Butyltin Compounds *In vitro* Inhibition of Human Platelet Epinephrine-Induced Aggregation and Blockade of Epinephrine Binding to Platelets

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Abstract: The effects of mono-, di-, and tributyltin, which are widely used in industry, on human platelet aggregation were studied *in vitro*. The results demonstrated that these organic butyltin compounds inhibit epinephrine-induced aggregation. The antiaggregating concentrations (IC_{50}) of the butyltin compounds were 10 nM for tributyltin, 10 μ M for dibutyltin and 500 μ M for monobutyltin. However, none of these compounds affected the platelet aggregation induced by others stimuli (ADP, arachidonate, and collagen). On the other hand, all of the butyltin compounds inhibited the binding of [3H]-DHEC to human platelets, and di- and tributyltin significantly inhibited the incorporation of [^{14}C]-epinephrine into gel-filtered platelets. These results indicate that organic butyltin compounds inhibit epinephrine-induced aggregation by blocking the epinephrine binding to human platelets.

Key words: Butyltin—Organotin—Alpha-adrenergic antagonist—Epinephrine binding—Platelet aggregation

INTRODUCTION

Organotin compounds are used in a variety of applications.^{1,2)} They are involved in such widely divergent fields as: 1) stabilizers for polyvinylchloride; 2) industrial and agricultural biocides; 3) wood-preserving and anti-fouling agents; and 4) catalytic agents in a variety of industrial processes. This wide use of organotin has led to the extensive study of the toxicology of these compounds. The inhibition of oxidative phosphorylation,³⁾ production of edematous lesions in the white matter of the central nervous system,⁴⁾ depression of thymus-dependent immunity,^{5,6)} and diabetic lipemia,⁷⁻⁹⁾ are among the biological

Abbreviations: ADP, adenosine diphosphate; DHEC, dihydroergocryptine; HHT, 12-L-hydroxy-5, 8, 10-heptadecatrienoic acid; HETE, 12-L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid; and TX, thromboxane.

effects of these agents which have been shown in mammalian systems.

Recent studies in our laboratory have shown that organotins inhibit the stimulus-reaction system of various cells.^{7,10-12} In this study, we have examined in detail the effects of butyltin, a representative organotin, on stimulus-induced human platelet aggregation *in vitro*. The results of this study show that butyltin compounds inhibit epinephrine-induced human platelet aggregation by blocking epinephrine binding to cell membrane.

MATERIALS AND METHODS

Preparation of platelet-rich plasma

Blood was collected from six healthy male subjects (mean age, $29 \pm$ S.D. 3 years) who had not received any medication for 4 weeks, using 21-gauge needles and sterile plastic syringes containing sodium citrate as an anticoagulant (1 part of 3.8% sodium citrate to 9 parts of blood). The citrated blood was gently mixed and centrifuged at $180 \times g$ for 20 min at 20°C and the supernatant (platelet-rich plasma; PRP) was carefully transferred to a plastic tube using a plastic pipette. The platelet concentration of PRP was adjusted to approximately $350,000/\mu\text{l}$ by the addition of platelet-poor plasma (PPP) prepared by centrifugation of the remainder of the blood at $3000 \times g$ for 20 min at 4°C . Platelet concentration was counted in a platelet counter (Towa Model PL 100, Tokyo, Japan).

Platelet aggregation

Platelet aggregation was examined at 37°C in a Lumi-Aggregometer (Chrono-log Corp., Havertown, PA) according to the method of Feinman *et al.*¹³ Aggregation was measured as percentage light transmission, using the light transmission of PRP as 0% and that of PPP as 100%. Added chemicals consisted of 10% of the final volume of the assay mixture. L-epinephrine bitartrate (Sigma Chem. Co., St. Louis, MO) was dissolved in saline containing 5 mM NaHSO_3 and 0.01 N HCl, and then diluted 100-fold or more with saline prior to the aggregation assay. Disodium ADP (10^{-2} M) was prepared in 100 mM veronal buffer (pH 6.8) and was further diluted in saline prior to the aggregation assay. Collagen reagent (HORM) was obtained from Hormon-Chemie (München, West Germany), and diluted in HORM buffer. Sodium arachidonate (Sigma) was prepared and kept under nitrogen.

