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Effects of Butyltin Compounds on Mitochondrial Respiration and Its Relation to Hepatotoxicity in Mice and Guinea Pigs

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Because the mechanisms responsible for the difference in toxicity between different experimental animal species remain unclear, the effects of tributyltin chloride (TBTC) and dibutyltin dichloride (DBTC) on mitochondrial respiration were compared among the livers of mice and guinea pigs *in vitro* and *in vivo*. Further, the levels of these butyltin compounds and their derivatives in the mitochondrial fractions of the hepatocytes were investigated in these animal species. Administration of TBTC and DBTC to mice resulted in the obvious elevation of serum enzymatic activities, as well as the inhibition of succinate-linked State 3 respiration in hepatic mitochondria at 24 h after administration. On the other hand, these metal compounds failed to induce such hepatotoxicity or to inhibit mitochondrial respiration in guinea pigs. There was no significant difference between mice and guinea pigs in the IC_{50} (metal concentration observed in 50% inhibition of mitochondrial respiration) of TBTC and DBTC against the succinate-linked State 3 respiration of hepatic mitochondria *in vitro*, although the mitochondrial respiration of succinate-linked State 3 was inhibited in the liver of mice treated with the metals *in vivo*. The levels of total butyltin compounds in the mitochondrial fractions of hepatocytes were higher in the mice than in the guinea pigs, and the main butyltin compound in the mitochondrial fractions was DBTC in both species at 24 h after TBTC or DBTC administration. The amount of sulfhydryl groups, which were capable of binding with DBTC, in mice hepatic mitochondria was twice as large as that in guinea pigs, and the affinity of DBTC for the isolated hepatic mitochondria was higher in mice than in guinea pigs *in vitro*. These results suggested that the induction of hepatotoxicity by TBTC and DBTC *in vivo* was closely associated with the depression of mitochondrial respiration and that the difference in susceptibility to the metal-induced mitochondrial damages between mice and guinea pigs might result from the high affinity of butyltin compounds, in particular DBTC, for hepatic mitochondria in mice containing higher levels of sulfhydryl groups, compared with guinea pigs.

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The toxicity of butyltin compounds has attracted special interest because of the expanding utilization of and pollution with organotin compounds in the form of plastic stabilizers, catalytic agents, industrial agricultural biocides, antifouling paints, and pesticides (Hoch, 2001; Snoeiji *et al*., 1987; Wilkinson, 1984). Depending on the number of organic moieties, butyltin compounds are classified as mono-, di-, tri-, or tetrabutyltins. In experimental animals, tributyltin chloride (TBTC) and dibutyltin dichloride (DBTC) have been shown to induce inflammation of the bile duct associated with hepatic lesions (Krajnc *et al*., 1984) and to cause hepatotoxicity, detected by serological criterion, after oral administration to mice, whereas monobutyltin trichloride (MBTC) did not induce liver injury (Ueno *et al*., 1994).

Metabolism of butyltin compounds by cytochrome P450 enzymes has been suggested to play an important role in the induction of biological effects: Tributyltin was found to undergo hydroxylation followed by dealkylation to produce dibutyltin, monobutyltin, and inorganic compounds in the presence of microsomes and nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro* (Casida *et al*., 1971; Fish, 1984; Fish *et al*., 1976; Kimmel *et al*., 1977). Moreover, several studies have shown a variety of metabolites in rat (Matsuda *et al*., 1993) and mouse liver (Ueno *et al*., 1997) formed during the metabolism of TBTC *in vivo*. Regarding the relation between the metabolism and hepatotoxicity of tributyltin compound *in vivo*, we have reported that inhibition of cytochrome P450 enzymes in the liver of mice prevents the hepatotoxicity caused by TBTC compounds (Ueno *et al*., 1995, 1997). Thus, hepatic metabolism of TBTC may be associated with the induction of hepatotoxicity by these butyltin compounds.

