DMD Fast Forward. Published on December 29, 2009 as doi:10.1124/dmd.109.031153

"DMD 31153"

Polychlorinated Biphenyl-Mediated Decrease in Serum Thyroxine Level in Rodents

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DMD Fast Forward. Published on December 29, 2009 as DOI: 10.1124/dmd.109.031153
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Running title: PCB-mediated decrease of serum T_4 level

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Number of text pages: 28

Number of tables: 3

Number of figures: 9

Number of references: 26

Number of words in the Abstract: 238

Number of words in the Introduction: 300

Number of words in the Discussion: 907

Abbreviations: PCB, polychlorinated biphenyl; KC500, Kanechlor-500; CB126, 3,3',4,4',5-pentachlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UDPGT, UDP-glucuronosyltransferase; T_4, thyroxine; T_3, triiodothyronine; TSH, thyroid-stimulating hormone; HPLC, high-performance liquid chromatography; TBG, thyroxine binding protein; TTR, transthyretin.

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Abstract

Effects of Kanechlor-500 (KC500), a commercial polychlorinated biphenyl mixture, on the levels of serum thyroid hormones such as total thyroxine (T_4) and triiodothyronine were examined in male mice, hamsters, rats and guinea pigs. Four days after a single intraperitoneal injection of KC500, significant decreases in the levels of the serum total T_4 and free T_4 occurred in all the animals examined, while a significant decrease in the level of serum triiodothyronine was observed only in guinea pigs among the animals examined. In addition, no significant change in the level of serum thyroid stimulating hormone was observed in any of the rodents examined. A significant increase in the activity of hepatic T_4-UDP-glucuronosyltransferase after the KC500 administration occurred only in guinea pigs, while the increase in the amount of biliary [^{125}I]T_4-glucuronide after an intravenous injection of [^{125}I]T_4 to the KC500-pretreated animals occurred only in rats. On the other hand, in all the rodents examined, KC500-pretreatment promoted the clearance of [^{125}I]T_4 from the serum and led to a significant increase in the steady state distribution volumes of [^{125}I]T_4. Likewise, its pretreatment raised the concentration ratio (Kp value) of the liver to serum and the liver distribution of [^{125}I]T_4 in all the rodents tested. The present findings for the first time indicate that the KC500-mediated decrease in the serum T_4 level in mice, hamsters, rats and guinea pigs occurs mainly through an increase in the accumulation level of T_4 in the liver.

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Introduction

There are known species differences among experimental animals in responses to polychlorinated biphenyl (PCB)-derived toxicities, including endocrine disruption, impairments of the reproductive and immune systems, and teratogenicity (Safe, 1994). The species differences might be attributed to the differences in the metabolic patterns of PCB congeners and/or the PCB-mediated induction of drug-metabolizing enzymes (Duigman et al., 1987, 1988).

In general, PCBs, including 3,3',4,4',5-pentachlorobiphenyl (CB126) and Aroclor 1254, have abilities to decrease serum thyroid hormone levels in rats and mice, and the decreases are thought to occur through the induction of thyroxine (T_4)-UDP-glucuronosyltransferases (UDPGTs) (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Craft et al., 2002), especially UGT1A1 and UGT1A6 (Visser, 1996). However, we have previously found that the PCB-mediated reduction of the serum T_4 level in rats and mice is not necessarily correlated with an increase in hepatic T_4 glucuronidation activity (Kato et al., 2003), that Kanechlor-500 (KC500)-treatment results in a significant decrease in the level of serum total T_4 not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007), and that the KC500-mediated decrease in rats occurs through an increase in the accumulation level of T_4 in the liver rather than an increase in hepatic T_4-UDPGT activity (Kato et al., 2007). To date, however, only limited data are available which would help to explain the mechanism of the PCB-mediated decrease in the level of serum thyroid hormone and its species difference.

In the present study, we examined the KC500-mediated biological alterations,

such as decreases in the levels of serum thyroid hormones, induction of hepatic T_4-UDPGT, and increase in hepatic accumulation level of T_4, in mice, hamsters, rats, and guinea pigs. On the basis of the obtained results, a mechanism underlying the PCB-mediated decrease in serum T_4 level was further discussed.

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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 (greater than 95% radiochemical pure as determined by HPLC, specific activity: 150 μCi /μg T_4), radiolabeled at the 5'-position of the outer ring, was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, 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 were obtained commercially at the highest grade of purity.

Animal Treatments. Male ddy mice (28-36 g), male Syrian hamsters (95-120 g), male Wistar rats (160-200 g), and male Hartley guinea pigs (400-540 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). They were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM to 8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%), and handled with animal care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received a single intraperitoneal injection of KC500 at the desired doses (6.25, 12.5, 25, 37.5, 50, and 100 mg/kg), and the other animals received an intraperitoneal injection of KC500 (37.5 or 100 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

In Vivo Study. All animals were killed by decapitation 4 days after the intraperitoneal 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°C until use. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at –50°C until use.

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

Hepatic microsomal T_4-UDPGT 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 UDPGT toward T_4 (T_4-UDPGT activity) was determined by the methods of Barter and Klaassen (1992).

Ex Vivo Study. Four days after intraperitoneal administration of KC500 the animals were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP8, SP10, and SP31; Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml). The bile duct was cannulated, and then the animal's

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body was warmed to 37°C. Fifteen minutes later, the animals were given intravenously [^{125}I]T_4 (15 μCi /ml) dissolved in saline containing 10 mM NaOH and 1 % normal animal serum. The doses of [^{125}I]T_4 were 0.1 ml for mice, 0.6 ml for hamsters, 1 ml for rats and 2 ml for guinea pigs, respectively. These dose of [^{125}I]T_4 administered was calculated on the base of the dose used for rats by Vansell and Klaassen (2001).

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

Biliary excretion of [^{125}I]T_4. After the administration of [^{125}I]T_4, bile was collected in glass tube on ice for 2 h at 30-min intervals. Bile volume was determined gravimetrically. For analysis of biliary total [^{125}I]T_4 level, two aliquots (10-30 μl each) were taken from each bile sample for determination of [^{125}I]T_4 level by a gamma counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences). The amount of biliary [^{125}I]T_4 glucuronide was determined with high-performance liquid chromatography (HPLC) as described by Vansell and Klaassen (2001). In brief, a portion (10-20 μl) of bile was added to 2 volumes of methanol and kept at -20°C for 1 h to precipitate protein. After the mixture was centrifuged at 12,000 g (4°C) for 10 min, the resultant supernatant was collected for HPLC analysis. The HPLC analysis was performed using a ChromSpher C18 column (10×0.3 cm) (Chrompack, Inc., Raritan,

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NJ) in combination with both a ChromSep reverse-phase guard column (10×2 mm) (Chrompack, Inc.) and Adsorbosphere C18 reverse-phase guard column (7.5×4.6 mm) (Alltech Associates, Deerfield, IL). Then, 0.02 M ammonium acetate, pH4.0, containing 16 to 45% of acetonitrile solution was used for elution of [^{125}I]T_4 glucuronide; 16% of acetonitrile was used as the initial solution for 6 min, and then the elution solution was changed by a linear increase to 27% over 12 min, held for 4 min, followed by a linear increase to 45% over 5 min and held for 11 min. The levels of biliary [^{125}I]T_4 glucuronide were determined by a Radioisotope Detector 171 (Beckman Coulter, Fullerton, CA).