Preparation of solutions of butyltin compounds

Monobutyltin trichloride, dibutyltin dichloride, and tributyltin chloride were obtained from Sankyo Organosynthesis Co., Ltd. (Kawasaki, Japan). The stock solutions of these butyltin compounds, containing 10^{-1} to 10^{-9} moles/l, were prepared by dissolving them in 97% ethanol. Each solution of these compounds

was diluted 100-fold with saline just before the experiments. In addition, tin (IV) chloride (Nitto Kasei Co., Ltd. Osaka, Japan) was dissolved in 0.1% ethanol.

Spectrophotometric analyses

L-epinephrine bitartrate was dissolved in 50 mM sodium phosphate buffer, pH 7.4. The stock solutions of butyltin compounds were diluted 100-fold with 50 mM sodium phosphate buffer, pH 7.4. After confirming the pH of the final solutions, epinephrine (10^{-4} M) was incubated with either 0.1% ethanol (control) or 10^{-3} M butyltin compounds at 37°C for 30 min. Absorbance and fluorescence spectra were obtained using 0.1% ethanol in phosphate buffer as the blank. Spectrophotometric measurements were performed on a Hitachi 124 Dual-beam Spectrometer and a Hitachi Fluorescence Spectrophotometer.

The incorporation of [^{14}C]-epinephrine into human platelets

DL-[7- ^{14}C]-epinephrine hydrochloride (48.2 mCi/m mol) was obtained from New England Nuclear (Boston, USA) and stored at -20°C . PRP (3.0×10^5 cells/ μl) was preincubated with tin (IV) chloride or butyltin compounds at 37°C for 15 min. After pre-incubation, 10 nmol or 30 nmol of [^{14}C]-epinephrine was added to 10 ml PRP, and incubated at 37°C. Samples of 330 μl were transferred at appropriate time intervals into microfuge tubes containing 50 μl of FL 20 silicone oil (Shinetsu Chemical Co., Ltd, Annaka, Japan), and centrifuged for 5 min in an Eppendorf microfuge (Model 1514, New York, USA). Because the specific gravity of FL 20 silicone oil is the same as that of human platelets, the latter were spun down with the silicone oil. After discarding the supernatant, the platelet fraction containing silicone oil was transferred into the vial of a scintillation counter and the radioactivity was assayed. The radioactivity of the samples without incubation was defined as the nonspecific [^{14}C]-epinephrine incorporation. The specific incorporations was calculated as total minus nonspecific incorporation. Preliminary experiments showed a linear relationship between the incubation time (1 min to 30 min) and the amount of incorporated radioactive epinephrine. Therefore, in the following experiments radioactive epinephrine was incubated with PRP for 1 min.

Preparation of gel-filtered platelets

Gel filtration of platelets was carried out as reported earlier¹⁴⁾ on siliconized glass columns measuring 2.5 by 45 cm (Pharmacia, Sweden). The columns were equipped with gel-supporting nets having a pore diameter of 40 μm and were packed to a height of 25 cm with Sepharose 2B (Pharmacia, Sweden). The columns were previously equilibrated with Ca^{2+} -free Tyrode's solution containing 1.6% human serum albumin (Sigma). The PRP was gently layered on top of the gel and eluted with the solutions with which the gel was equilibrated. The most concentrated fractions of gel-filtered platelets appearing in the void volumes were collected. The collected fractions were at once diluted with Ca^{2+} -free

Tyrode's solution containing 1.6% human serum albumin and 5 mM HEPES buffer (N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid, Sigma), pH 7.35. After the addition of CaCl_2 (final concentration, 4 mM), an alpha-adrenergic binding study was done as described below.