It has been well known that the toxicity of organotin differs greatly among experimental animal species. For instance, pre-

vious studies have shown that TBTC induced hepatotoxicity in rats and mice but not in guinea pigs and rabbits (Boyer, 1989) and that DBTC did not induce any toxicity in guinea pigs (Barnes and Magee, 1958). However, the reason for the difference in the toxicity between these experimental animals remains unclear. We have recently shown differences in the effect of TBTC and DBTC on the induction of hepatotoxicity between mice, rats, and guinea pigs, and reported that these differences were in part related to differences in hepatic metabolism and distribution of butyltin compounds within cell organelles because remarkable morphological changes were induced in the mitochondria of hepatocytes by TBTC and DBTC in mice, whereas no such changes were observed in the mitochondria of rats and guinea pigs (Ueno *et al*., 2003). In this study, these preliminary observations have been extended by examining the effects of TBTC and DBTC on the mitochondrial respiration in the hepatocytes of mice and guinea pigs *in vitro* and *in vivo*, using polarography. In addition, the levels of butyltin metabolites in hepatic mitochondria *in vivo* and the affinity of DBTC for hepatic mitochondrial fractions *in vitro* were also investigated to elucidate the mechanism of mitochondrial damages caused by butyltin compounds in these animals. Our results illustrated that the differences in hepatotoxicity of TBTC or DBTC between mice and guinea pigs might be associated with the differences in the depression of mitochondrial respiration, possibly due to the higher affinity of DBTC for sulfhydryl groups in hepatic mitochondria of mice.

MATERIALS AND METHODS

Animals and administration. Male albino mice of the same colony (ddY strain) weighing about 30 g (8 weeks) and male albino guinea pigs (Hartley strain) weighing about 400 g (8 weeks) were used throughout the study. These experimental animals were housed in plastic cages in a group and maintained on a standard laboratory diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water and exposed to a 12-h light (7:00 A.M. to 7:00 P.M.) and 12-h dark (7:00 P.M. to 7:00 A.M.) cycle. Ambient temperature during the study was maintained at about 21°C. All procedures employed in this study followed the guidelines established for animal treatment by Kitasato University and were in accordance with its principles of laboratory animal care.

Treatment. TBTC and DBTC (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) were dissolved in corn oil (SIGMA, St. Louis, MO) and administered orally (10 ml/kg for mice, 1 ml/kg for guinea pigs). The dosage of TBTC or DBTC in our experiments was 360 μmol/kg (117.2 mg/kg) or 120 μmol/kg (36.6 mg/kg), respectively. These dosages of butyltins are equivalent to twice the minimum dosage that induced hepatotoxicity in mice (Ueno *et al*., 1994).

Serological evaluation of liver injury. To evaluate the liver injury induced by the butyltin compounds, the activities of ornithine carbamyl transferase (OCT; EC: 2.1.3.3), aspartic acid aminotransferase (AST; EC: 2.6.1.1), and alanine aminotransferase (ALT; EC: 2.6.1.2) in serum were observed at 24 h after administration. OCT activity was measured as previously described (Ueno *et al*., 1994) and was defined as 1 IU/l, the amount necessary to catalyze the formation of 1 μmol citrulline/min/l serum. The activities of AST and ALT were determined by blood chemistry autoanalyzer AU-550 (Olympus Company, Tokyo, Japan) using a kit (Boehringer Mannheim Co. Ltd., Mannheim, Germany) based on UV method.

Preparation of mitochondrial fraction. The animals were killed by decapitation, and livers were removed immediately. One gram of the livers was homogenized in 9 ml of 10-mM Tris–HCl buffer (pH 7.4) containing 0.21 M mannitol, 0.07 M sucrose, and 0.1 mM EDTA. The homogenate was centrifuged at 600 x g for 10 min at 4°C, and the supernatant was centrifuged at 9,000 x g for 10 min at 4°C. After the supernatant was removed by decantation, the mitochondria-rich pellet was gently resuspended in 9 ml of the buffer, and the centrifugation procedure was repeated. The mitochondrial fraction obtained was suspended in 1 ml of 10-mM Tris–HCl buffer (pH 7.4) containing 225 mM mannitol, 75 mM sucrose, 10 mM KCl, and 5 mM KH_2PO_4 (Trounce *et al*., 1996).

