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Pharmacological property of tributyltin in vivo and in vitro

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Abstract

Tributyltin (TBT), an assumed endocrine-disrupting chemical, is widely known to show harmful effects in invertebrates including the dioecious snails. As for mammals, there are several reports concerning TBT toxicology, but few indications about general pharmacology of TBT. In the present study, we comprehensively examined the pharmacological effects of TBT both in vivo and in vitro. TBT (0.3 or 1.0 mg/kg) attenuated the small intestinal propulsive activity measured by the charcoal method in vivo, and caused concentration-dependent relaxation of isolated guinea-pig ileum in vitro (1.0×10^{-8}–3.0×10^{-6} M). TBT induced concentration-dependent relaxation of guinea-pig trachea, which was not inhibited by pre-treatment with a β-adrenoceptor antagonist. TBT caused a concentration-dependent contraction of rat aortae, and also evoked endothelium-dependent relaxation in the presence of an α-adrenoceptor antagonist. The relaxation was inhibited by a muscarinic receptor antagonist. TBT reduced the electrically evoked, sympathetic contractile responses of rat vas deferens, which were slightly prevented by an α_2-adrenoceptor antagonist. These results suggest that TBT possesses diverse pharmacological properties in mammalian organs.

Keywords: Tributyltin; Organotin; α-Adrenoceptor; Muscarinic receptor

1. Introduction

Tributyltin (TBT) has been widely utilized in the production of biocides and in the stabilization of polyvinyl chloride (Piver, 1973) and has recently received much attention as an assumed endocrine-disrupting chemical because of its environmental and health hazards (Snoeij et al., 1987; Fent, 1996; Kannan and Falandysz, 1997). Particularly, the compound develops a concentration- and time-dependent 'imposex', which consists of a superimposition of male characteristics onto a reproductive anatomy in female animals of the dioecious snail such as *Nassarius obsoletus* Say (Smith, 1981) and *Neogastropod molluscs* (Horiguchi et al., 1998). The anatomical aberration is assumed to result from the inhibition by TBT of aromatase cytochrome P450, which catalyzes the aromatization of androgens to estrogens (Bettin et al., 1996; Sumpter, 1998).

As for mammals, many previous studies on organotins, including TBT, revealed their toxicity including immunodeficiency (Snoeij et al., 1987; Pieters et al., 1994; Fent, 1996). Experimentally, it has been widely used as an apoptosis inducer in various types of cells, including thymocytes (Raffray et al., 1993; Zucker et al., 1994; Sumpter, 1998), hepatocytes (Reader et al., 1999), neurons (Thompson et al., 1996) and pheochromocytoma PC12 (Funahashi et al., 1980; Aw et al., 1990; Viviani et al., 1995). However, most of these studies considered TBT as a poison, but few approaches with the pharmacological scope have been conducted. Therefore, the present study comprehensively investigated the pharmacological property of TBT in mammalian organs, using mice, rats, guinea pigs and their isolated tissue.

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2. Materials and methods

2.1. Animals

In the present study, we used male animals because the data of female animals that have the estrus cycle are sometimes difficult to be interpreted. Male Std-ddY mice (5–18 weeks, 24–35 g, SLC, Shizuoka, Japan), male Hartley guinea pigs (5–7 weeks, 300–600 g, SLC, Shizuoka, Japan) or male Wistar-ST rats (7–9 weeks, 200–250 g, SLC) were kept under temperature- and humidity-controlled conditions (22 ± 1°C, 55 ± 15%, respectively) and were housed three to five animals per cage (mouse, $140 \times 280 \times 420$ mm; guinea pig, $220 \times 400 \times 500$ mm; rat, $200 \times 240 \times 320$ mm). They had free access to TBT-free food and water. All efforts were made for the care and use of animals as per the guideline of Graduate School of Pharmaceutical Sciences, The University of Tokyo.