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

Analysis of [^{125}I]T_4 bound to serum proteins. The levels of serum [^{125}I]T_4-thyroxine binding protein (TBG), [^{125}I]T_4-albumin, and [^{125}I]T_4-transthyretin ([^{125}I]T_4-TTR) complexes were determined according to the method of Davis et al. (1970). In brief, serum was diluted in 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and the diluted serum was subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels (PAG Mid "Daiichi" 4/20; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The electrophoresis was performed at 4°C for

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11 h at 20 mA in 0.025 M Tris buffer, pH 8.4 containing 0.192 M glycine. The human albumin and TTR incubated with [^{125}I]T_4 were also applied on the gel as references. After the electrophoresis, the gel was dried and autoradiographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd.). The levels of [^{125}I]T_4-TBG, [^{125}I]T_4-albumin, and [^{125}I]T_4-TTR in serum were determined by counting the corresponding gel fractions identified with the Bio Imaging Analyzer (BAS-2000II IP Reader; Fuji Photo Film Co., Ltd.).

Tissue distribution of [^{125}I]T_4. Tissue distribution of [^{125}I]T_4 was assessed according to the modified method of Oppenheimer et al. (1968). In brief, at 60 min after administration of [^{125}I]T_4 to KC500-pretreated animals, blood was sampled from the abdominal aorta. Then, the cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, cecum, 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 gamma counter (COBRA II AUTO-GAMMA 5002; Perkin Elmer Life and Analytical Sciences), and amounts of [^{125}I]T_4 in the tissues were calculated as ratios to the amount in serum.

Statistics. The data obtained were statistically analyzed according to the Student's *t* test or Dunnett's test after analysis of variance. In addition, clearance of [^{125}I]T_4 from the serum, 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 the Newman-Keuls test after

analysis of variance. The pharmacokinetic parameters of [^{125}I]T_4 were estimated with noncompartmental methods as described previously (Tabata et al., 1999).

RESULTS

Serum Hormone Levels. A dose effect of KC500 on the level of serum total T_4 was first examined in mice 4 days after the chemical treatment (Figure 1). Serum total T_4 levels were significantly decreased by the treatment with KC500 at doses of over 25 mg/kg, and the decrease occurred in a dose-dependent fashion up to 100 mg/kg. The 50% effective dose (ED_{50}) of KC500 for decreasing the level of serum total T_4 was about 37.5 mg/kg. Therefore, 37.5 and/or 100 mg/kg were selected as doses of KC500 in the present experiments.

Treatments of mice, hamsters, rats and guinea pigs with KC500 at a dose of 37.5 mg/kg decreased the total T_4 levels to 63%, 43%, 35% and 27%, respectively, of the corresponding controls (Figure 2). Treatment at a dose of 100 mg/kg resulted in a more effective decrease in the rodents, with the exception of guinea pigs. Likewise, the serum free T_4 level was markedly decreased by KC500 (37.5 mg/kg) in all the rodents examined: 56% of control in mice, 43% of control in hamsters, 24% of control in rats, and 24% of control in guinea pigs. The decrease in each animal species was greater when the KC500 treatment was used at the higher dose (100 mg/kg).

A significant decrease in serum total T_3 level by the treatment with KC500 at a dose of 37.5 mg/kg occurred only in guinea pigs among the species of animals examined (Figure 3). On the other hand, no significant change in the level of serum TSH by the KC500 treatment was observed in any animals examined (Figure 3).

Hepatic T_4-UDPGT. The effects of KC500 on hepatic microsomal T_4-UDPGT activity were examined in mice, hamsters, rats and guinea pigs. A significant increase in the

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activity of hepatic T_4-UDPGT by the treatment with KC500 at a dose of 37.5 mg/kg was observed in only guinea pigs among the rodents examined (Figure 4).

Biliary Excretion of [^{125}I]T_4 Glucuronide. Effects of pretreatment of KC500 (37.5 mg/kg) on biliary excretion of T_4-glucuronide were examined in mice, hamsters, rats and guinea pigs. After intravenous injection of [^{125}I]T_4 to the KC500 -pretreated animals, biliary excretion levels of T_4-glucuronide were measured. A significant increase in the biliary excretion level was observed only in rats among the species of animals examined (Figure 5).

Clearance of [^{125}I]T_4 from Serum. After an intravenous administration of [^{125}I]T_4 to the KC500 (37.5 or 100 mg/kg)-pretreated mice, hamsters, rats and guinea pigs, serum concentrations of [^{125}I]T_4 in the animals were measured at the indicated times (Figure 6). KC500 (100 mg/kg)-pretreatment clearly enhanced the clearance of [^{125}I]T_4 from the serum in all the animals tested. Within 5 min after the administration of [^{125}I]T_4, concentration of serum [^{125}I]T_4 in mice, hamsters, rats and guinea pigs were about 69%, 32%, 28% and 54% of the corresponding control levels, respectively, and the decreases remained up to 120 min later. When the animals were pretreated with KC500 at a dose of 37.5 mg/kg, clear promotion of the clearance of [^{125}I]T_4 was observed in the animals with the exception of mice.

The serum pharmacokinetic parameters of the [^{125}I]T_4 estimated from these data (Figure 6) were summarized in Table 1. The mean total body clearance of [^{125}I]T_4 and steady-state volumes of distribution in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats and guinea pigs increased, as compared with the corresponding control

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animals, although these increases were observed in the KC500 (37.5 mg/kg)-pretreated rats and guinea pigs but not in the mice and hamsters. The steady-state volumes of distribution in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats and guinea pigs increased to 1.5-times, 4.0-times, 4.1-times and 1.8-times over the corresponding control animals, respectively (Table 1).

Tissue Distribution of [^{125}I]T_4. Effects of KC500 (100 mg/kg)-pretreatment on the tissue-to-serum concentration ratio (Kp value) and the distribution level of [^{125}I]T_4 in tissues after the administration of [^{125}I]T_4 were examined in mice, hamsters, rats and guinea pigs. In all animals examined, the thyroid gland, liver, kidney, stomach, duodenum and jejunum had Kp values over 1 (Figure 7), and the Kp value was the greatest in the thyroid gland and liver among the examined tissues, with the exception of kidney in guinea pigs. Pretreatment with KC500 resulted in significant increases in the Kp values of thyroid gland, liver, kidney and duodenum in all the animals examined (Figure 7).

In KC500-untreated (control) each species of animals, the accumulation level of [^{125}I]T_4 in the liver was the highest among the tissues examined (Figure 8). In all the animals examined, pretreatment with KC500 (100 mg/kg) resulted in significant increases in the accumulation level of [^{125}I]T_4 in the liver. More than 34%, 55%, 58% and 17% of the [^{125}I]T_4 dosed were accumulated in the liver in the KC500-pretreated mice, hamsters, rats and guinea pigs, respectively (Figure 8). In addition, the accumulation level per g of liver was also increased in the KC500-pretreated mice, hamsters, rats and guinea pigs, as compared with the corresponding control animals (Table 2). Furthermore, KC500-pretreatment led to significant increases in the liver

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weight in mice, rats, and guinea pigs, but not in hamsters (Table 3).

Serum Proteins Bound to [^{125}I]T_4. The effects of pretreatment with KC500 (100 mg/kg) on the binding of [^{125}I]T_4 to serum proteins, such as TBG, albumin, and TTR, were examined in mice, hamsters, rats and guinea pigs (Figure 9). In KC500-pretreated hamsters, the level of serum [^{125}I]T_4-TTR complex slightly decreased, while the binding level of [^{125}I]T_4 to serum albumin slightly increased. In mice, no such effects of KC500-pretreatment were observed. In rats and guinea pigs, the KC500 pretreatment resulted in significant decreases in the level of [^{125}I]T_4-TTR complex, while it led to significant increases in the level of [^{125}I]T_4 bound to albumin. In addition, most of [^{125}I]T_4 released from [^{125}I]T_4-TTR complex in KC500-pretreated animals, with the exception of guinea pigs, bound to serum albumin. In the KC500-pretreated guinea pigs, most of the released [^{125}I]T_4 was detected as free (serum protein-unbound) [^{125}I]T_4.