Polarography. According to the method of Morikawa *et al*. (1996), mitochondrial respiration was measured at 25°C with an oxygen monitor using a Clark-type oxygen electrode in the same medium used to suspend the mitochondria. Respiratory substrates were used at final concentrations of 5 mM succinate (plus a 0.33 μM rotenone). Mitochondria (1 mg protein) and 125 nmol of adenosine-5′-diphosphate (ADP) were added to initiate phosphorylating oxygen consumption (State 3). Because the respiratory control index (RCI; State 3/State 4) reflects the degree of coupling of respiration with adenosine-5′-triphosphate (ATP) production, the RCI was calculated by dividing the rate of oxygen consumption in the presence of ADP by that in the absence of ADP. In the *in vitro* study, after State 3 respiration was measured, butyltin compounds dissolved in dimethyl sulfoxide (DMSO; 5 μl) were added to the reaction mixture (1 ml) at State 4 respiration, and after a few minutes, the same amount of ADP was added again to measure State 3 respiration. The IC_{50} values (the concentration that decreases the oxygen consumption rates in State 3 respiration) were calculated graphically. After it had been confirmed that the addition of DMSO to the incubation mixture did not affect State 3 respiration, different concentrations of TBTC or DBTC were tested. Nonlinear regression analysis was carried out to obtain the concentration-response curves for the oxygen consumption rates in the presence or absence of the butyltin compounds using data analysis software (SigmaPlot 2001: Sigmoid, 3 parameter) from SPSS Science software products (Chicago, IL).

Measurement of butyltin metabolites in the mitochondrial fractions of hepatocytes. The butyltin compounds were extracted and purified from the mitochondrial fractions by the method of Suzuki *et al*. (1994) The levels of each butyltin compound in the samples were determined by the method of Suzuki *et al*. (1994), using an HP Model 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a split/splitless injection port interfaced to an HP Model 5921A atomic emission detector equipped with a turbo makeup gas valve. All standards were purchased or prepared as reported previously (Suzuki *et al*., 1994). The chemical names, their abbreviations, and detection limits are shown in Table 1.

TABLE 1
Standards, Abbreviations, and Their Detection Limits

Standard	Abbrev.	Detection limit[a] (ng/g)
Tri-*n*-butyltin chloride	TBTC	0.5
Di-*n*-butyl(3-hydroxybutyl)tin chloride	T3OH	1.0
Di-*n*-butyl(4-hydroxybutyl)tin chloride	T4OH	0.8
Di-*n*-butyl(3-oxybutyl)tin chloride	T3CO	0.8
Di-*n*-butyl(3-carboxypropyl)tin chloride	TCOOH	3.0
Di-butyltin dichloride	DBTC	0.4
N-butyl(3-hydroxybutyl)tin dichloride	D3OH	1.0
N-butyl (4-hydroxybutyl)tin dichloride	D4OH	0.8
N-butyl (3-oxobutyl)tin dichloride	D3CO	1.0
N-butyl (3-carboxybutyl)tin chloride	DCOOH	3.0
N-butyltin trichloride	MBTC	1.0

[a]Calculated as three times the SD of the noise levels of peak height.

