, ip, once daily for 10 days), and levels of serum thyroid hormones were measured as described in *Materials and Methods.* Constitutive levels: total T_4, 4.29 ± 0.38 (Wistar, N=5) and 5.80 ± 0.32 μg/dl (Gunn, N=5); free T_4, 2.17 ± 0.16 (Wistar, N=5) and 2.71 ± 0.17 ng/dl (Gunn, N=5); total T_3, 0.34 ± 0.03 (Wistar, N=6) and 0.96 ± 0.05 ng/ml (Gunn, N=4); TSH, 4.89 ± 0.33 (Wistar, N=5) and 7.48 ± 1.14 ng/ml (Gunn, N=5). Each column represents the mean ± SE (vertical bars) for five to six animals. **P*<0.01, significantly different from each control.

FIG. 2. Effects of KC500 on the activity of hepatic microsomal UDP-glucuronyltransferase in Wistar and Gunn rats. Each column represents the mean ± SE (vertical bars) for five to six animals. Constitutive levels: T_4-UDP-GT (pmol/mg protein/min), 14.17 ± 1.11 (Wistar) and 6.36 ± 1.34 (Gunn). **P*<0.01, significantly different from each control.

FIG. 3. Representive Western blot profiles for hepatic microsomal UGT isoforms in the KC500-treated Wistar and Gunn rats.

FIG. 4. Effects of KC500 on levels of hepatic microsomal UGT isoforms in Wistar and Gunn rats. The isolated bands responsible for UGT isoforms, which are shown in

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Figure 3, were densitometrically quantified as described in *Materials and Methods*. The data are represented as the mean ± SE (vertical bars) for five to six animals. **P*<0.05, significantly different from each control. ND, Not detectable.

FIG. 5. Effects of KC500 on the binding of [^{125}I]T_4 to serum proteins in Wistar rats. Amounts of [^{125}I]T_4 bound to the serum proteins were assessed by the method as described in *Materials and Methods*. Each column represents the mean ± SE (vertical bars) for three to six animals. **P*<0.05, significantly different from each control.

FIG. 6. Effects of KC500 on the binding of [^{125}I]T_4 to serum proteins in Gunn rats. Amounts of [^{125}I]T_4 bound to the serum proteins were assessed by the method as described in *Materials and Methods*. Each column represents the mean ± SE (vertical bars) for four to five animals. **P*<0.05, significantly different from each control.

FIG. 7. Effects of KC500 on the clearance of [^{125}I]T_4 from serum in Wistar and Gunn rats. 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 four to eight animals. **P*<0.001, significantly different from each control. －○－, control; －●－, KC500.

FIG. 8. Tissue-to-serum concentration ratio (Kp value) of [^{125}I]T_4 in various tissues after administration of [^{125}I]T_4 to the KC500-pretreated Wistar and Gunn rats

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KC500 (10 mg/kg) was given i.p. to animals once daily for 10 days, and then, the animals were administered i.v. with [^{125}I]T_4. At 60 min after administration of [^{125}I]T_4, the radioactivity in each tissue was measured. Each column represents the mean ± S.E. (vertical bars) for three to six animals. *P<0.05, significantly different from each control. □, control; ■, KC500.

FIG. 9. Tissue distribution of [^{125}I]T_4 after administration of [^{125}I]T_4 to the KC500-pretreated Wistar and Gunn rats. The experimental conditions were the same as described in Figure 8. Each column represents the mean ± S.E. (vertical bars) for four to six animals. *P<0.05, significantly different from each control. □, control; ■, KC500.

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Table 1. Effects of KC500 on the activity of hepatic microsomal alkoxyresorufin *O*-dealkylases in Wistar and Gunn rats

Alkoxyresolfin *O*-dealkylase activity (nmol/mg protein/min)	Wistar		Gunn	
	Control	KC500	Control	KC500
Substrates				
7-Benzyloxyresolfin	0.07 ± 0.01	3.34 ± 0.33*	0.03 ± 0.003	1.08 ± 0.27*
7-Pentoxyresolfin	0.03 ± 0.003	0.43 ± 0.05*	0.02 ± 0.003	0.22 ± 0.05*
7-Ethoxyresolfin	0.14 ± 0.01	9.02 ± 0.09*	0.21 ± 0.01	2.21 ± 0.29*

Animals were killed at 4 days after the final administration of KC500 (10 mg/kg, i.p., once daily for 10 days). The values shown are expressed as the mean ± S.E. for four to five animals. *$P<0.05$, 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 KC500-pretreated Wistar and Gunn rats

Animal	Treatment	Mean total body clearance × 100 (ml/min)	Distribution volume (ml)
Wistar	Control	7.82 ± 0.59	17.91 ± 0.52
	KC500	18.85 ± 3.49*	51.51 ± 6.34*
Gunn	Control	8.44 ± 0.22	20.21 ± 1.79
	KC500	13.84 ± 0.88*	48.91 ± 3.50*

The experimental conditions were the same as described in Figure 7. The values shown are expressed as the mean ± S.E. for four to seven animals. *P<0.05, significantly different from each control.