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Discussion

In the present study, we found that treatment with KC500 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 not only in rats but also in mice, hamsters, and guinea pigs. Incidentally, we have previously reported the KC500-induced decreases in the level of serum total T_4 in the Wistar and Gunn rats (Kato et al., 2004, 2007).

As a possible explanation for the PCB-induced decrease in serum thyroid hormones, a hepatic T_4-UDPGT-dependent mechanism is generally considered because T_4-UDPGT inducers, such as Aroclor 1254, TCDD, and CB126, show strong activities for decreasing the levels of serum total thyroid hormones in rats (Barter and Klaassen, 1994; Schuur et al., 1997; Van Birgelen et al., 1995). However, between mice and rats treated with a T_4-UDPGT inducer, the differences in magnitude of the decreases in the level of serum total T_4 is not necessarily correlated with that of hepatic T_4-UDPGT activity (Craft et al., 2002, Hood et al., 2003, Kato et al., 2003). More recently, we have demonstrated that KC500 treatment resulted in significant decreases in the level of serum total T_4 not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007) and further indicated that the KC500-mediated decrease in rats occurred through an increase in the accumulation of T_4 in several tissues, especially the liver, rather than through an increase in hepatic T_4-UDPGT activity (Kato et al., 2007). In addition to the previous results, we herein showed that the activity of hepatic T_4-UDPGT was changed very little by KC500-treatment in mice, hamsters, and rats, although a KC500-mediated decrease in the serum total T_4 level was observed in

all the species of animals examined. In addition, no significant changes in the excretion level of biliary T_4-glucuronide were observed in KC500-pretreated mice, hamsters, and guinea pigs. All the results obtained herein strongly suggest that the KC500-induced decrease in the serum T_4 level in mice, hamsters, rats, and guinea pigs primarily occurs in a T_4-UDPGT-independent manner.

KC500 treatment led to no significant change in the level of serum TSH in mice, hamsters, rats, and guinea pigs, although serum TSH is considered as one of the factors regulating the level of serum total T_4. These results are similar with those in previous reports on the effect of PCBs on the level of serum TSH in rats (Hallgren et al., 2001; Hood et al., 1999; Liu et al., 1995, Kato et al., 2004, 2007).

The factors regulating the level of serum total T_4, hepatic type-I iodothyronine deiodinase and sulfotransferase are also known. However, hepatic type-I iodothyronine deiodinase activity was significantly decreased by KC500 in rats and hamsters, and furthermore, no significant change in the enzyme activity by treatment with KC500 occurred in either mice or guinea pigs (data not shown). No significant change in the activity of hepatic sulfotransferase was also observed in the KC500-treated mice, hamsters, rats, and guinea pigs (data not shown). Therefore, a KC500-mediated decrease in the serum T_4 level seems to occur in the type-I iodothyronine deiodinase- and sulfotransferase-independent pathways.

As another possible mechanism for the PCB-induced decrease in the level of serum total T_4, a TTR-associated pathway might also be considered because PCB and its ring-hydroxylated metabolites act as T_4 antagonists to TTR (Lans et al., 1993; Brouwer et al., 1998; Meerts et al., 2002; Kato et al., 2004). Thus competitive inhibition by PCB and/or its metabolites might decrease the level of serum total T_4 through an

increase in the level of free T_4 and promotion of T_4 excretion. However, no such competitive inhibition by KC500 was observed in ddy mice, although the significant 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 occurred in KC500-pretreated hamsters, rats, and guinea pigs. In hamsters, rats, and guinea pigs, but not mice, KC500-mediated inhibition of the T_4-TTR formation might lead to changes in the tissue distribution of T_4. Therefore, to clarify this, we administered [^{125}I]T_4 to KC500-pretreated mice, hamsters, rats and guinea pigs and measured the levels of [^{125}I]T_4 in their tissues. Marked increases in the mean total body clearance of [^{125}I]T_4 and in the steady state distribution volume of [^{125}I]T_4 were observed in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats, and guinea pigs. Similar increases were demonstrated to occur in KC500 (intraperitoneal injection at a dose of 10 mg/kg once daily for 10 days)-pretreated rats (Kato et al., 2007). The tissue-to-serum concentration ratio (Kp value) was greater in the tissues, especially thyroid gland, liver, kidney, stomach and small intestine, of KC500-pretreated animals than in those of the corresponding control animals. In addition, more than 34%, 55%, 58% and 17% of the [^{125}I]T_4 dosed were accumulated in the liver of the KC500-pretreated mice, hamsters, rats, and guinea pigs, respectively.

In conclusion, we demonstrate for the first time that a KC500-mediated decrease in serum T_4 occurs not only in rats (Kato et al., 2004, 2007), but also in mice, hamsters and guinea pigs and further propose a hypothesis that the PCB-induced decrease occurs through an increase in accumulation (transportation from serum to liver) of T_4 in the liver rather than through induction of hepatic T_4-UDPGT. Furthermore, we suggest that the increased accumulation in the liver is attributed, at least in part, to the PCB- and its metabolite(s)-mediated inhibition of formation of the

serum T_4-TTR complex.

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Footnotes

This work was supported in part by the Grant-in-Aid for Scientific Research (C) (no. 20510070, Y.K.) and for Scientific Research (B) (no. 19310042, K.H., Y.K.) from Japan Society for the Promotion of Science.

Legends for figures

Fig. 1. Effect of graded doses of KC500 on the level of serum total T_4 in mice. Mice were killed 4 days after the intraperitoneal administration of KC500 at the various doses indicated, and the level of serum total T_4 was measured as described in *Materials and Methods*. Constitutive level: 3.24 ± 0.25 μg/dl (*n*=5). Each point represents the mean ± S.E. (vertical bars) for four to six mice. **P*<0.05, significantly different from the control.

Fig. 2. Effects of KC500 on the levels of serum total T_4 and free T_4 in animals. Animals were killed 4 days after the intraperitoneal administration of KC500 (37 and 100 mg/kg), and levels of serum thyroid hormones were measured as described in *Materials and Methods*. Constitutive levels: total T_4, 2.98 ± 0.17 (mice, *n*=8), 2.67 ± 0.17 (hamsters, *n*=8), 3.73 ± 0.32 (rats, *n*=6) and 2.05 ± 0.16 μg/dl (guinea pigs, *n*=5); free T_4, 0.43 ± 0.05 (mice, *n*=8), 1.08 ± 0.08 (hamsters, *n*=8), 1.47 ± 0.11 (rats, *n*=6) and 1.44 ± 0.16 ng/dl (guinea pigs, *n*=5). Each column represents the mean ± S.E. (vertical bars) for four to eight animals. **P*<0.05, significantly different from each control.

Fig. 3. Effects of KC500 on the levels of serum total T_3 and TSH in animals. Animals were killed 4 days after the intraperitoneal administration of KC500 (37 mg/kg), and levels of serum thyroid hormones were measured as described in *Materials and Methods*. Constitutive levels: total T_3, 0.38 ± 0.05 (mice, *n*=6), 0.46 ± 0.02 (hamsters, *n*=6), 0.59 ± 0.09 (rats, *n*=6) and 0.25 ± 0.02 ng/ml (guinea pigs, *n*=6); TSH, 3.50 ±

0.19 (mice, *n*=6), 3.46 ± 0.22 (hamsters, *n*=6), 5.45 ± 0.61 (rats, *n*=6) and 2.40 ± 0.07 ng/ml (guinea pigs, *n*=5). Each column represents the mean ± S.E. (vertical bars) for five to six animals. **P*<0.05, significantly different from each control.