Measurement of sulfhydryl groups in the mitochondrial fractions of hepatocytes. The contents of sulfhydryl groups in each mitochondrial fraction were measured according to the method of Elman (1959). Fifty μl of mitochondrial suspension was added to 1.85 ml of 100-mM Tris–HCl buffer (pH 7.2) containing 1 mM EDTA; then 0.1 ml of 10-mM 5,5′-dithio-2-nitrobenzoic acid (DTNB) solution was added. Absorbance at 412 nm of the centrifuged supernatant (9,000 x g for 10 min) was measured at 20 min after DTNB, using the same mixture without DTNB as a blank sample. To measure the total contents of sulfhydryl groups in the mitochondrial fractions, the absorbance of the denatured sample was monitored with the same procedure, using 100-mM Tris–HCl buffer (pH 7.2) containing 1 mM EDTA and 2% sodium diodecyl sulfate (SDS) (Baillie and Horowitz, 1976). The contents of sulfhydryl groups were conveniently calculated using absorptivity of thionitrobenzoate anion, 13,000 cm^{-1} M^{-1}.

Affinity of DBTC for the mitochondrial fractions isolated from hepatocytes. To compare the affinity of DBTC for mitochondrial fractions, the purified mitochondrial fractions (5–10 mg protein/ml) were exposed to DBTC at 50 μg/ml for 5 min, then the contents of DBTC were measured as above. Furthermore, we also observed the effects of iodoacetamide, a sulfhydryl blocker, on the affinity of DBTC for these fractions. After the mitochondrial fractions were pretreated with 1 mM of iodoacetamide for 5 min, the fractions were exposed to the same conditions of DBTC, and the contents of DBTC were measured.

Statistical analysis. Because the serum enzyme activities were not normally distributed, the statistical differences in these data were evaluated by the Mann-Whitney *U* test. The data obtained from polarography *in vivo* were analyzed using one-way ANOVA, and a p value less than 0.05 was considered statistically significant. Differences between treatment and control groups were compared with Dunnett test. Data from studies with only two groups were analyzed by the Student's t test for equal variance or the Welch t test for unequal variance after Bartlett's test.

RESULTS

Hepatotoxicity of Butyltin Compound

Table 2 shows the activities of enzymes in the serum of mice and guinea pigs at 24 h after TBTC administration. The median of OCT activity in the mice treated with corn oil alone as a control was 3.8 IU/l ($n = 5$), and an obvious elevation of activities of this enzyme was observed in the mice treated with TBTC (median = 180, $n = 5$). The median of AST and ALT activities in the control mice was 72 and 25 IU/l, respectively, and all these activities were significantly increased by the TBTC treatment. On the other hand, TBTC showed no significant effects on these activities in guinea pigs.

In the case of DBTC, the median of OCT, AST, and ALT activities in the serum of mice treated with DBTC was significantly increased to 137, 4545, and 1350 IU/l, respectively. As was the case in TBTC-treated animals, DBTC treatment did not induce any obvious hepatotoxicity in guinea pigs.

Effects of Butyltin Compounds on Mitochondrial Respiration in Vivo

Table 3 shows the respiratory responses of mitochondria from the livers of the experimental animals treated with TBTC or DBTC at 24 h after oral administration. The treatment of mice with TBTC or DBTC resulted in a significant decrease of the succinate-linked State 3 respiration, as well as of RCI, although no significant influence was observed in guinea pigs. Thus, the *in vivo* treatment of TBTC or DBTC in mice specifically inhibited the mitochondrial State 3 respiration of liver.

Effects of Butyltin Compounds on the State 3 Respiration in Vitro

The IC_{50} values of butyltin compounds to directly inhibit State 3 respiration of the mitochondria isolated from the livers

TABLE 2
Effects of TBTC or DBTC on the Activities of Serum Enzymes at 24 h after Oral Administration to Mice and Guinea Pigs

Animal	Dose		OCT (IU/l)	AST (IU/l)	ALT (IU/l)
Mice	Control	Median	3.8	73	25
		First quartile	2.7	61	21
		Third quartile	7.0	88	39
	TBTC	Median	180.6*	333**	120*
	360 μmol/kg	First quartile	145.3	235	92
		Third quartile	183.5	351	141
	DBTC	Median	137.6*	4545*	1350*
	120 μmol/kg	First quartile	136.6	3688	1135
		Third quartile	138.7	5418	1785
Guinea pigs	Control	Median	4.6	67	36
		First quartile	3.2	56	33
		Third quartile	4.8	77	45
	TBTC	Median	2.8	99	65
	360 μmol/kg	First quartile	2.0	90	59
		Third quartile	4.0	101	66
	DBTC	Median	4.8	67	64
	120 μmol/kg	First quartile	4.7	66	62
		Third quartile	5.8	71	86

Note. OCT, ornithine carbamyl transferase; AST, aspartic acid aminotransferase; ALT, alanine aminotransferase.
*Significant increase compared with the control values (Mann-Whitney *U* test, $n = 5$, $p < 0.01$).