Alpha-adrenergic binding assays

Human platelets first aggregate and undergo a release reaction when incubated with catecholamines. Indirect evidence indicates that these events are mediated through alpha-adrenergic receptors.¹⁵⁾ We used [^3H]-DHEC, an alpha-adrenergic antagonist, to determine the effects of butyltin compounds on the binding of epinephrine to platelet membrane, because it has been established that DHEC binds to the alpha-adrenergic receptor of human platelets.^{15,16)} Gel-filtered platelets were used in these binding assays. Alpha-adrenergic binding assays were done by the method of Newman *et al.*¹⁶⁾ A one-tenth volume of [^3H]-DHEC taken up in distilled water (26.2 Ci/mmol, New England Nuclear, Boston, USA) was incubated at 37°C with gel-filtered human platelets. Binding reaction was terminated by the addition of 4 ml of buffer, pH 7.4 at 37°C containing 10 μM phentolamine (Ciba-Geigy Corp. New Jersey, USA). Samples were then filtered over glass-fiber filters (Whatman GF/C) and washed within 20 seconds with 20 ml of 50 mM Tris-HCl buffer at 37°C. Dried filters were then counted in a liquid scintillation system. Nonspecific binding, determined by incubation of platelets with [^3H]-DHEC and 10 μM phentolamine, generally amounted to 25 to 40% of the total bound radioactivity from the labeled substance. Specific [^3H]-DHEC (0.5 to 40 nM) binding to platelets at 37°C reached equilibrium by 30 min. Therefore, all equilibrium binding studies were carried out for 30 min.

Chromatographic separation of arachidonic acid and its metabolites

Samples of 50 ml of PRP were incubated at 37°C for 2 h with 2 μCi of [^{14}C]-arachidonic acid (52.1 mCi/mmol) (Amersham Corp.). At the end of the incubation period, EDTA was added to the medium to make a final concentration of 2 mmol, and the platelets were pelleted by centrifugation at $3000 \times g$ for 20 min at 4°C. The pellet was resuspended gently in 50 ml NaCl/Tris-HCl (120 mM/30 mM) buffer, pH 7.4 (Tris/NaCl buffer).

One-milliliter samples of the labeled platelets (1×10^9 cells/ml) were preincubated with either 0.1% ethanol (control) or tributyltin chloride (1×10^{-5} M) for 15 min at 37°C. Collagen or epinephrine was added to give a final concentration of 20 $\mu\text{g}/\text{ml}$ or 2 μM respectively, and the mixture was incubated at 37°C for 5 min. Lipid extraction of the whole incubation mixture was done according to the method of Billah *et al.*¹⁷⁾ Dried lipids were dissolved in chloroform and spotted on silica gel G plates (E.M. Merck, Darmstadt, West Germany).

The solvent used for separation was the upper phase from a mixture of ethyl-acetate-2, 2, 4-trimethylpentane-acetic acid-water (90 : 50 : 20 : 100, by vol.).

Radioactive spots were visualized by iodide staining and autoradiography, and the radioactivity in each spot was determined by liquid scintillation counting. Standard HETE, HHT and TXB₂ were made available by members of the Experimental Chemistry Unit of the Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

RESULTS

Effects of tin compounds on human platelet aggregation induced by various stimulants

As shown in Table 1, mono-, di-, and tri-butyltin (10 μ M) significantly inhibited epinephrine-induced aggregation while these compounds did not have any inhibitory effects on ADP-, collagen-, or arachidonate-induced aggregation. In addition, ADP-, collagen-, and arachidonate-induced aggregation were not inhibited by these butyltin compounds at any concentration up to 500 μ M (data not shown). Butyltin compounds inhibited epinephrine-induced aggregation in a dose-dependent manner (Fig. 1). Among butyltin compounds tested in this experiment, tributyltin had the strongest inhibitory activity on epinephrine-induced aggregation (Fig. 1). The anti-aggregating concentrations (IC₅₀) of butyltin compounds were 10 nM for tributyltin, 10 μ M for dibutyltin and 500 μ M for monobutyltin, respectively. Inorganic tin (IV) did not inhibit either epinephrine-induced aggregation or other types of stimulant-induced aggregation at any concentration up to 10⁻³ M.