Fig. 4. Effect of KC500 on the activity of hepatic microsomal T_4-UDP-GT in animals. Animals were killed 4 days after the intraperitoneal administration of KC500 (37 mg/kg), and hepatic microsomes from individual animals were used for the T_4-UDP-GT enzyme assay, as described in *Materials and Methods*. Constitutive levels: T_4-UDP-GT, 21.22 ± 1.02 (mice, *n*=6), 21.83 ± 2.36 (hamsters, *n*=6), 15.39 ± 2.96 (rats, *n*=6) and 25.15 ± 1.04 pmol/mg protein/min (guinea pigs, *n*=6). Each column represents the mean ± S.E. (vertical bars) for five to six animals. **P*<0.05, significantly different from each control.

Fig. 5. Effect of KC500 on the amount of the biliary [^{125}I]T_4-glucuronide in animals. KC500 (37.5 mg/kg) was intraperitonealy given to animals, and 96 h after the KC500-treatment, a portion of [^{125}I]T_4 (15 μCi/ml) was further intravenously administered to animals, as described in *Materials and Methods*. The level of [^{125}I]T_4-glucuronide excreted was measured in bile collected at 30-min intervals after the intravenous administration of [^{125}I]T_4. Each point represents the mean ± S.E. (vertical bars) for three to seven animals. **P*<0.05, significantly different from each control. —○—, control; —●—, KC500.

Fig. 6. Effects of KC500 on the clearance of [^{125}I]T_4 from serum in animals. KC500 (37.5 and 100 mg/kg) was intraperitonealy given to animals, and 96 h after the

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KC500-treatment, a portion of [^{125}I]T_4 (15 μCi/ml) was further intravenously administered to the animals, as described in *Materials and Methods*. The amount of serum [^{125}I]T_4 was measured at the indicated times after the intravenous administration of [^{125}I]T_4. Each point represents the mean ± S.E. (vertical bars) for four to eight animals. **P*<0.05, significantly different from each control. —○—, control; —●—, KC500 37.5 mg/kg; —▼—, KC500 100 mg/kg.

Fig. 7. Tissue-to-serum concentration ratio (Kp value) of [^{125}I]T_4 in various tissues after administration of [^{125}I]T_4 to KC500-pretreated animals. KC500 (100 mg/kg) was intraperitonealy given to animals, and 96 h after the KC500-treatment, [^{125}I]T_4 was further intravenously administered to the animals. At 60 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 three to six animals. **P*<0.05, significantly different from each control. □ , control; ■ , KC500.

Fig. 8. Tissue distribution of [^{125}I]T_4 after administration of [^{125}I]T_4 to KC500-pretreated animals. Experimental protocols were the same as those described in the legend of Fig. 7. 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. Effect of KC500 on the binding of [^{125}I]T_4 to serum proteins in animals. KC500 (100 mg/kg) was intraperitonealy given to animals, and 96 h after the KC500-treatment, [^{125}I]T_4 was further intravenously administered to the animals. The amounts of [^{125}I]T_4 bound to the serum proteins 60 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 three to four animals. *P<0.05, significantly different from each control.

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Table 1. Pharmacokinetic parameters for [^{125}I]T_4 after the administration of [^{125}I]T_4 to the KC500-pretreated animals

Animal	Pretreatment	Dose (mg/kg)	Mean total body clearance × 100 (ml/min)	Distribution volume (ml)
Mice	Control		1.56 ± 0.15	4.46 ± 0.25
	KC500	37.5	1.65 ± 0.20	4.69 ± 0.31
	KC500	100	2.66 ± 0.22*	6.52 ± 0.51*
Hamsters	Control		3.63 ± 0.32	10.46 ± 0.53
	KC500	37.5	5.35 ± 0.93	25.76 ± 1.21*
	KC500	100	8.19 ± 1.24*	42.04 ± 4.50*
Rats	Control		7.83 ± 0.39	15.62 ± 0.68
	KC500	37.5	13.23 ± 1.46*	31.81 ± 3.31*
	KC500	100	30.75 ± 3.27*	64.06 ± 4.58*
Guinea pigs	Control		13.73 ± 0.91	35.70 ± 2.77
	KC500	37.5	20.65 ± 1.54*	54.70 ± 4.70*
	KC500	100	23.42 ± 2.17*	65.42 ± 4.98*

The pharmacokinetic parameters of [^{125}I]T_4 were calculated from the data in Figure 6 with noncompartmental methods as described previously (Tabata et al., 1999). The values shown expressed as the mean ± S.E. for four to eleven animals. *P<0.05 significantly different from each control.

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Table 2. Accumulation of [^{125}I]T_4 in the KC500-pretreated mice, hamsters, rats and guinea pigs livers

	[^{125}I]T_4 (% of dose/g liver)	
Animal	Control	KC500
Mice	10.03 ± 0.13	15.34 ± 0.90*
Hamsters	6.93 ± 0.59	10.41 ± 1.03*
Rats	2.75 ± 0.12	3.95 ± 0.33*
Guinea pigs	0.45 ± 0.02	0.76 ± 0.05*

The radioactivity in the liver was measured at 60 min after the [^{125}I]T_4-administration, as described in *Materials and Methods*. Accumulation levels of the liver in the control mice, hamsters, rats, and guinea pigs were 292620 ± 8873 (*n*=4), 577975 ± 51307 (*n*=4), 568665 ± 16375 (*n*=6), and 121496 ± 4234 (*n*=4) cpm/g wet liver, respectively. The values shown are expressed as the mean ± S.E. for four to six animals. *P<0.05, significantly different from each control.

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Table 3. Liver weights after the administration of KC500 to animals

	Relative liver weight (% of body weight)	
Animal	Control	KC500
Mice	5.08 ± 0.09	5.74 ± 0.21*
Hamsters	4.27 ± 0.30	4.64 ± 0.33
Rats	3.74 ± 0.08	4.65 ± 0.17*
Guinea pigs	3.81 ± 0.17	4.36 ± 0.11*

Animals were killed 4 days after the intraperitoneal administration of KC500 (100 mg/kg), and the liver weight was measured. The values shown are expressed as the mean ± S.E. for four to six animals. *P<0.05, significantly different from each control.