TABLE 3

Effects of TBTC (360 μmol/kg) or DBTC (120 μmol/kg) on Succinate-Linked State 3 or 4 Respiration of Mitochondria Isolated from Mice and Guinea Pigs at 24 h after Oral Administration

	Oxygen consumption[a]		
Succinate	State 3	State 4	RCI[b]
Mice			
Control	100.9 ± 20.3	25.0 ± 3.1	4.03 ± 0.47
TBTC	50.6 ± 27.1**	21.9 ± 3.7	2.21 ± 0.83**
DBTC	65.8 ± 17.1*	26.0 ± 3.4	2.61 ± 1.89*
Guinea pigs			
Control	66.3 ± 13.0	13.9 ± 2.2	4.78 ± 0.69
TBTC	61.3 ± 9.1	15.8 ± 3.1	3.91 ± 0.37
DBTC	60.0 ± 8.6	13.8 ± 4.6	4.62 ± 1.22

Note. Data represent the mean ± SD ($n = 4$).

[a]Oxygen consumption was expressed as nanoatoms O/min/mg protein.

[b]RCI, respiratory control index; calculated as the ratio of State 3 rate of oxidation to State 4 rate of oxidation.

*,**Significant inhibition compared with the control values (one-way ANOVA and Dunnett test, $p < 0.05$ or 0.01).

of the experimental animals are shown in Table 4. In contrast to the *in vivo* experiments, the butyltin compounds inhibited the State 3 respiration linked by succinate in hepatic mitochondria isolated from both mice and guinea pigs, and there was no significant difference between these animals in the IC_{50} of TBTC or DBTC. Therefore, the *in vitro* inhibitory effects of butyltin compounds on mitochondrial respiration in mice were similar to those in guinea pigs, although the *in vivo* effects were observed only in mice. It should be noted that the *in vitro* inhibitory effects of TBTC were stronger than those of DBTC, because the IC_{50} of TBTC to inhibit succinate-linked State 3 respiration were 10^{-7} M in the order of concentration, and those of DBTC to inhibit State 3 respiration were 10^{-5} M in the order of concentration, respectively.

Metabolites of Butyltin Compounds in the Mitochondrial Fractions of Hepatocytes

To understand the differences between the *in vivo* and *in vitro* effects of these butyltin compounds in the mitochondrial respiration, we examined the levels of total butyltin and their derivatives in the mitochondrial fractions of hepatocytes of mice and guinea pigs at 24 h after oral administration with TBTC or DBTC. Our previous study showed that butyltin compounds had a greater tendency to accumulate in the liver mitochondrial fraction of mice than in other animals (Ueno *et al*., 2003). Similarly in this study, the total levels of butyltin compounds in the mitochondrial fractions of mice and guinea pigs were 3068 ppb and 1544 ppb, respectively. The main metabolites of TBTC in the hepatic mitochondria were DBTC and MBTC in mice, and DBTC and TCOOH in guinea pigs; however, the levels of DBTC in the mitochondrial fraction were about three times greater in the mice than in the guinea pigs.

In the case of DBTC, as shown in Table 5, the total butyltin levels in hepatic mitochondria were also greater in the mice than in the guinea pigs. With respect to metabolites in hepatic mitochondria, DBTC showed very little degradation in these animals, even at 24 h after the administration, because about 90% of butyltin compounds were distributed as DBTC in the mitochondrial fraction of both mice and guinea pigs. As was the case with TBTC, the levels of DBTC in the fraction were about three times greater in the mice than in the guinea pigs.