Table 1. Effects of tin compounds on human platelet aggregation induced by various stimulants

Tin compounds (μ M)	Tin (IV) chloride		Monobutyltin trichloride		Dibutyltin dichloride		Tributyltin chloride		Control
Stimulants	1.0	10	1.0	10	1.0	10	1.0	10	
Epinephrine (1 μ M)	75 $\pm$ 5.7	71 $\pm$ 4.1	68 $\pm$ 9.2	58 $\pm$ 2.8*	69 $\pm$ 8.6	48 $\pm$ 4.0*	22 $\pm$ 5.0*	9.5 $\pm$ 2.0*	75 $\pm$ 3.0
ADP (10 μ M)	70 $\pm$ 2.2	72 $\pm$ 3.7	70 $\pm$ 4.5	67 $\pm$ 6.1	70 $\pm$ 5.2	64 $\pm$ 6.9	68 $\pm$ 4.1	68 $\pm$ 4.0	70 $\pm$ 3.2
Collagen (20 μ g/ml)	74 $\pm$ 4.1	75 $\pm$ 1.9	75 $\pm$ 4.0	73 $\pm$ 3.0	71 $\pm$ 4.1	75 $\pm$ 3.3	70 $\pm$ 4.2	72 $\pm$ 2.6	75 $\pm$ 2.7
Sodium Arachidonate (1.7 mM)	76 $\pm$ 1.5	74 $\pm$ 3.9	73 $\pm$ 2.9	75 $\pm$ 3.1	75 $\pm$ 3.0	72 $\pm$ 2.7	75 $\pm$ 2.1	74 $\pm$ 1.8	76 $\pm$ 4.0

The extent of aggregation is expressed as percentage light transmission. Values indicate the means $\pm$ S.D. of three or five different experiments performed in PRP preincubated for 15 min with butyltin compounds and SnCl₄. Controls were preincubated with 0.01% ethanol solution.

* $p < 0.01$, as compared with control.

Fig. 1 Dose-response curves for the inhibition of epinephrine (1 μ M)-induced aggregation by butyltin compounds.

The extent of aggregation is expressed as percentage light transmission. Each point represents the mean $\pm$ S.D. of three or six different experiments performed in PRP preincubated for 15 min with either butyltin compounds or tin(IV) chloride.

* $p < 0.01$, as compared with control (tin compound-free control).

Δ : Tin (IV) chloride (SnCl₄)

$\circ$: Monobutyltin trichloride

$\bullet$: Dibutyltin dichloride

$\blacktriangle$: Tributyltin chloride

Counteractive effect of epinephrine itself against the inhibitory action of butyltin compounds on epinephrine-induced aggregation

PRP was preincubated for 15 min with either 0.1% ethanol (control) or butyltin compounds (10^{-5} M) and then exposed to various doses of epinephrine. As shown in Fig. 2, epinephrine induced a complete platelet aggregation at a concentration of $1 \mu\text{M}$ in the control. In platelets pretreated with mono-, di-, and tri- butyltin compounds, the aggregating concentration became higher than that of the control. Aggregating concentrations for platelets pretreated with butyltin compounds were approximately $2 \mu\text{M}$ for monobutyltin and approximately $20 \mu\text{M}$ for dibutyltin, respectively. Only 50% aggregation was seen in tributyltin-treated platelets even when stimulated with $20 \mu\text{M}$ of epinephrine. However, aggregation occurred dose-dependently with increased epinephrine concentrations. These results indicate that epinephrine itself has a counteractive effect against the inhibitory activity of butyltin compounds.