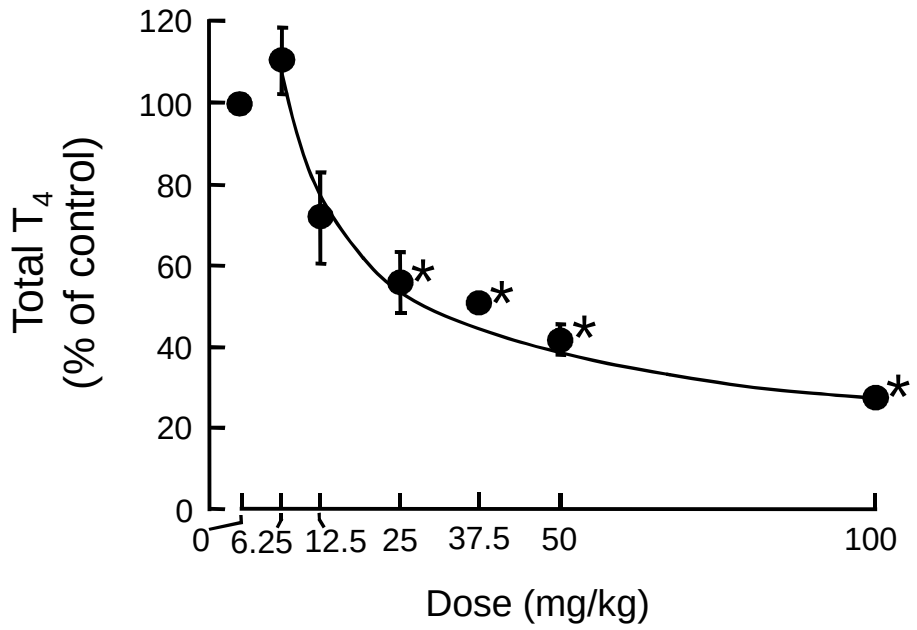

Fig. 1

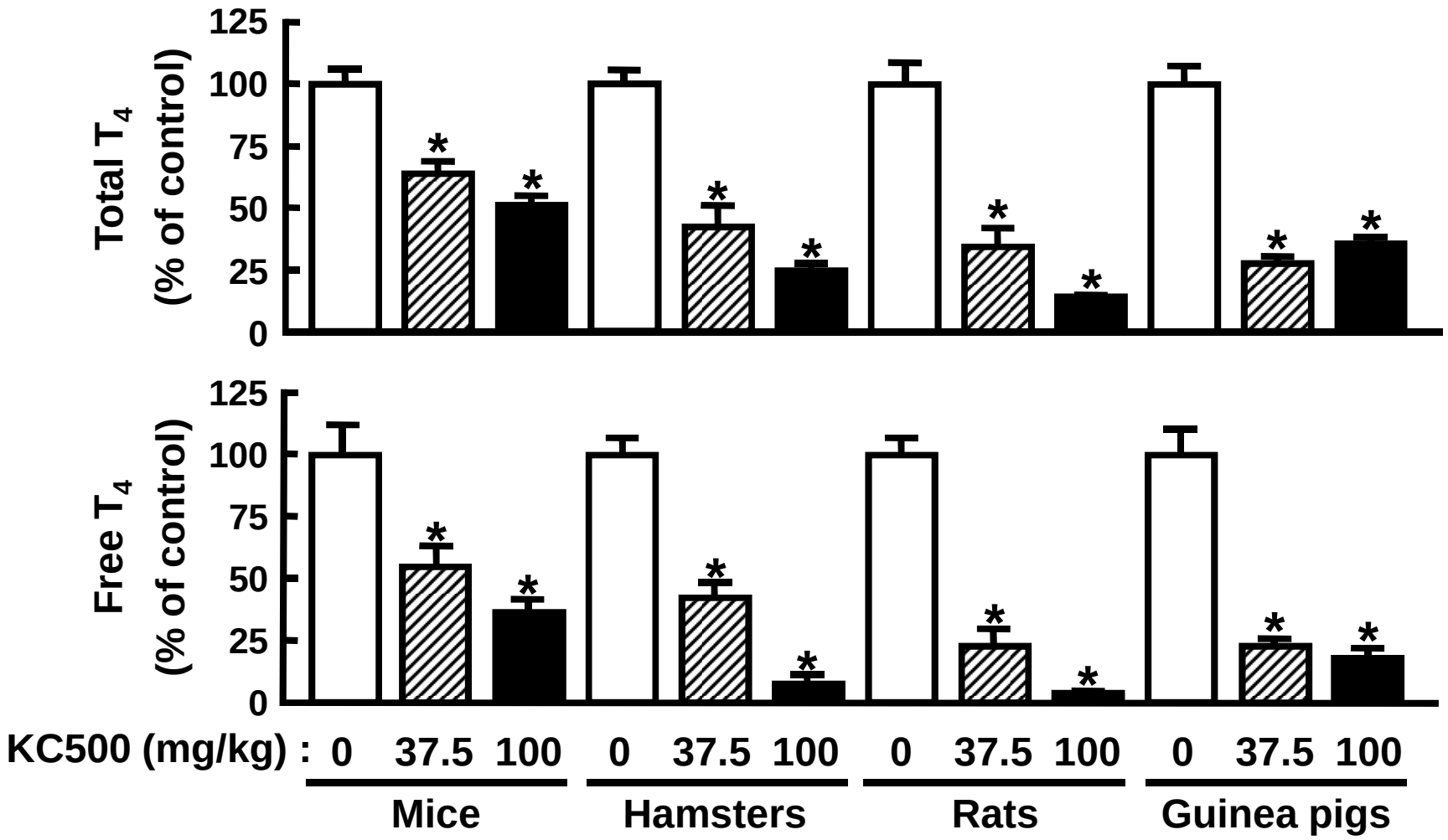

Fig. 2

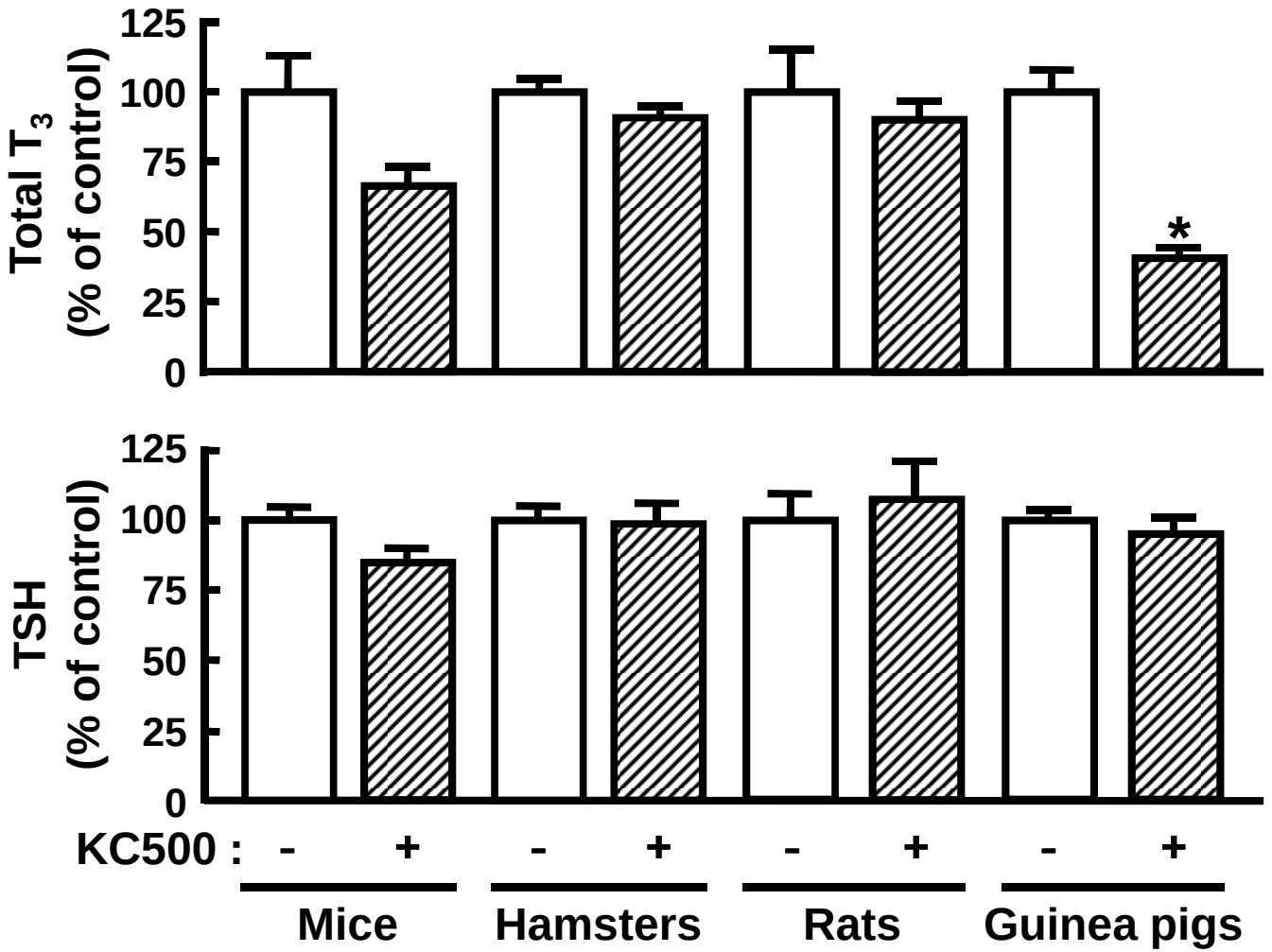