Contents of Sulfhydryl Groups in the Mitochondrial Fractions of Hepatocytes

The contents of sulfhydryl groups in mitochondria isolated from mice and guinea pigs are shown in Table 6. When the mitochondrial fractions were denatured by SDS, there was no difference in the contents of total sulfhydryl groups, which produced thionitrobenzoate anion reacting with DTNB, among these animals. On the other hand, without the denaturation by SDS, about 40% of total sulfhydryl groups in the mitochondria fractions of mice reacted with DTNB, whereas only 17% reacted in guinea pigs, indicating that the contents of sulfhydryl groups (the reduced form) in mice without the denaturation were two times greater than those in guinea pigs.

Affinity of DBTC for the Mitochondrial Fractions Isolated from the Hepatocytes

The affinity of DBTC for the hepatic mitochondrial fractions isolated from mice and guinea pigs was measured *in vitro*. As shown in Table 7, the affinity of DBTC for mice mitochondria was significantly higher than that of guinea pigs. When the isolated mice mitochondria fractions were pretreated with iodoacetamibe as a sulfhydryl blocker, the affinity of DBTC for the fractions was significantly decreased by one third, compared with that of unpretreated mitochondria, whereas the pretreatment had no significant effect on the affinity for DBTC in the mitochondria isolated from guinea pigs.

TABLE 4

Effects of TBTC or DBTC on Succinate-Linked State 3 Respiration of the Mitochondria Isolated from Mice and Guinea Pigs *in Vitro*

Organotin	Animal	IC_{50} of organotin compound
TBTC	Mice	8.9×10^{-7} M
	Guinea pigs	9.1×10^{-7} M
DBTC	Mice	1.7×10^{-5} M
	Guinea pigs	2.2×10^{-5} M

Note. Results represent the IC_{50} of each organotin compound to inhibit State 3 respiration (rate of oxygen consumption) of the mitochondria.

TABLE 5
Typical Distributions of Butyltin Metabolites in the Mitochondrial Fractions of Hepatocytes Isolated from Mice and Guinea Pigs at 24 h after Oral Administration of TBTC or DBTC

	Butyltin metabolites (ng/g wet weight)									
	MBTC	DCOOH	D3CO	D3OH	DBTC	TCOOH	T3CO	T3OH	TBTC	Total
TBTC										
Mouse	667	75	302	40	1885	51	0	0	48	3068
Guinea pig	33	60	13	25	572	317	22	40	462	1544
DBTC										
Mouse	638	4	11	8	4986	0	0	0	16	5663
Guinea pig	180	0	0	0	1658	0	0	0	60	1898

DISCUSSION

Previous studies have shown that tri- and dibutyltin compounds induced the lesions in liver of mice and rats, but these compounds did not show such toxicity in guinea pigs or rabbits (Boyer, 1989). There is a possibility that differences in bioavailability or clearance between animal species might contribute to the observed differences in the hepatotoxicity of butyltins. However, there are few pharmacokinetic analyses reported for these compounds comparing the differences among these animals because of the difficulty in analytical technique. Therefore, we attempted to investigate the relation between mitochondrial distribution and the functional influence of butyltin compounds *in vivo*.