2.2. In vivo experiments

2.2.1. Spontaneous locomotor activity

Mice were intraperitoneally treated with TBT (0, 0.3, 1.0 or 3.0 mg/kg) dissolved in polyethylene glycol 400, and their locomotor activities were assessed 30 min, 1, 2, or 3 months after the injection. Briefly, 5 min after a mouse was placed in the doughnut-shaped apparatus (outer diameter, 320 mm; inner diameter, 160 mm; wall height, 130 mm) (AT-320, Toyo Sangyo Co. Ltd., Toyama, Japan), the numbers of horizontal movement actions and rearing actions, movement time, movement distance or average speed for 30 min were automatically monitored with 144 infrared sensors at scanning rate of 10 Hz. Rearing actions were detected by the sensors that were fixed 70 mm in height from the cage bottom. LD50 of TBT was calculated by Brownlee's method.

2.2.2. Potentiation of hexobarbital-induced anesthesia

Hexobarbital (70 mg/kg) was intraperitoneally administered 30 min after subcutaneous injection of TBT (0, 0.3, 1.0 and 3.0 mg/kg) or chlorpromazine (2.0 mg/kg, as a positive control) dissolved in polyethylene glycol 400. The reason for the injection routes is to avoid a direct interaction of the drugs. Hexobarbital-induced anesthesia of mice was estimated by the duration of righting reflex loss.

2.2.3. Blood pressure

Rats were anesthetized with a mixture of urethane (1.0 mg/kg) and α-chloralose (25 μg/kg), and arterial blood pressure was measured via a pressure transducer (LPU-0.5-290-0-II, ToyoMeas. Instruments Co. Ltd., Japan). Because TBT was not able to be resolved in physiological aqua-solution suitable for an intravenous injection, the animal received subcutaneous administrations of TBT (1.0 and 3.0 mg/kg) dissolved in polyethylene glycol 400. Within 20–40 min after arterial cannulation for stabilization of the preparation, a change in the blood pressure was measured after TBT injection.

2.2.4. Intestinal propulsive activity

TBT was not intraperitoneally but subcutaneously applied, so that TBT did not directly act on the intestine. Thirty minutes after the injection of TBT (0, 0.3, 1.0 and 3.0 mg/kg) or a muscarinic receptor antagonist atropine (10 mg/kg, as a positive control) dissolved in polyethylene glycol 400, the mice received oral administration of activated charcoal powder suspension at 0.3 ml. Thirty minutes later the animal was sacrificed with an overdose of anesthetics, and the intestine was quickly eviscerated. Gastrointestinal transit was assessed by measuring the passage of charcoal in the small intestine. Transit index was defined as the ratio of the distance traveled by charcoal (the length from the pylorus to the front of the transited powder) to the total length of the small intestine (the length from the pylorus to the cecum).

2.3. In vitro experiments

2.3.1. Guinea-pig ileum

Guinea pigs were decapitated and an incision was made in the abdominal wall. The ileum was removed and placed in a dish filled with modified Krebs–Henseleit solution (physiological salt solution, PSS). The tissue was cut into 3–5 cm in length and one end of the preparation was tied to a fixed pin and then mounted in the organ bath containing PSS. Acetylcholine (final concentration, 1.0×10^{-5} M) or TBT (1.0×10^{-8}–3.0×10^{-6} M) dissolved in < 0.5% dimethyl sulfoxide were applied, and changes in length of the preparation was measured with an isotonic transducer (Type 45347, NEC San-ei Instruments Ltd., Japan). PSS used in the present experiment was composed of NaCl 120.0 mM, $NaHCO_3$ 25 mM, KCl 4.7 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.2 mM and glucose 11.1 mM. The solution was aerated with a mixture of 95% O_2–5% CO_2 and was kept at 37°C.

2.3.2. Guinea-pig trachea

Following decapitation, the trachea were dissected and transferred to a dish containing PSS. They were cut transversely between the segments of cartilage into rings and then tied together so as to form a chain. The chain consisting of five to six rings was mounted in the organ bathes containing PSS. Acetylcholine (1.0×10^{-5} M) or TBT (1.0×10^{-8}–3.0×10^{-6} M) dissolved in < 0.5% dimethyl sulfoxide was added to the organ bath. In some experiments, in order to investigate the

involvement of β-adrenoceptor, propranolol (1.0×10^{-6} M) was added 3 min prior to the treatment with TBT. Changes in tension of the preparation were measured isometrically with a force-displacement transducer (Model TB-612T, Nihonkkoden Co., Japan).