Fig. 3

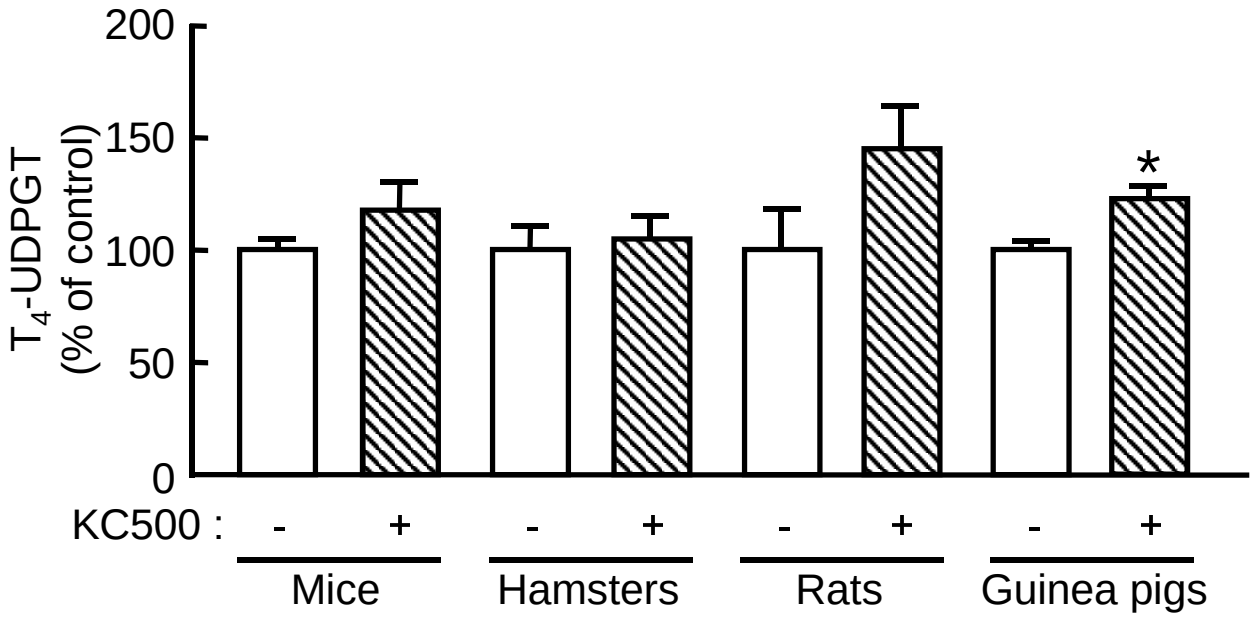

Fig. 4

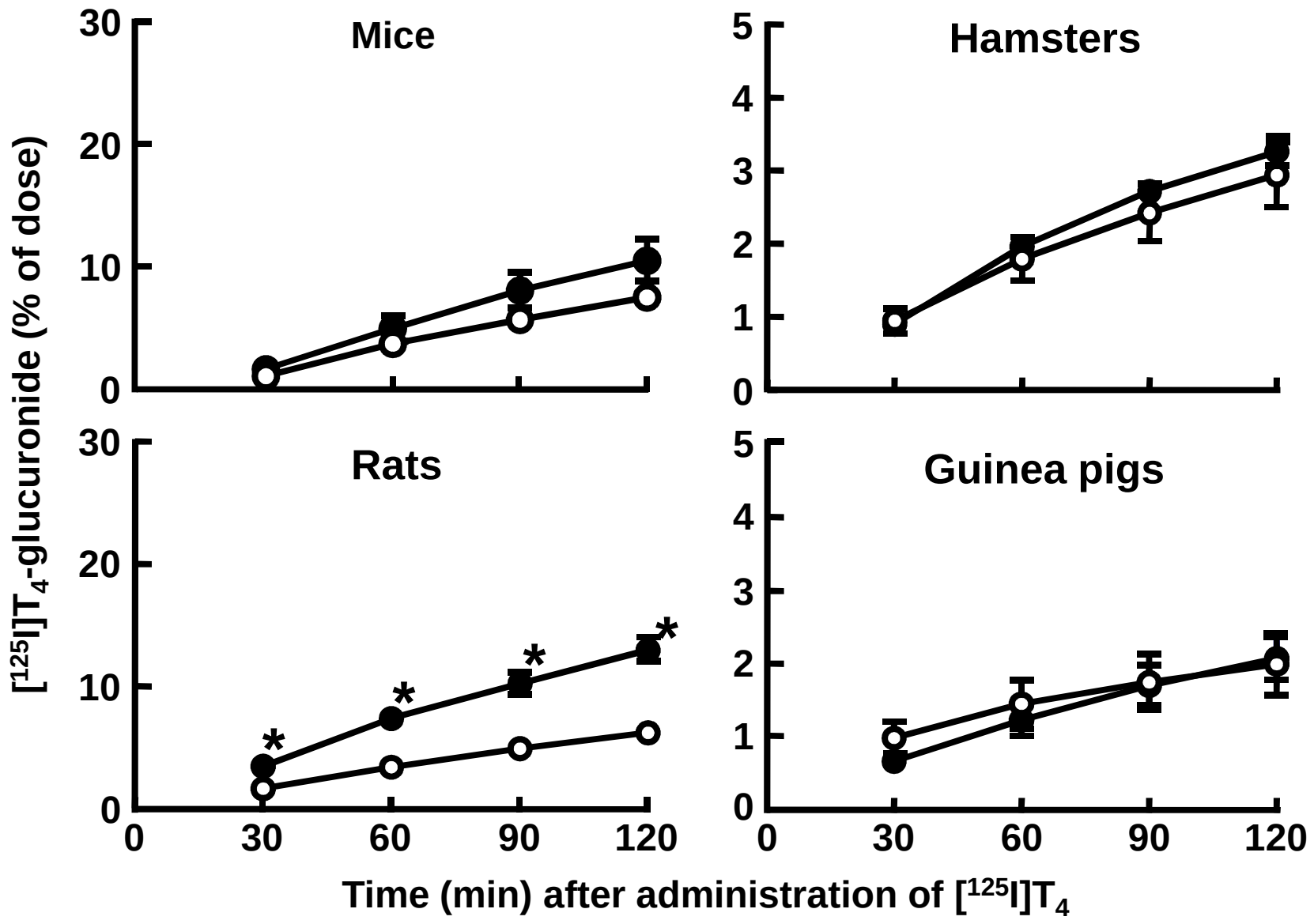

Fig. 5