This and our previous studies also showed that mice were more sensitive to hepatotoxicity than guinea pigs and that guinea pigs were insensitive to hepatotoxicity caused by butyltin compounds, because the administration of TBTC or DBTC resulted in a significant increase in the activity of OCT, ALT, and AST enzymes in mice, whereas these enzyme activities were not affected in guinea pigs (Table 2). Under the same experimental conditions, although the total butyltin levels in liver have been shown to be greater in guinea pigs, compared with mice (Ueno *et al*., 2003), these serological results were consistent with the histopathological results in which swelling and collapse of mitochondria were observed in mice livers but not in livers of guinea pigs (data not shown). In addition, this study suggested that the swelling of mitochondria induced by TBTC or DBTC in mice might lead to an uncoupling oxidative phosphorylation by means of the electron transport system, because the administration of TBTC (180 μmol/kg) or DBTC (60 μmol/kg) showed a statistically significant facilitation of State 4 respiration at 48 h in mice liver *in vivo* (succinate as substrate: control, 27.6 $\pm$ 2.1; TBTC, 39.3 $\pm$ 5.5; DBTC, 43.3 $\pm$ 4.0 nano atoms O/min/mg protein). Thus, these results strongly indicated that the influences of TBTC and DBTC on the mitochondria in liver might play a critical role in the induction of hepatotoxicity by these organotin compounds *in vivo*.

Previous studies have reported inhibitory effects on isolated mitochondria respiration by TBTC and DBTC *in vitro* (Aldridge, 1976; 1977). Similarly, our *in vitro* study also showed that TBTC and DBTC suppressed State 3 respiration linked by succinate as the substrates (Table 4). It is interesting to note that the *in vivo* inhibition against hepatic mitochondrial respiration by TBTC and DBTC was observed only in mice (Table

TABLE 6
Contents of Sulfhydryl Groups in the Mitochondria Isolated from Livers of Mice and Guinea Pigs

	Contents of sulfhydryl groups (nmol/mg protein)		
Animal	Undenatured mitochondria	Denatured mitochondria	Ratio (%) (undenatured/denatured)
Mice	31.6 $\pm$ 1.8	79.0 $\pm$ 5.2	40.3 $\pm$ 5.0
Guinea pigs	14.4 $\pm$ 0.8*	86.1 $\pm$ 4.4	16.8 $\pm$ 1.1*

Note. Sulfhydryl groups were measured as the production of thionitrobenzoate anion by Elman method. Data represent the mean $\pm$ SD (n=4).

*Significant decrease compared with the mice values ($p < 0.01$).

TABLE 7
The Effects of Pretreatment with Iodoacetamide, a Sulfhydryl Blocker, on the Amounts of DBTC Combined with the Mitochondrial Fractions Isolated from Livers of Mice and Guinea Pigs

	DBTC content (μg DBTC/mg protein)	
	Unpretreated mitochondria	Pretreated with iodoacetamide
Mice	3.137 $\pm$ 0.272	1.223 $\pm$ 0.094**
Guinea pigs	2.701 $\pm$ 0.221*	1.878 $\pm$ 0.751*·***

The isolated mitochondrial fractions were treated with or without 1mM iodoacetamide for 5 min, then exposed to DBTC at 50 μg/ml for 5 min. The mitochondrial fractions were washed twice by the buffer, and the concentration of DBTC was determined in each fraction.

**·*Significant decrease compared with the unpretreated mice values ($p <$ 0.01 or 0.05).

***Significant increase compared with the unpretreated guinea pigs values ($p < 0.05$).

3), whereas there was no significant difference between mice and guinea pigs in the IC_{50} of TBTC and DBTC on State 3 respiration *in vitro* (Table 4). Furthermore, the inhibitory effects of TBTC on State 3 respiration of the isolated mitochondria were 10 times or more as strong as those of DBTC *in vitro* (Table 4), although there was no significant difference between the treatment of mice with TBTC (360 μmol/kg) and DBTC (120 μmol/kg) in the inhibition of the hepatic mitochondrial respiration *in vivo*. It is possible that the differences between *in vivo* and *in vitro* experiments may result from the differences in the levels of TBTC and DBTC in hepatic mitochondria of mice and guinea pigs, because our previous studies showed that the hepatotoxicity of TBTC was closely related to its metabolism in the liver *in vivo* (Ueno *et al*., 1995; 1997). The present analysis of metabolites of TBTC in the mitochondrial fraction revealed that the main metabolite of TBTC was DBTC in both mice and guinea pigs *in vivo* (Table 5). With respect to DBTC treatment, DBTC showed very little degradation in the mitochondrial fractions of both species. Interestingly, the mitochondrial levels of DBTC in mice treated with either TBTC or DBTC were three times greater than those in guinea pigs. On the other hand, the mitochondrial levels of TBTC in liver of the TBTC-treated guinea pigs was about 10 times greater than those in mice; nevertheless, no mitochondrial injuries were observed in guinea pigs *in vivo*. Although the critical metabolites that are responsible for mitochondrial damages caused by these butyltin compounds are not clear, these results strongly indicated that the mitochondrial damages induced by TBTC or DBTC *in vivo* may be closely associated with the levels of DBTC but not TBTC in this fraction of hepatocytes.