2.3.3. Rat vas deferens

Animals were decapitated and the abdomens were incised. The vas deferens was cut just above the epididymis and also at the point where it joins the urethra, and one end was tied to an electrode inserted into the seminal duct of the preparation and the other end was tied to a transducer. It was then mounted in the organ bath containing PSS and nerve stimulation (100-μs duration, 10 Hz for 2 s) was applied at intervals of 30 s. TBT (1.0×10^{-8}–3.0×10^{-6} M) dissolved in $<$ 0.5% dimethyl sulfoxide was added to the organ bath. In some experiments, in order to investigate the involvement of α_2-adrenoceptor, yohimbine (1.0×10^{-6} M) was added 3 min prior to the treatment with TBT. Neurogenic responses were isometrically recorded.

2.3.4. Guinea-pig cardiac atria

Guinea pigs were decapitated and their chests were opened. The hearts were immediately removed and all other tissues were removed until nothing was left except the auricles. The preparation was tied with a thread at each tip of the auricle, and mounted in the organ bath containing PSS. TBT (1.0×10^{-8}–3.0×10^{-6} M) dissolved in $<0.5\%$ dimethyl sulfoxide was applied to the preparation. The average systolic tension in spontaneous atrial beats was measured with an isometric transducer.

2.3.5. Rat aorta

Animals were decapitated, and their thoraxes were opened. The aorta was cut through near the heart, dissected and then cut spinally so as to produce a continuous strip approximately 3–5 mm wide and 3 cm long. A thread was attached to each end and the preparation was mounted in the organ bath. Norepinephrine (1.0×10^{-5} M) or TBT (1.0×10^{-8}–3.0×10^{-6} M) dissolved in $<0.5\%$ dimethyl sulfoxide was applied to the preparation. In some experiments, in order to investigate the involvement of α_1-adrenoceptor or the vascular endothelium, prazosin (1.0×10^{-6} M), hemoglobin (1.0×10^{-5} M), atropine (1.0×10^{-6} M) or botulinum toxin (1.0×10^{-10} M) was added prior to the treatment with TBT. Isometric changes in tension of the aortic strip were measured with a force-displacement transducer.

2.4. Drugs

The following drugs were used, tributyltin(IV) chloride (95% purity) (Wako Pure Chemical Industries, Ltd., Saitama, Japan), polyethylene glycols 400 (Wako), atropine (Tokyo Kasei Co. Ltd., Tokyo, Japan), hexobarbital (Teikoku Chemical Industries Ltd., Tokyo, Japan), chlorpromazine (Sigma Chemical, St. Louis, MO, USA), acetylcholine (Wako), propranolol (Wako), DMSO (Wako), yohimbine (Sigma), norepinephrine (Wako), prazosin (Sigma), bovine hemoglobin (Wako), and activated charcoal powder (Wako).

3. Results

3.1. In vivo experiments

First, we investigated the acute toxicity of TBT and its effect on the general behavior of mice. LD_{50} of intraperitoneally injected TBT, calculated by Brownlee's method, was 5.2 mg/kg by the day after the injection ($N = 19$) and 0.75 mg/kg by 1 month after the injection ($N = 15$). Incidentally, the mice treated with TBT (> 0.75 mg/kg) died within the first week. The mice given TBT displayed hypokinesia in a dose-dependent manner (Table 1). In the locomotor activity test, the number of movements (total detected actions) of mice treated with TBT at 1.0 and 3.0 mg/kg was significantly less than those of the control mice 30 min after the injection. However, the hypokinesia disappeared gradually after the administration, and locomotor activities of the mice became almost normal 3 months after the treatment with TBT (Table 1). In this experiment, four out of the four mice given TBT 3.0 mg/kg and three of the four mice given TBT 1.0 mg/kg were dead within the first month. Furthermore, we examined the effect of TBT on the hypnoid action of hexobarbital 70 mg/kg in mice, but TBT did not potentiate hexobarbital-induced anesthesia at 0.3, 1.0 or 3.0 mg/kg; the total sleeping time is 13.0 ± 1.8 min (control), 19.0 ± 3.9 min (TBT 0.3 mg/kg), 16.8 ± 2.2 min (TBT 1.0 mg/kg) and 20.2 ± 3.9 min (TBT 3.0 mg/kg) (mean $\pm$ S.E.M. of four cases). We observed no abnormality in the behavior of the anesthetized animals that had received the treatment with TBT. In this experiment, chlorpromazine as a positive control significantly potentiated hexobarbital-induced anesthesia (total sleeping time, 31.3 ± 7.0 min).