Fig. 2. Counteractivity of epinephrine itself against the inhibitory effects of butyltin compounds on epinephrine-induced aggregation.

Human platelets pretreated with butyltin compounds ($10 \mu\text{M}$) were exposed to various doses of epinephrine. Controls were pretreated with 0.01% ethanol solution for 15 min at 37°C . Each point represents the mean $\pm$ S.D. of three experiments.

- : Control
- : Monobutyltin trichloride
- : Dibutyltin dichloride
- ▲ : Tributyltin chloride

Effects of butyltin compounds on absorbance and fluorescence spectra of epinephrine

Epinephrine (10^{-4} M) was incubated with either 0.1% ethanol (control) or 10^{-3} M tri-, di-, or monobutyltin at 37°C for 30 min. Epinephrine (control) had two absorption peaks at 205 nm and 279 nm at pH 7.4 before incubation. After incubation, there was no difference between the absorbance spectrum of the control and that of epinephrine incubated with butyltin compounds. Also, no new peaks were detected (data not shown). In addition fluorometric analyses showed that the fluorescence spectrum of epinephrine was not changed by incubation with butyltin compounds (10^{-3} M) (data not shown). These results suggest that epinephrine is not destroyed by butyltin compounds, and also that no epinephrine-butyltin adducts are formed during incubation.

Effects of butyltin compounds on the incorporation of [^{14}C]-epinephrine into intact platelets

[^{14}C]-Epinephrine was incubated with PRP (3.0×10^5 cells/ μl) pretreated with inorganic tin (SnCl_4) or butyltin compounds at 37°C for 60 sec. After 5 min centrifugation, the incorporation of [^{14}C]-epinephrine into platelets was determined. Specific [^{14}C]-epinephrine incorporation was defined as total minus non-specific incorporation. As can be seen in Table 2, tributyltin ($> 1 \times 10^{-7}$ M) and dibutyltin ($> 1 \times 10^{-5}$ M) significantly inhibited the incorporation of [^{14}C]-

Table 2. Effects of tin compounds on the incorporation of [^{14}C]-epinephrine into intact human platelets

	Final concentration (M)	[^{14}C]-epinephrine	
		1 μM	3 μM
Tributyltin chloride	1×10^{-11}	78.7 ± 3.67	206 ± 20.0
	1×10^{-9}	63.4 ± 8.00	186 ± 24.7
	1×10^{-7}	$56.2 \pm 2.80^*$	$163 \pm 11.7^*$
	1×10^{-5}	$51.0 \pm 3.73^*$	$140 \pm 21.0^*$
Dibutyltin dichloride	1×10^{-11}	80.0 ± 5.07	222 ± 28.9
	1×10^{-9}	70.7 ± 8.00	211 ± 12.7
	1×10^{-7}	64.0 ± 10.0	182 ± 14.0
	1×10^{-5}	52.3 ± 7.00	$176 \pm 10.0^*$
Monobutyltin trichloride	1×10^{-11}	89.2 ± 4.00	236 ± 20.5
	1×10^{-9}	90.3 ± 5.25	244 ± 19.0
	1×10^{-7}	82.5 ± 2.11	233 ± 13.4
	1×10^{-5}	74.1 ± 4.08	218 ± 21.1
Tin (IV) chloride	1×10^{-5}	89.4 ± 3.40	241 ± 12.1
Control	—	86.0 ± 2.67	238 ± 20.3

The specific [^{14}C]-epinephrine incorporation is expressed as radioactivity ($\text{dpm} \times 10^{-3}$) per 10^8 platelets. Values indicate the mean $\pm$ S.D. of three experiments in PRP preincubated for 15 min with butyltin compounds and inorganic tin (SnCl_4). Controls were preincubated with 0.01% ethanol solution.