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Table 3. Liver weights after the administration of KC500 to Wistar and Gunn rats

	Liver weight (% of body weight)	
Animal	Control	KC500
Wistar	3.07 ± 0.04	3.81 ± 0.17*
Gunn	3.25 ± 0.08	3.38 ± 0.10

Animals were killed at 4 days after the final administration of KC500 (10 mg/kg, i.p., once daily for 10 days). The values shown are expressed as the mean ± S.E. for four to six animals. *P<0.01, significantly different from each control.

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Table 4. Accumulation of [^{125}I]T_4 in the KC500-pretreated Wistar and Gunn rat livers

	[^{125}I]T_4 (% of dose/g liver)	
Animal	Control	KC500
Wistar	3.86 ± 0.18	6.01 ± 0.24*
Gunn	4.74 ± 0.43	6.33 ± 0.62

The experimental conditions were the same as described in Figure 8. The values shown are expressed as the mean ± S.E. for four to six animals. *P<0.001, significantly different from each control.

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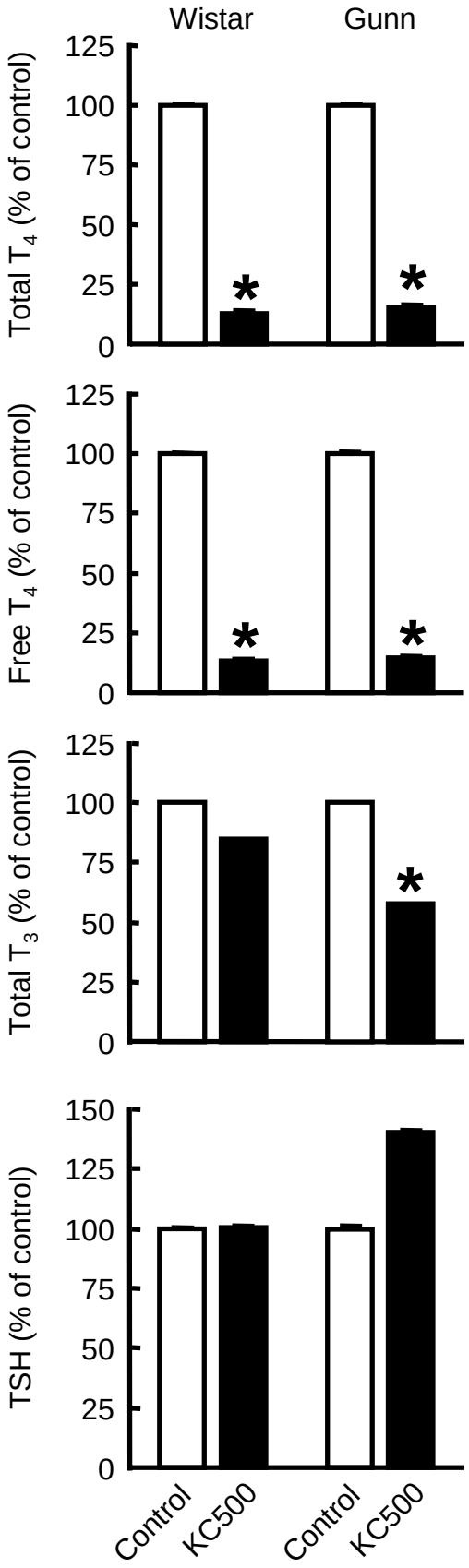