Next, the effect of TBT on mean arterial blood pressure was evaluated in the anesthetized rats. But, no marked change in blood pressure was observed at least within the 4 h following the treatment with TBT at 1.0 or 3.0 mg/kg; the average blood pressure 120 min after TBT injection is 66.3 ± 4.0 mmHg (control), 74.5 ± 7.1 mmHg (TBT 1.0 mg/kg) and 72.8 ± 2.9 mmHg (TBT 3.0 mg/kg) (mean $\pm$ S.E.M. of four cases).

Finally, we investigated the effect of TBT on the gastrointestinal transit in mice and found that this

compound significantly suppressed the intestinal function at 0.3 or 1.0 mg/kg (Fig. 1), as did atropine (10 mg/kg).

3.2. In vitro experiments

Since TBT reduced the intestinal propulsive activity in vivo, we examined its effect on guinea-pig isolated ileal preparations. When TBT was applied at concentrations of 1.0×10^{-8}–3.0×10^{-6} M, the ileum was elongated in a concentration-dependent manner. The relaxant effect was maximal at $> 3.0 \times 10^{-7}$ M, and the maximal response to TBT was about 20% of the contractive response to acetylcholine 1.0×10^{-5} M (Fig. 2A).

To examine whether TBT has influences on the muscles of other tissues, we performed several lines of experiments with the tracheal smooth muscle, vas deferens smooth muscle, atrial muscle or aortic smooth muscle. In experiments with guinea-pig isolated trachea, when TBT was applied to the preparation at 1.0×10^{-8}–3.0×10^{-6} M, it caused relaxation of the tracheal rings in a concentration-dependent manner (Fig. 2B). The maximal change in tension of the preparation treated with TBT was almost comparable with the responses to acetylcholine 1.0×10^{-5} M. The effect of TBT was not inhibited by pre-treatment with β-adrenoceptor antagonist propranolol 1.0×10^{-6} M ($F(1, 59) = 0.10$, $P = 0.76$: two-way ANOVA).

In experiments with rat vas deferens, TBT caused a concentration-dependent inhibition of the twitch response of the preparation to nerve stimulation (Fig. 2C) without a change in the background tension. The relaxant effect of TBT was slightly antagonized by treatment

Table 1
Locomotor activities of mice given TBT[a]

TBT (i.p., mg/kg)	0		0.3		1.0		3.0	
	N	Mean ± S.E.M.	*N*	Mean ± S.E.M.	*N*	Mean ± S.E.M.	*N*	Mean ± S.E.M.
Total movement (number of counts)								
30 min	7	3180 ± 864	4	2105 ± 345	4	763 ± 332*	4	558 ± 76*
1 month	7	4011 ± 925	4	2698 ± 1152	1	2648	0	–
2 months	7	3862 ± 435	4	2648 ± 358	1	1662	0	–
3 months	7	4522 ± 581	4	4078 ± 477	1	3190	0	–
Horizontal action (number of counts)								
30 min	7	91.1 ± 18.8	4	91.3 ± 6.8	4	51.8 ± 21.7	4	61.3 ± 5.3
1 month	7	145.6 ± 11.0	4	85.5 ± 24.8	1	111.0	0	–
2 months	7	146.4 ± 11.0	4	106.3 ± 12.9	1	98.0	0	–
3 months	7	140.4 ± 12.2	4	133.0 ± 11.8	1	155.0	0	–
Rearing action (number of counts)								
30 min	7	212.7 ± 84.9	4	86.3 ± 28.2	4	32.8 ± 32.1	4	3.3 ± 3.0
1 month	7	206.7 ± 29.4	4	171.8 ± 100.0	1	107.0	0	–
2 months	7	213.4 ± 34.3	4	143.8 ± 31.8	1	99.0	0	–
3 months	7	232.4 ± 37.7	4	246.3 ± 42.6	1	154.0	0	–
Movement time (s)								
30 min	7	769 ± 162	4	549 ± 103	4	316 ± 120	4	249 ± 20*
1 month	7	1011 ± 91	4	660 ± 251	1	669	0	–
2 months	7	972 ± 89	4	684 ± 81	1	468	0	–
3 months	7	1021 ± 133	4	1014 ± 98	1	787	0	–
Movement time distance (cm)								
30 min	7	4354 ± 1268	4	2168 ± 419	4	1052 ± 368	4	834 ± 119
1 month	7	4664 ± 752	4	3166 ± 1484	1	2733	0	–
2 months	7	4308 ± 511	4	2812 ± 424	1	1489	0	–
3 months	7	4599 ± 828	4	4610 ± 1328	1	2913	0	–
Average speed (cm/s)								
30 min	7	5.04 ± 0.79	4	3.93 ± 0.35	4	3.83 ± 0.63	4	3.37 ± 0.39
1 month	7	4.50 ± 0.37	4	4.42 ± .0.37	1	4.10	0	–
2 months	7	3.73 ± 0.18	4	4.08 ± 0.21	1	3.20	0	–
3 months	7	4.40 ± 0.25	4	4.50 ± 0.35	1	3.70	0	–