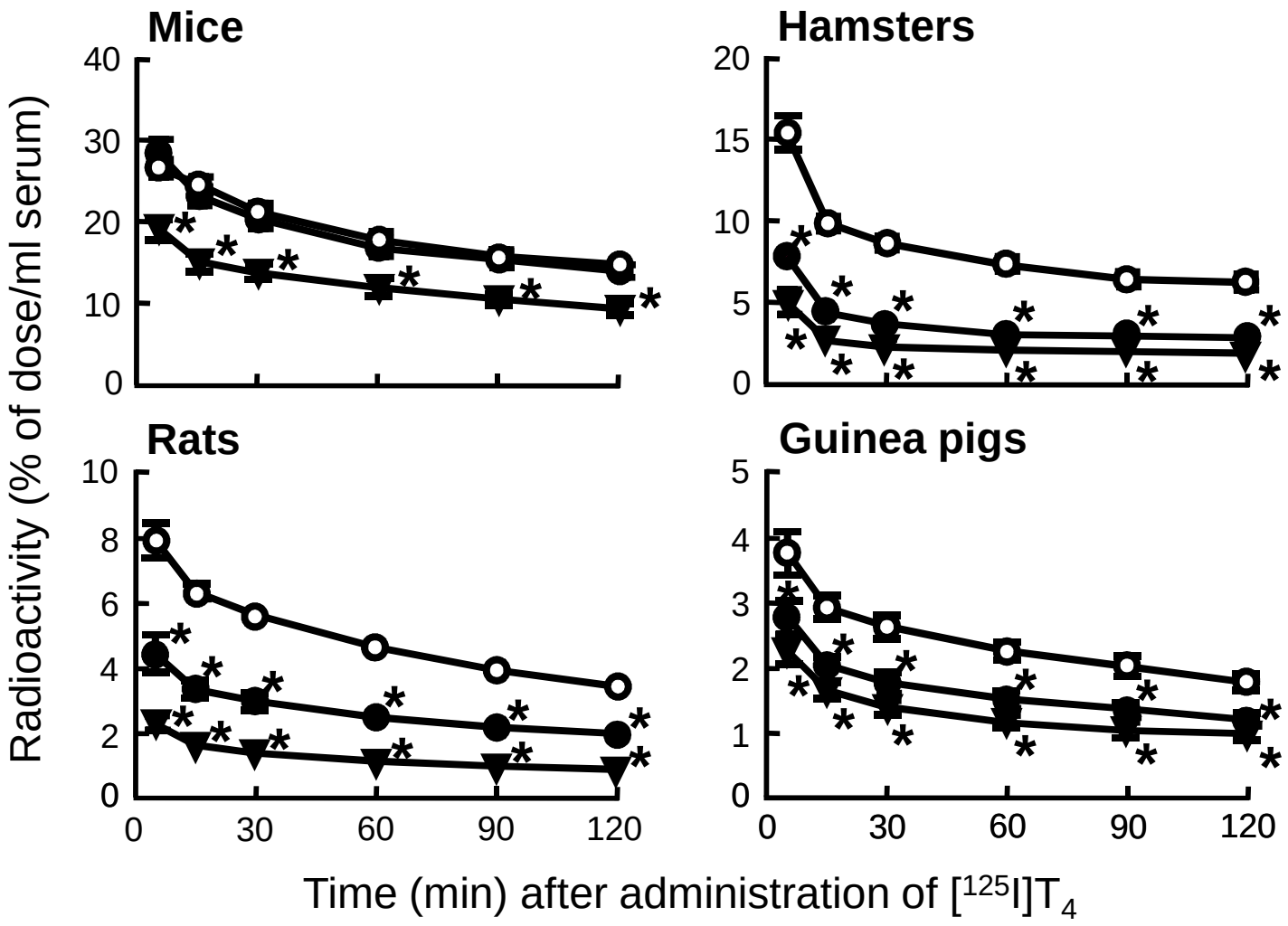

Fig. 6

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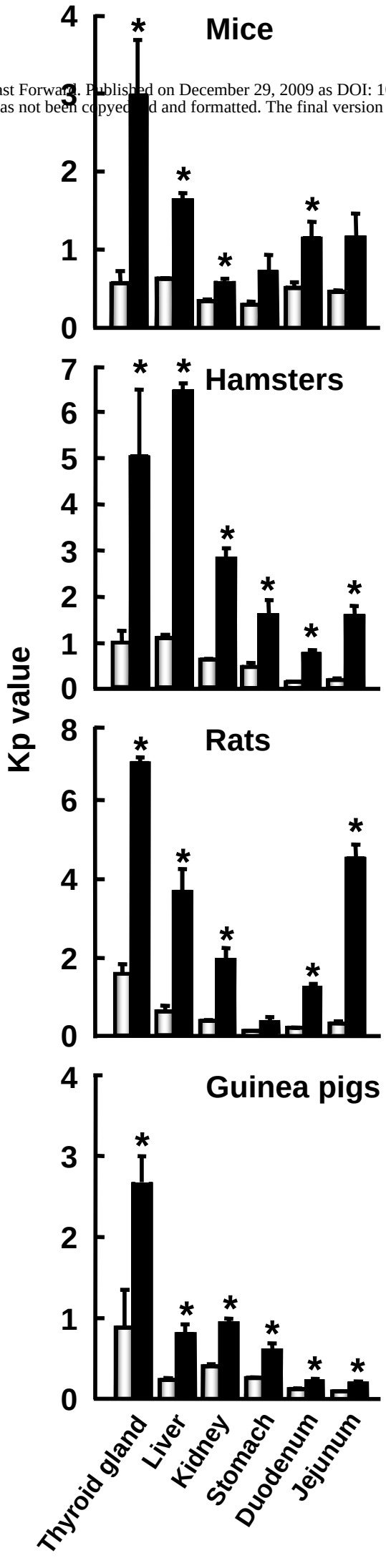