Fig. 1

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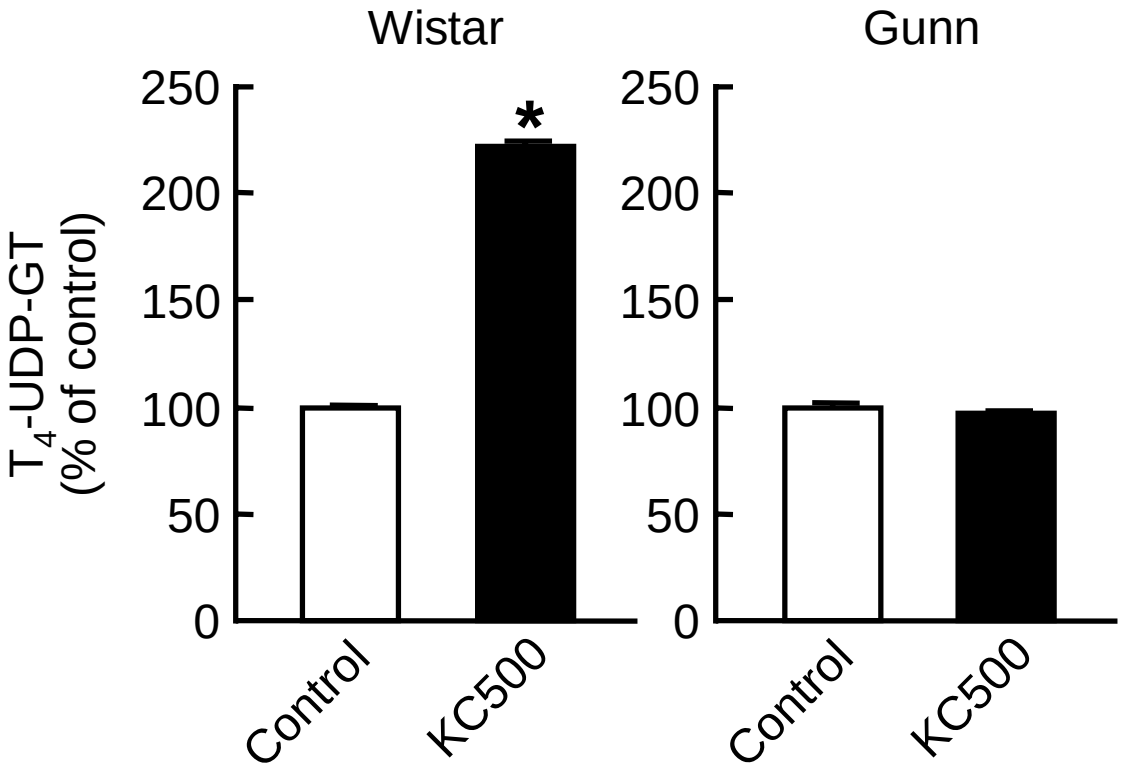

Fig. 2

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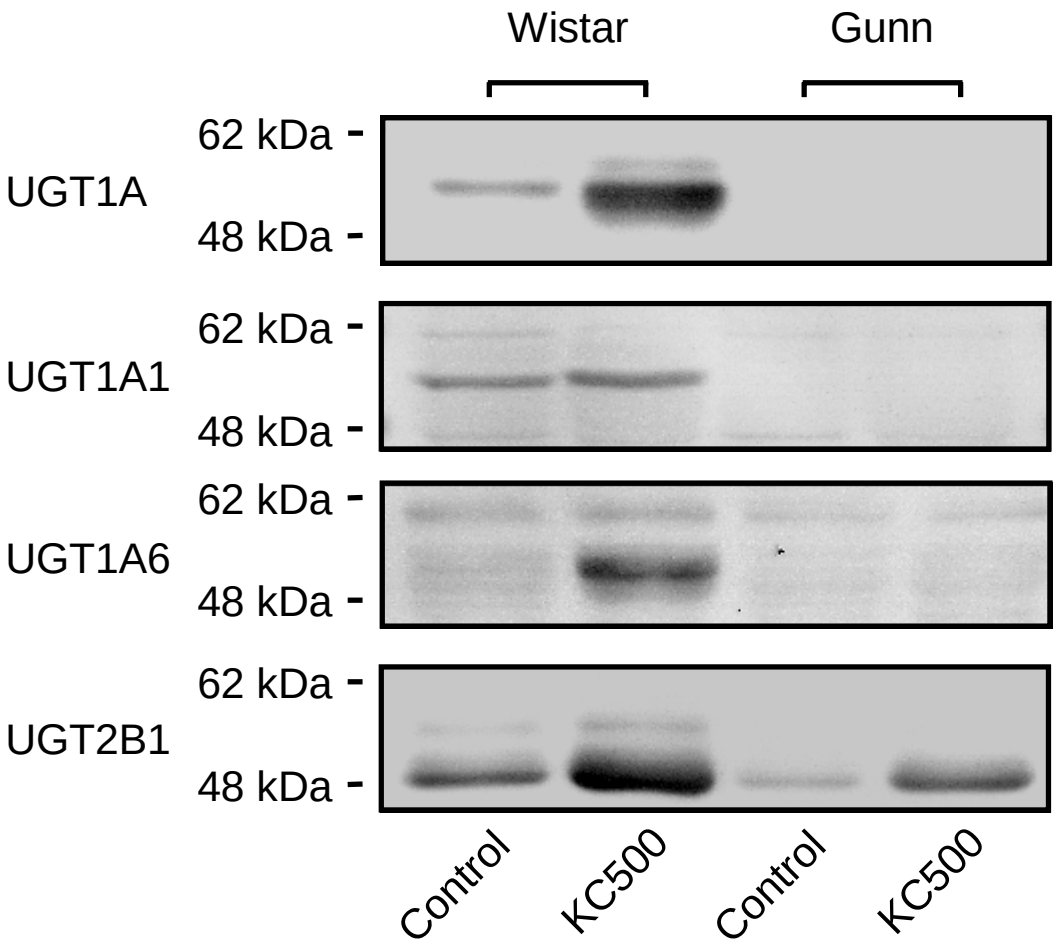