[a] The locomotor activity for 30 min was measured 30 min, 1, 2 and 3 months after the intraperitoneal injection of TBT (0, 0.3, 1.0 and 3.0 mg/kg). The numbers of horizontal movement actions and rearing actions, movement time, movement distance or average speed were recorded as the indicators of locomotor activities. Each value represents the mean ± S.E.M. of *N* cases. *, $P < 0.05$ vs. vehicle, analysis of variance (ANOVA) followed by Tukey's test.

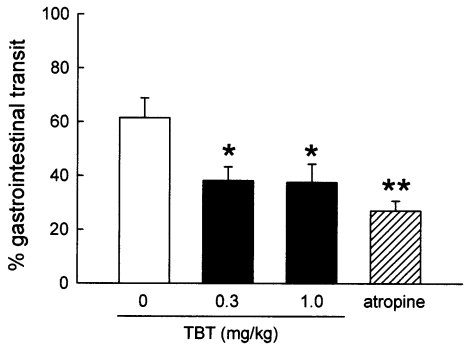

Fig. 1. Effect of subcutaneous injection of TBT on the intestinal propulsive activity of mice. TBT 0 mg/kg (open column), 0.3, 1.0 (closed column) or atropine 10 mg/kg (a positive control, hatched column) was injected 30 min before an oral administration of activated charcoal powder. The ordinate indicates the percentage of the distance traveled by charcoal for the total length of the small intestine. Each value represents the mean ± S.E.M. of four mice. *, $P < 0.05$; **, $P < 0.01$ vs. vehicle, Tukey's test following one-way ANOVA.

with α_2-adrenoceptor antagonist yohimbine 1.0×10^{-6} M ($F(1, 77) = 1.68$, $P = 0.19$, two-way ANOVA).

In experiments with guinea pig atria, TBT reduced the systolic contraction of the preparation in a concentration-dependent manner (Fig. 2D).

In experiments with rat aortic strips, the application of TBT caused a concentration-dependent contraction of the preparation (Fig. 3). The contractile response of the aorta was completely abolished by treatment with α_1-adrenoceptor antagonist prazosin 1.0×10^{-6} M. Rather, in the presence of prazosin, TBT provoked relaxation of the preparation, which suggests that TBT induced the relaxation as well as α_1-adrenoceptor-mediated contraction of the aorta. It is well known that the relaxation of the aortic smooth muscle is mediated by muscarinic receptor-stimulated release of nitrogen oxide from the vascular endothelial cells (Rapoport and Murad, 1983; Ignarro, 1989). Thus, we examined the possible involvement of this endogenous vascular relaxation system in the relaxation induced by exogenous TBT. The relaxant effect of TBT was not observed in the preparation without the vascular endothelium, and was inhibited in the presence of nitrogen-oxide chelator hemoglobin 1.0×10^{-5} M or muscarinic receptor antagonist atropine 1.0×10^{-6} M, but not by an application of synaptic vesicle-docking inhibitor botulinum toxin (1.0×10^{-10} M). These data suggest that TBT may act directly on muscarinic receptor on the vascular endothelium. This idea was supported by the result that