* $p < 0.01$, as compared with control.

epinephrine ($1\ \mu\text{M}$ and $3\ \mu\text{M}$) into human intact platelets. Although a decreasing tendency of incorporation was seen in platelets pretreated with monobutyltin, there was no significant decrease even at $1 \times 10^{-5}\ \text{M}$. In addition, inorganic tin (SnCl_4) did not show any inhibitory effects on the incorporations of ^{14}C -epinephrine into platelets even at $1 \times 10^{-5}\ \text{M}$.

Inhibition of ^3H -DHEC binding by butyltin compounds

Specific bindings of ^3H -DHEC (0.5 to $40\ \mu\text{M}$) to intact platelets at 37°C reached equilibrium within approximately 30 min. Therefore, all equilibrium binding studies were carried out for 30 min. Gel-filtered platelets were used in these experiments. Binding of ^3H -DHEC to platelets was apparently inhibited by butyltin compounds (Fig. 3). Binding of ^3H -DHEC to platelets pretreated with monobutyltin was saturable with increasing concentration of the ligand while binding of ^3H -DHEC to platelets pretreated with di-, and tributyltin compounds did not reach plateau levels of binding even at a ligand concentration

Fig. 3. The inhibition of ^3H -DHEC binding to platelets by butyltin compounds. ^3H -DHEC was incubated with gel-filtered platelets pretreated with butyltin compounds ($1\ \mu\text{M}$) and a control (0.01% ethanol) for 30 min at 37°C . The binding of ^3H -DHEC to platelets is expressed as specific binding of DHEC per 10^7 platelets.

- : Control
- : Monobutyltin trichloride
- : Dibutyltin dichloride
- ▲ : Tributyltin chloride

of 30 μ M. These results, however, seem to agree with those suggesting that epinephrine itself has a counteractive effect against the inhibitory action of butyltin compounds (Fig. 2).

Effects of tributyltin compounds on arachidonate metabolism

To determine the effects of butyltin compounds on stimulus-induced arachidonate metabolism, tributyltin was selected for this experiment in view of its powerful inhibitory activity on platelet aggregation as shown in Fig. 1. Tributyltin did not have any effect on collagen-induced arachidonate metabolism (Fig. 4). Epinephrine-induced formation of arachidonate metabolites was not visualized by autoradiography because of low radioactivity. Therefore, the spots were visualized by iodide staining, and the radioactivity was determined by liquid scintillation counting. As can be seen in Table 3, a significant decrease in all the determined arachidonate metabolites was detected in tributyltin-pretreated platelets as compared with the control. These results suggest not only that tributyltin, one of the most powerful inhibitors of epinephrine-induced aggregation, does not

Fig. 4. Thin-layer chromatographic separation of radioactive phosphatidic acid, arachidonate and its metabolites.

Human platelets were prelabelled with [14 C]-arachidonic acid and incubated with (A) 0.01% ethanol (control) or (B) tributyltin (1×10^{-5} M) for 15 min at 37°C and then exposed to 2 μ g/ml collagen. Radioactive spots were visualized by autoradiography.

Abbreviations: PA, phosphatidic acid; TXB₂, thromboxane B₂; HHT, 12-L-hydroxy-5, 8, 10-heptadecatrienoic acid; HETE, 12-L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid; AA, arachidonic acid.

Table 3. Effects of tributyltin on epinephrine-induced formation of arachidonate metabolites

	Arachidonate metabolites (cpm)		
	TXB ₂	HHT	HETE
Tributyltin chloride (1×10^{-5} M)	158 ± 15*	169 ± 18*	366 ± 27*
Control	511 ± 44	605 ± 51	1801 ± 200

Human platelets prelabeled with [¹⁴C]-arachidonic acid were incubated with 0.01% ethanol (control) or tributyltin chloride (1×10^{-5} M) for 15 min at 37°C and then exposed to 2 μ M epinephrine. Radioactive spots were visualized by iodide staining, and the radioactivity was determined using a liquid scintillation counter.