Fig. 3

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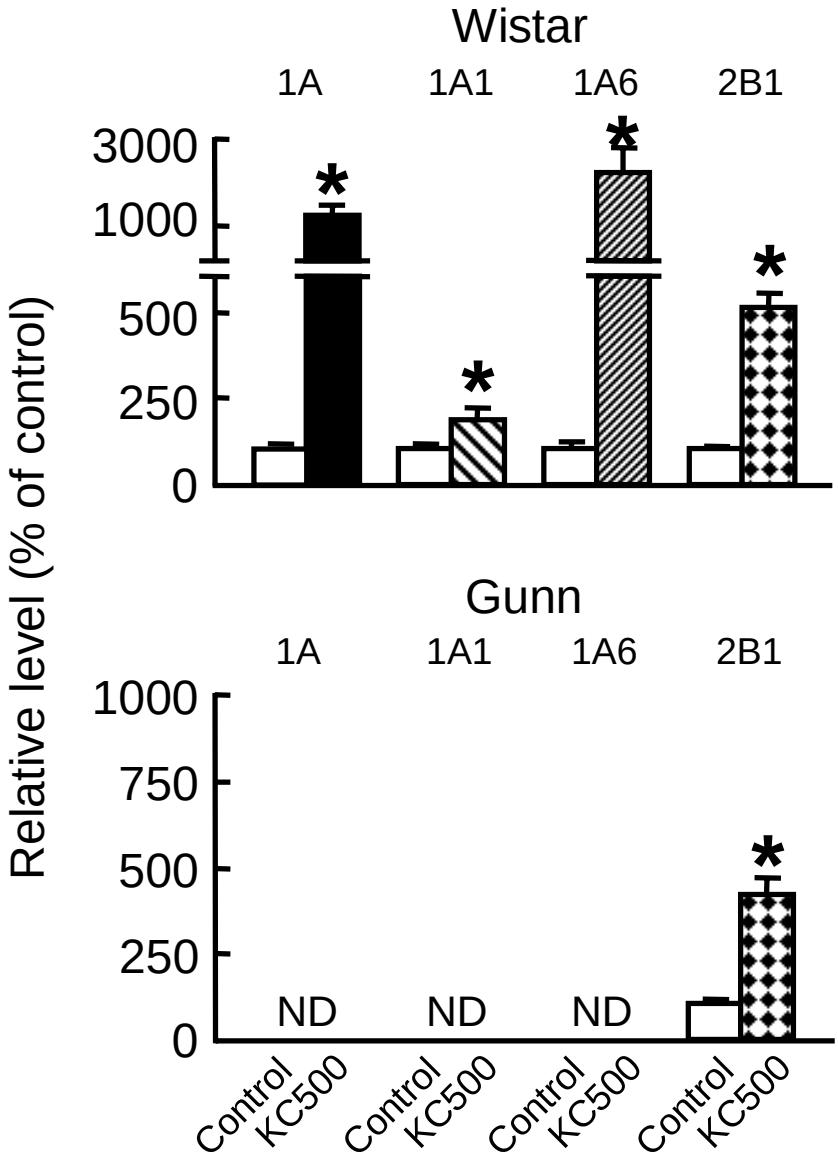