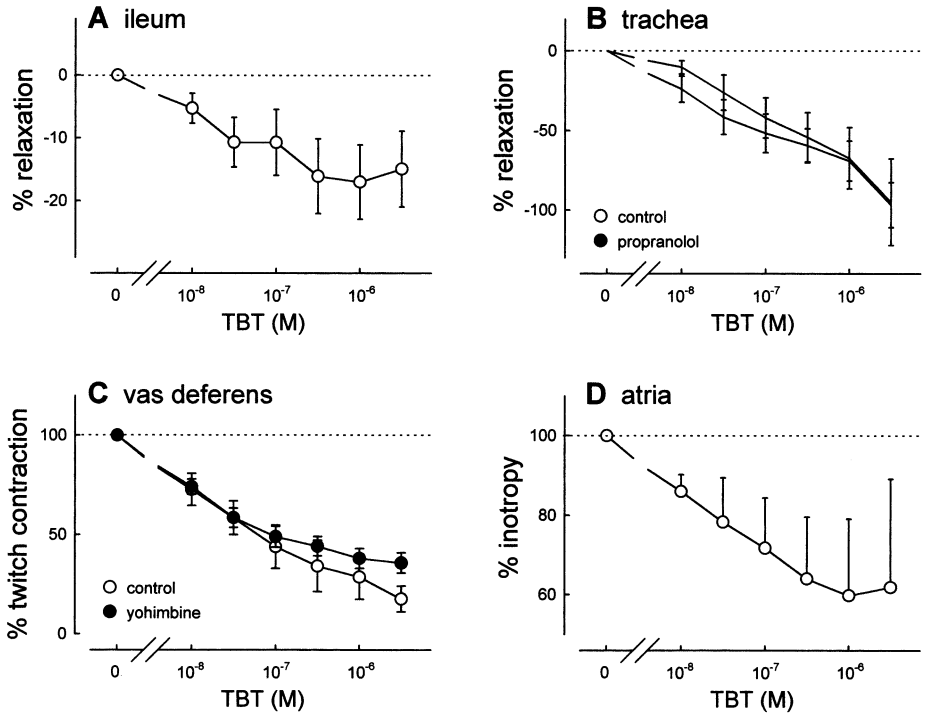

Fig. 2. (A) Relaxant effect of TBT on guinea-pig ileum. The ordinate indicates the ratio of change in length of the ileum treated with TBT (1.0×10^{-8}–3.0×10^{-6} M) to responses to acetylcholine 1.0×10^{-5} M. Each value represents the mean ± S.E.M. of eight cases. (B) Relaxant effect of TBT on guinea-pig trachea. The ordinate indicates the ratio of change in tension of the preparation treated with TBT to responses to acetylcholine 1.0×10^{-5} M. ○, control ($N = 6$); ●, the group pretreated with propranolol 1.0×10^{-6} M ($N = 4$). (C) Inhibitory effect of TBT on electrically evoked contractile responses of rat vas deferens. The ordinate indicates the ratio of twitch response after treatment with TBT to that before TBT application. ○, control; ●, the group pre-treated with yohimbine 1.0×10^{-6} M. Each value represents the mean ± S.E.M. of seven cases. (D) Relaxant effect of TBT on guinea-pig atria. The ordinate indicates the ratio of mean inotropic tension after treatment with TBT to that before the application of TBT. Each value represents the mean ± S.E.M. of seven cases.