Our previous analysis of cellular distributions of DBTC in the liver after the metal administration *in vivo* showed that the levels of DBTC in mitochondrial fractions in mice hepatocytes were greater than those in guinea pigs (Ueno *et al*., 2003). As expected, higher levels of organotin compounds in the mitochondrial fractions of hepatocytes were also observed in the mice than in the guinea pigs at 24 h after administration of either TBTC or DBTC (Table 5). Therefore, the butyltin compounds appear to become more concentrated in the mitochondrial fractions of mice hepatocytes than in those of guinea pigs. Because sulfhydryl groups (reduced form), which organize dithiol structure, have been shown to have a high affinity for DBTC (Aldridge, 1977; Merkord *et al*., 2000), we compared the contents of sulfhydryl groups in mitochondrial fractions of livers between these animals (Table 6) to understand the difference in distribution of DBTC in the mitochondrial fractions. When the mitochondrial fractions were denatured by SDS, there was no difference between mice and guinea pigs in the content of total sulfhydryl groups (reduced and oxidized form). On the other hand, the concentrations of sulfhydryl groups (reduced form) in the mitochondrial fractions without the denaturation were about two times higher in mice than in guinea pigs. Thus, the levels of sulfhydryl groups (reduced form) may be higher in the mitochondrial fractions of mice than in those of guinea pigs. Moreover, the affinity of DBTC for the mitochondria of mice hepatocytes was significantly higher than that in guinea pigs, and the pretreatment of the isolated mitochondria with a sulfhydryl blocker such as iodoacetamide reduced the affinity of DBTC only in mice (Table 7). These results indicated that the differences in the mitochondrial levels of DBTC between mice and guinea pigs *in vivo* might be due to the differences in the levels of sulfhydryl groups in the respective mitochondria. Our preliminary *in vitro* experiments showed that the amount of sulfhydryl groups of glutathione, which could be detected with DTNB, were not affected by the treatment with DBTC, whereas the amount of sulfhydryl groups of dimercapto-propanol, which organize dithiol structure, was reduced by DBTC (data not shown). Thus, it is possible that the mitochondrial levels of sulfhydryl groups, in particular dithiol, in mice liver may be higher than those in guinea pigs.

In conclusion, this study indicated that the difference in susceptibility to butyltin compounds between mice and guinea pigs may be closely associated with the inhibition of mitochondrial respiration, possibly due to the higher affinity of butyltin compounds, in particular DBTC, for hepatic mitochondria in mice. The results also suggest that DBTC in the mitochondrial fractions might be one of the main critical forms responsible for mitochondrial damages caused by TBTC and DBTC *in vivo*. Recently, Stridh *et al*. (1999) reported that low concentrations of TBTC triggered an immediate depletion of intercellular ATP followed by necrotic death in Jurket cells and showed that the mode of cell death was typically apoptotic when ATP levels were maintained by the addition of glucose. Moreover, depending on the situations of the cells, such as resting cells or CD3-stimulated cells, they also reported that TBTC could induce apoptosis or necrosis in human peripheral blood lymphocytes (Stridh *et al*., 2001). Thus, the effects of butyltin compounds on mitochondrial function may play an important role in both cell necrosis and apoptosis *in vitro*. Our present results should provide useful clues for future research concerned with the toxicity of butyltin compounds *in vivo*.

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