Fig. 4

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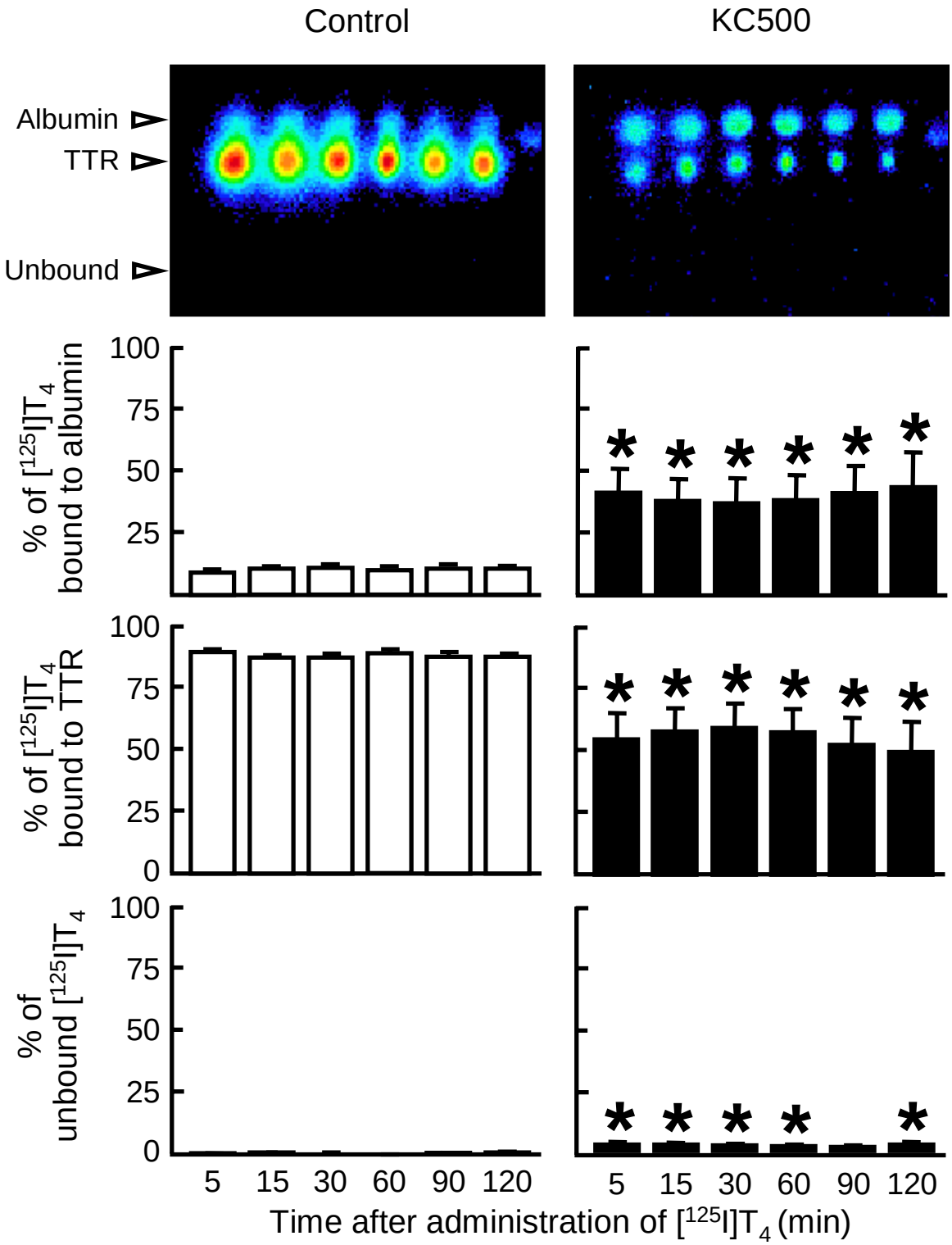