* $p < 0.01$, as compared with control.

influence arachidonate metabolism, but also that this compound inhibits arachidonate release from the platelet membrane only when epinephrine is used as a stimulant.

DISCUSSION

The results of the present study demonstrate that mono-, di-, and tributyltin inhibit epinephrine-induced human platelet aggregation *in vitro*. It is interesting to note that these butyltin compounds did not inhibit the platelet aggregation induced by other stimuli (ADP, arachidonate, and collagen) (Table 1). Butyltin compounds, furthermore, apparently inhibited the binding of [³H]-DHEC, a potent alpha-adrenergic antagonist, to human platelets (Fig. 3). This result indicates that butyltin compounds inhibit the binding of epinephrine, an alpha-adrenergic agonist, to alpha-adrenergic receptors of platelets, because it has been established that DHEC competes for alpha-adrenergic agonists including epinephrine.^{15,16} In accordance with this result, dibutyltin ($> 1 \times 10^{-5}$ M) and tributyltin ($> 1 \times 10^{-7}$ M) significantly inhibited the incorporation of [¹⁴C]-epinephrine into intact platelets while no significant decrease of [¹⁴C]-epinephrine incorporation was observed in platelets pretreated with monobutyltin (Table 2). These results indicate that butyltin compounds are potent inhibitors of epinephrine-platelet binding, and also that the inhibition of epinephrine-induced aggregation by butyltin compounds is due to the inhibitory actions of these compounds on the binding of epinephrine to platelet membrane. On the other hand, inhibitory actions of butyltin on [³H]-DHEC binding and epinephrine-induced aggregation were counteracted by overdoses of [³H]-DHEC or epinephrine (Figs. 2 and 3). These results might suggest that butyltin compounds are potent competitive inhibitors of the alpha-adrenergic receptor, because there was no evidence that butyltin compounds destroy epinephrine or that butyltin compound-epinephrine adducts are formed during incubation.

It is generally accepted that metabolites of arachidonic acid are important for platelet function. In the results obtained from this study, tributyltin, the

most powerful inhibitor of epinephrine-induced aggregation among the butyltin compounds tested, did not show any inhibitory effects on collagen-induced arachidonate metabolites (Fig. 4). However, tributyltin did inhibit the production of arachidonate metabolites by epinephrine (Table 3). These results suggest that butyltin compounds do not affect the metabolism of arachidonic acid, and that arachidonate release from platelet membrane lipids is inhibited only when platelets are stimulated by epinephrine. These results are compatible with the idea that butyltin compounds are inhibitors of the alpha-adrenergic receptor.

It has been reported that organotin compounds including tributyltin affect the tissue catecholamine levels in animals *in vivo*. Robinson¹⁸⁾ reported that heart epinephrine levels are increased by di-, and tributyltin in rats while adrenal epinephrine and norepinephrine levels are significantly reduced. In his report, the above author mentioned that the increase of epinephrine and norepinephrine in heart after administration of these organotin compounds tends to parallel the release of these catecholamines from the adrenals. This evidence may suggest that butyltin compounds produce a catecholamine-resistant state by inhibiting the binding of catecholamine to tissues, as the results of our experiments indicate that butyltin compounds are alpha-adrenergic antagonists.

Recent studies in our laboratory have shown that organotin compounds inhibit the stimulus-reaction system of platelets,¹⁰⁾ granulocytes^{11,12)} and pancreatic islets.⁷⁻⁹⁾ In the present experiment, we have shown that butyltin compounds inhibit epinephrine-induced aggregation by acting on the membrane alpha-adrenergic receptor. Other organotin compounds such as triphenyltin and tricyclohexyltin did not have any effects on human platelet aggregation induced by epinephrine (unpublished data). These findings suggest that butyltin compounds possess a special characteristic in their mode of action on mammalian cells. In this connection, the inhibition of epinephrine-induced aggregation by butyltin compounds might be useful as an index of exposure to these compounds in human subjects.

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