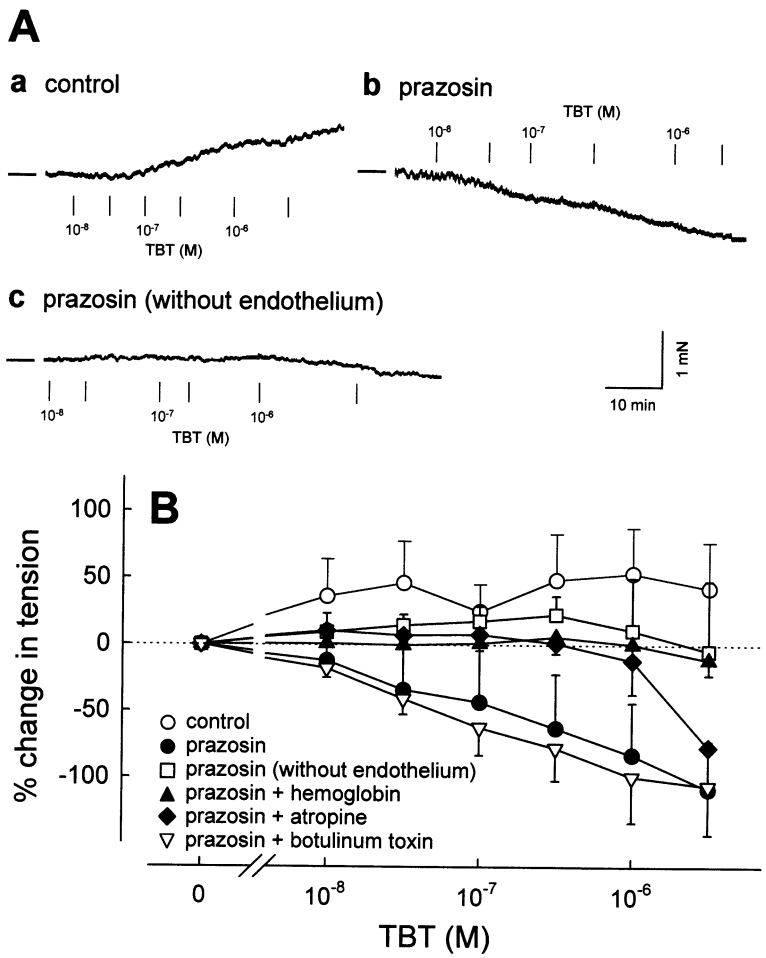

Fig. 3. Bi-directional effects of TBT on rat aorta. (A) Typical traces show responses of the intact aortic strips to TBT in the (a) absence or (b) presence of prazosin 1.0×10^{-6} M, and responses of the preparation without the vascular endothelium to TBT in the (c) presence of prazosin 1.0×10^{-6} M. Numbers and vertical line indicators below the traces represent the concentrations of TBT and the time of TBT application, respectively. The horizontal lines on the left side of the traces indicate the baseline tension of the preparation prior to TBT application. When the preparation increases the tension (contraction) or decreases the tension (relaxation), the trace goes to upside or downside to the baseline, respectively. (B), The ordinate indicates the ratio of change in tension of the preparation treated with TBT to that induced by norepinephrine 1.0×10^{-5} M. ○, control ($N = 6$); ●, pre-treatment with prazosin 1.0×10^{-6} M ($N = 5$); □, endothelium-denuded preparation treated with prazosin 1.0×10^{-6} M ($N = 3$); ▲, co-treatment with prazosin 1.0×10^{-6} M and hemoglobin 1.0×10^{-5} M ($N = 3$); ◆, co-treatment of prazosin 1.0×10^{-6} M and atropine 1.0×10^{-6} M ($N = 6$); △, co-treatment with prazosin 1.0×10^{-6} M and botulinum toxin 1.0×10^{-10} M ($N = 4$). Each value represents the mean ± S.E.M. of N cases.

the relaxant effect of TBT was not detected in the aorta that had fully relaxed by pre-treatment with acetylcholine ($N = 4$, data not shown), which suggests that TBT and acetylcholine share some common downstream mechanisms underlying the vasodilatation.

4. Discussion

In the present study, toxicological and pharmacological characteristics of TBT, an assumed environmental endocrine-disrupting chemical (Snoeij et al., 1987; Fent, 1996; Kannan and Falandysz, 1997), were exhaustively evaluated to clarify the influences of this compound on organisms.

With respect to the in vivo experiments, aqueous solution of TBT injected systemically may precipitate at the injection site because TBT has high lipid-solubility. However, subcutaneous injection of TBT affected the intestinal propulsive activity, and our preliminary study indicates that intraperitoneal injection of TBT causes severe neuron loss in the central nervous system (unpublished data). These indicate that systemically injected TBT is actually distributed in the whole body, even in the brain.