Fig. 5

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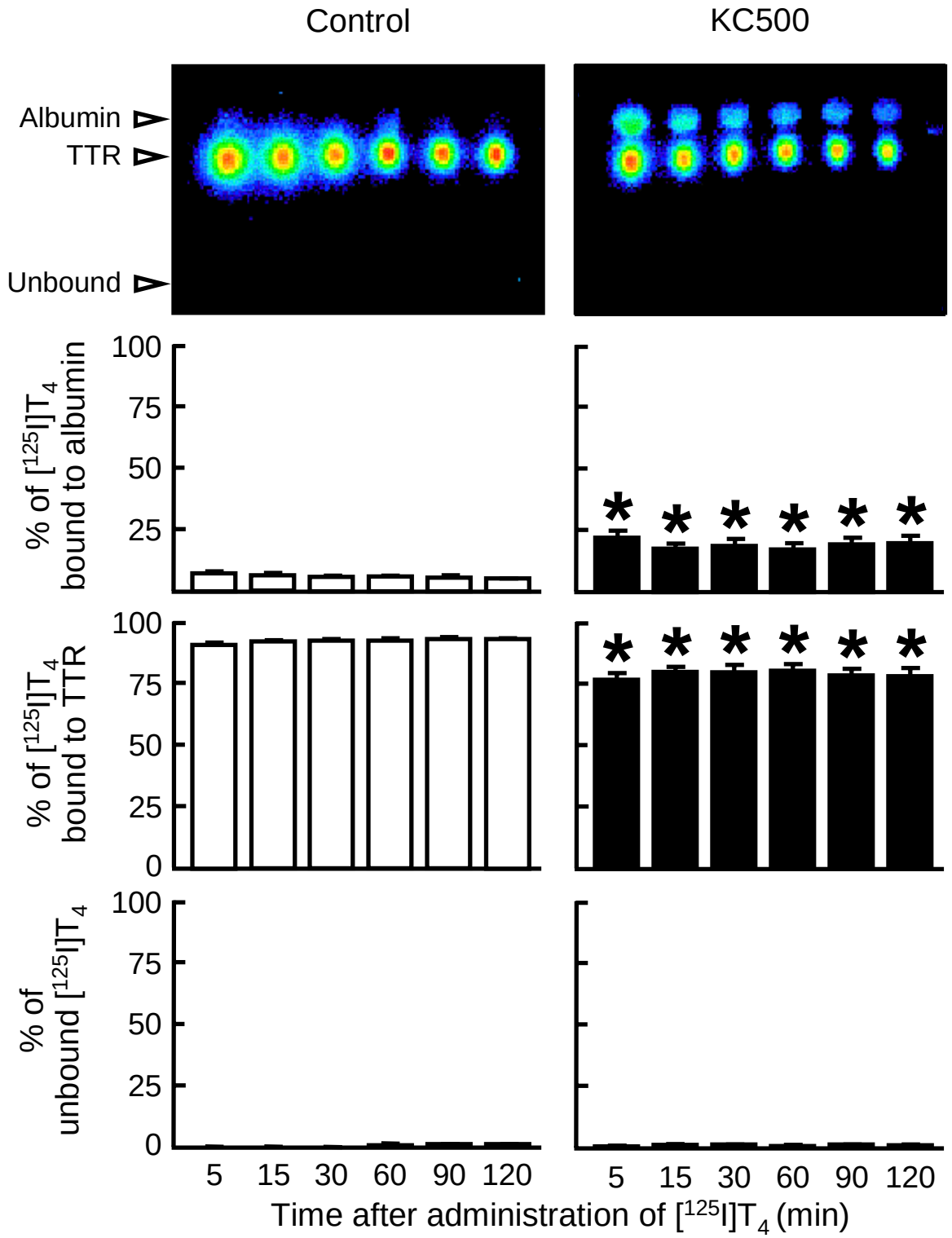

Fig. 6

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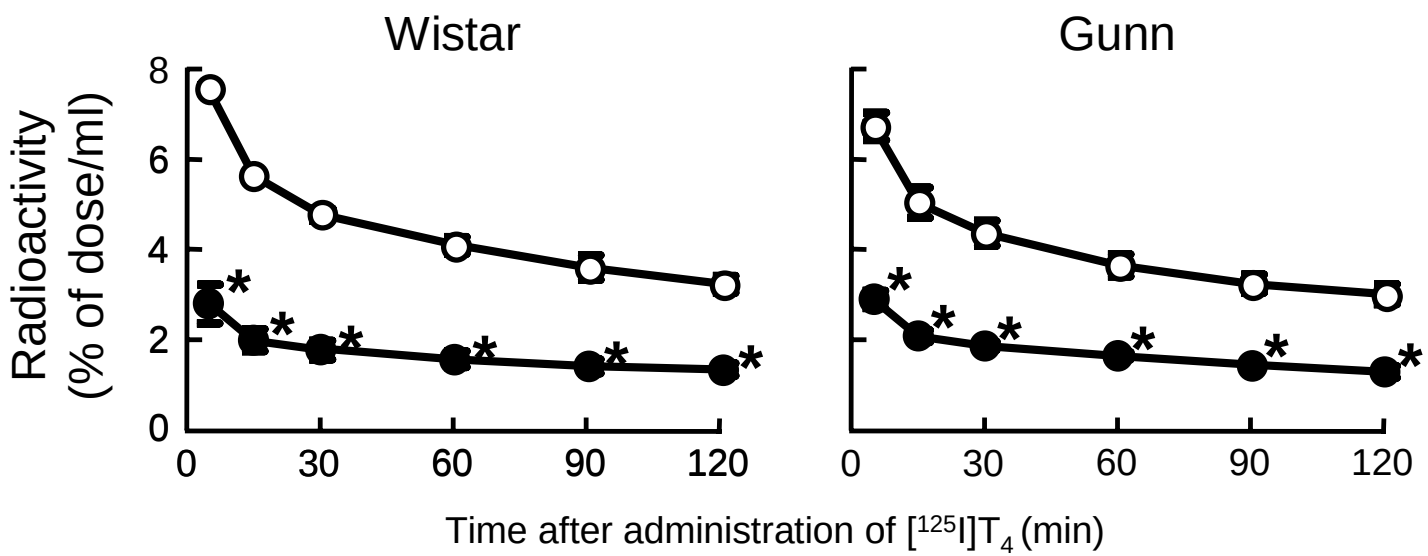