Fig. 7

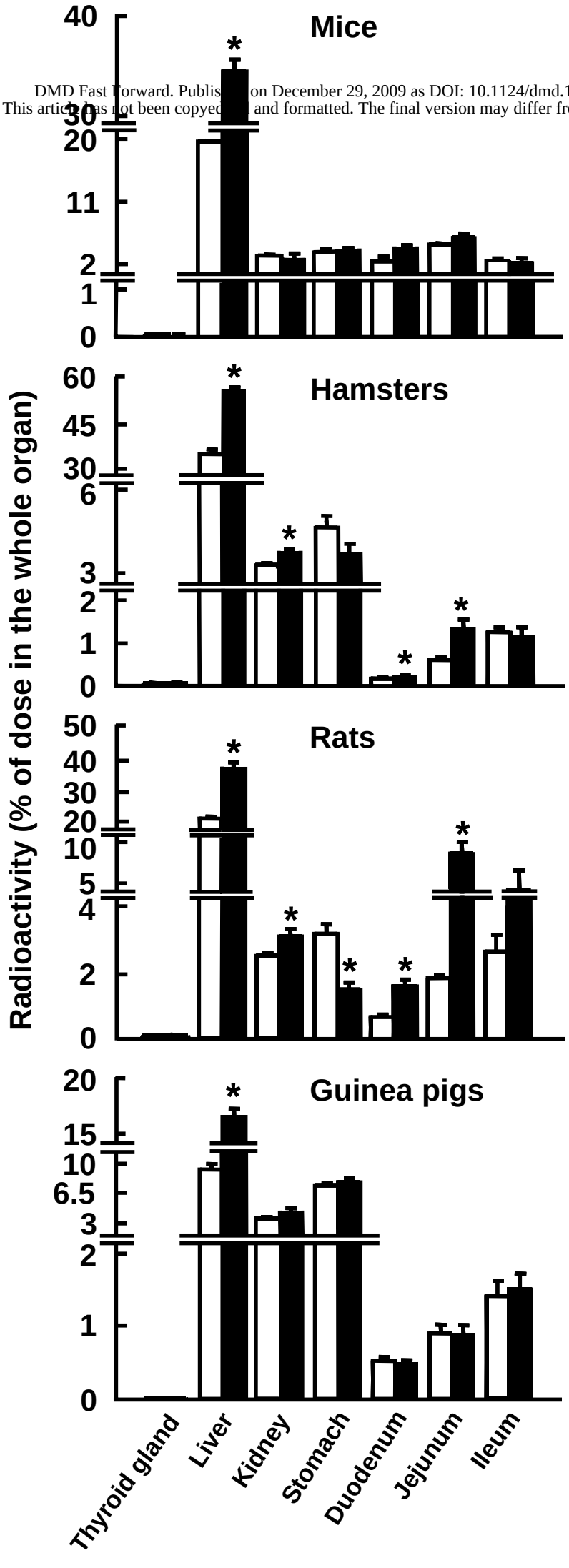

Fig. 8

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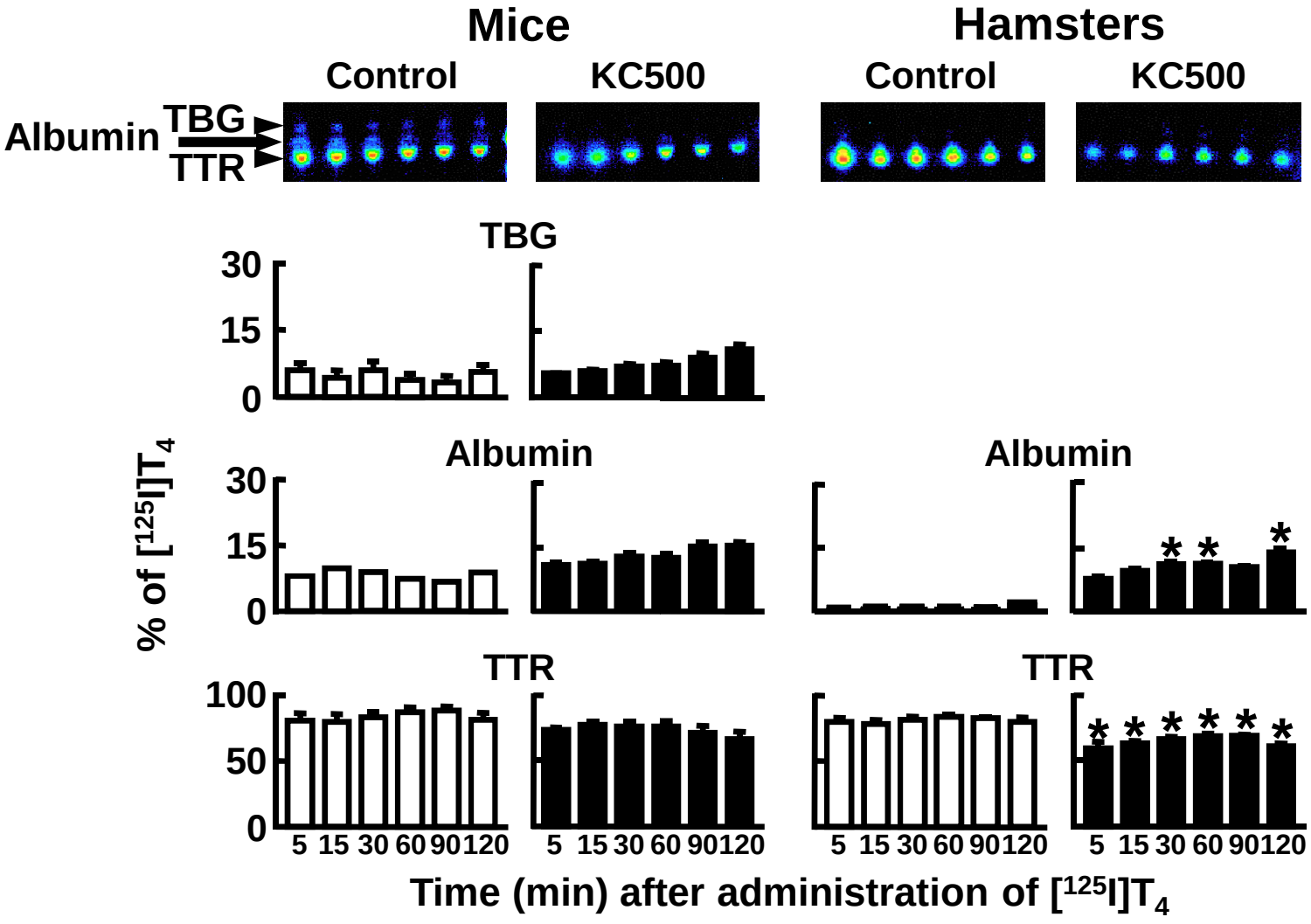

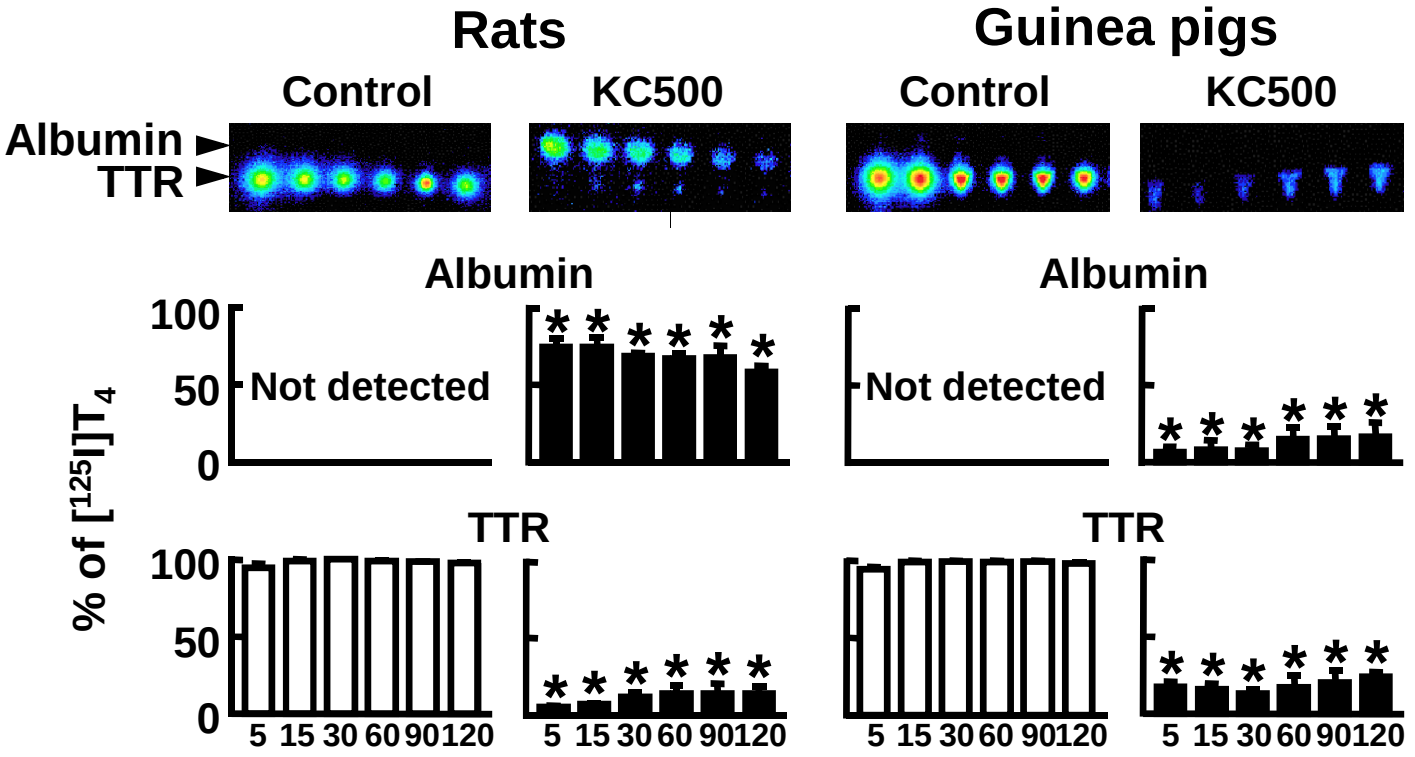

Fig. 9