As a macroscopic behavioral alternation, we found that the mice given TBT showed a decrease in the locomotor activities. Since TBT failed to change the hypnoid potency of hexobarbital in mice, the hypokinesia was probably distinguished from sleep, cataplexy or

narcolepsy, and may be explained by the action of TBT on the peripheral neuromuscular system, and not on the central nervous system. The alternation in locomotor activities almost fully reverted at least 3 months after the injection of TBT. The TBT-induced behavioral disturbance is, therefore, unlikely mediated by irreversible substantial damages of the tissue structures, but it may be attributable to transient disorders of physiological function of the tissues exposed to TBT. Indeed, TBT is metabolized in the liver (Iwai et al., 1981) and the recovery may hence be explained by the elimination or excretion of TBT from the body.

In the experiments with aortic strip preparation, TBT demonstrated bi-directional actions; TBT caused both the contraction and relaxation of the aorta. Since the relaxant effect of TBT was observed only in the presence of α_1-adrenoceptor antagonist, the contractive actions of TBT are probably mediated by α_1-adrenoceptor activation and are more predominant than the relaxant effect under naive conditions. On the other hand, the relaxant effect of TBT was inhibited entirely by the removal of vascular endothelium or by an application of nitrogen-oxide chelator. These results indicate that TBT-induced relaxant of the aorta depends on the endogenous vascular relaxation system. Interestingly, pharmacological blockade of muscarinic receptors also prevented the relaxant effect of TBT but botulinum toxin, a vesicle-fusion inhibitor that hinders acetylcholine release from parasympathetic terminals, failed to repress the relaxation. These results strongly suggest that TBT directly activates muscarinic receptors to produce the vasorelaxing effect. Taken together, TBT may serve as an agonist for α_1-adrenoceptor and muscarinic receptors. However, there is still a discrepancy in our study because TBT altered neither the blood pressure in vivo nor the background tension of the vas deferens in vitro, both of which were strictly controlled by α_1-adrenergic innervation. As a possible explanation for the contrariety, we consider that systemically injected TBT may not be distributed uniformly in tissues and so it fails to affect the blood pressure. Another possibility is that the distribution of α_1-adrenoceptor or muscarinic receptor subtypes that TBT can modulate is different among tissues and organs. Further studies to investigate what underlies the apparent conflict, are now underway in our laboratory.

TBT induced relaxation in the ileum and the trachea; it also reduced contractive responses of the vas deferens evoked by nerve stimulation and it also weakened the systolic contraction of the spontaneous heartbeat. The mechanisms underlying these effects of TBT remain unclear while the reduction in twitch responses of the vas deferens was slightly inhibited by α_2-adrenoceptor blocker. These actions appear to be a consequence of tissue dysfunction evoked by non-specific toxicity of TBT rather than the pharmacological effect. Thus, the effects of TBT may be unaccountable with the classical pharmacology. However, Yallopragada et al. (1991) indicated that a low concentration of TBT decreases the enzyme activities of Ca^{2+}-ATPase and phosphodiesterase through the inhibition of calmodulin (IC_{50} value was 0.63 μM). Therefore, such broad-spectrum toxicity of TBT may be mediated by alternation in the activities of widely distributed proteins such as calmodulin.

Our present study revealed various pharmacological properties of TBT in mammalian organs. Horiguchi et al. (1994) reported that TBT concentrations in the rock shells living in the polluted sea area reach 1–5 μM. Therefore, it is possible that TBT is accumulated in mammals as a result of ecological magnification. Indeed, Kannan and Falandysz (1997) indicated that a significant concentration of butyltin was detected in human liver. Although, the relevance of the experimental doses and injection routes of TBT remained to be resolved because of few available data for the distribution and metabolism of TBT in mammals, the present study might give a warning about unconfirmed toxicity of this environmental pollutant for mammals. Particularly, TBT activates pharmacological receptors in a more specific fashion than our expectation, and thereby, may aggravate certain types of diseases such as autonomic nervous system dysfunction.

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