Fig. 7

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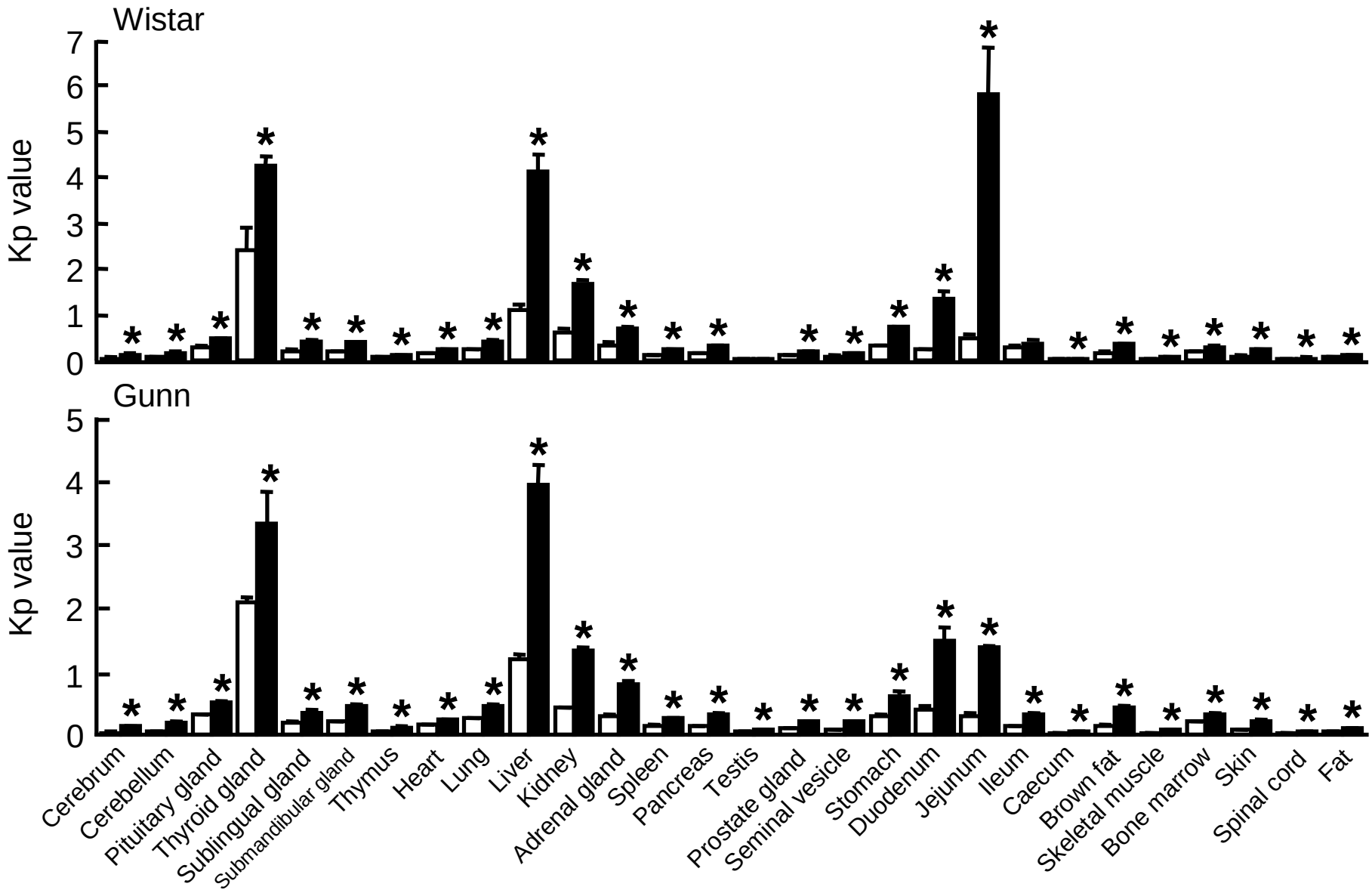

Fig. 8

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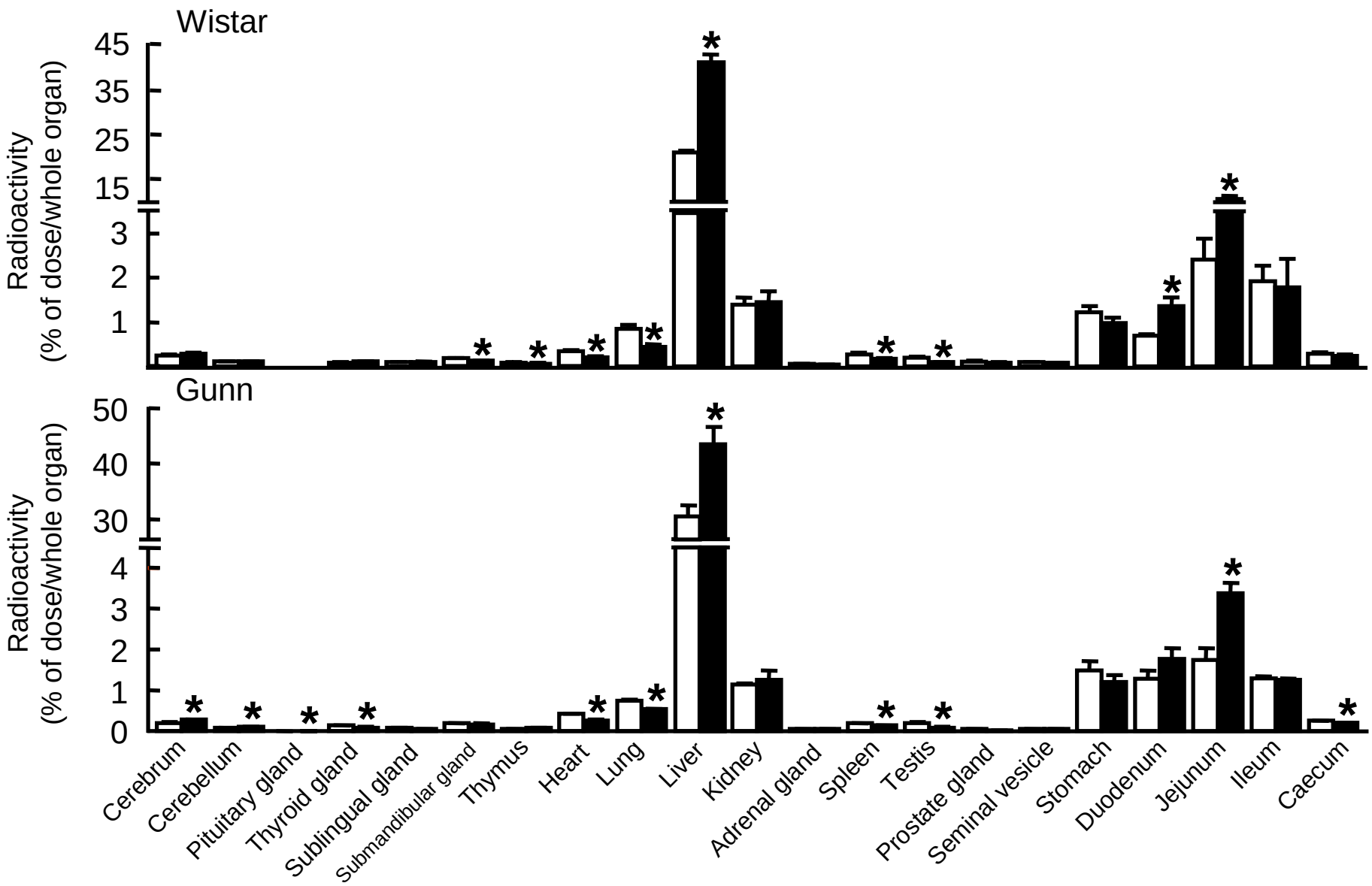

Fig. 9

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