Characterization of the Triphenyltin-Induced Increase in Intracellular Ca\(^{2+}\) of Mouse Thymocytes: Comparison with the Action of A23187

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ABSTRACT—The properties of triphenyltin (TPT) in increasing intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) of thymocytes was studied, in comparison with those of A23187, by the use of fluorescent dyes to monitor membrane potential and [Ca\(^{2+}\)]\(_i\). Both 1 \(\mu\)M TPT and 30 nM A23187 increased the [Ca\(^{2+}\)]\(_i\), associated with the hyperpolarization mediated by Ca\(^{2+}\)-dependent K\(^+\) conductance. The time course for the TPT-induced increase in the [Ca\(^{2+}\)]\(_i\) was much slower than that of A23187. When the external Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_o\)) was removed, TPT produced a slight, but persistent, increase in the [Ca\(^{2+}\)]\(_i\), while A23187 caused only a transient increase in the [Ca\(^{2+}\)]\(_i\). Reintroduction of Ca\(^{2+}\) to the external solution produced an increase in [Ca\(^{2+}\)]\(_i\) in both cases. Therefore, these results suggested that the increase in the [Ca\(^{2+}\)]\(_i\) of thymocytes induced by TPT and A23187 was dependent on the presence of [Ca\(^{2+}\)]\(_o\) and an intracellular Ca store. The potency of TPT in increasing the [Ca\(^{2+}\)]\(_i\), was greater than those of diphenyltin and monophenyltin, suggesting an involvement of the lipophilic property of organotins in increasing [Ca\(^{2+}\)]\(_i\). The TPT-induced increase in the [Ca\(^{2+}\)]\(_i\) may be partly responsible for the toxicity of TPT on organs and/or organ systems.

Keywords: Triphenyltin, Diphenyltin, Monophenyltin, Intracellular Ca\(^{2+}\), Cytotoxicity

Organotins are widely-used as heat stabilizers of polyvinyl chloride polymers, industrial catalysts in variety of chemical reactions, and industrial and agricultural biocides (1-3). Along with the expansion of technical applications of organotins, the possible enviromental and health effects of organotins have become a subject of concern. Although the mammalian toxicities, such as neurotoxicity, immunotoxicity, and hepatotoxicity, of organotins have been studied on target organs and/or organ systems, there is little information on the cellular basis for their toxicities except for some biochemical studies (4).

We have found that triphenyltin (TPT), one of the organotins, increases the intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) of mouse thymocytes by the use of respective fluorescent dyes for [Ca\(^{2+}\)]\(_i\) and membrane potential, suggesting the cytotoxic action of TPT (5). A similar effect of TPT on the [Ca\(^{2+}\)]\(_i\) has been observed in dissociated mammalian cerebellar neurons (6). Since it was thought to be a common feature of TPT cytotoxicity, we have investigated the properties of TPT in increasing [Ca\(^{2+}\)]\(_i\), in comparison with those of A23187 (Ca\(^{2+}\)-ionophore), diphenyltin and monophenyltin.

MATERIALS AND METHODS

Experimental methods used here were similar to the previously described ones (5, 6). In brief, the thymocytes were dissociated from the thymus of 4-week-old mice in Tyrode’s solution or Ca\(^{2+}\)-free Tyrode’s solution. Tyrode’s solution contained 148 mM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\) and 10 mM glucose; The pH of the solution was adjusted to 7.3 by adding 10 mM HEPES and an appropriate amount (about 2 mM) of NaOH. Ca\(^{2+}\)-free Tyrode solution was prepared by substituting equimolar MgCl\(_2\) for CaCl\(_2\) and adding 2 mM EGTA. The respective solution containing thymocytes was filtered through a mesh (diameter of 53 \(\mu\)m) to remove the residues of the thymus. The
cell suspension was incubated at the at 33–36°C for at least 60 min before any fluorescent measurement.

The photochemical measurement of membrane potential was made with bis-(1,3-dibutylbarbituric acid) trimethine oxonol (di-BA-C_4; Molecular Probe, Inc., U.S.A.) as previously reported (7). The oxonol dye di-BA-C_4 was added into the cell suspension to achieve a final concentration of 100 nM at least 3 min before the fluorescence measurement. For monitoring the [Ca^{2+}]_i, 1-[2-amino-5-(2,7-dichlor-6-hydroxy-3-oxy-9-xanthenyl) phenoxy]-2-(2'-amino-5'-methylphenoxy) ethane-N,N,N',N'-tetraacetic acid (fluo-3) was used (8). To load fluo-3 to the cells, the thymocytes were incubated with the penta-acetoxymethyl ester of fluo-3 (fluo-3-AM, Dojin do Lab., Japan) at a concentration of 150 to 300 nM for 60 min. The fluorescence measurements for di-BA-C_4 and fluo-3 were done on a flow-cytometer equipped with an argon laser (Cyto-ACE 150, Japan Spectroscopic Co., Ltd., Japan). The excitation wavelength used for these dyes was 488 nm, and the emission was detected at the wavelength of 530 ± 20 nm by a bandpass filter. A histogram representing the fluorescence distribution was obtained from a programmed number of thymocytes (4000 cells), using the software developed by Japan Spectroscopic Co., Ltd. on a personal computer (PC9801RX, Nippon Electronics Company, Japan). A change to increased or decreased fluorescent intensity corresponds to depolarization or hyperpolarization of the membrane potential for di-BA-C_4 and an increase or decrease in the [Ca^{2+}]_i for fluo-3, respectively.

Tentative calibration for the di-BA-C_4 fluorescence was made according to the previously reported method (9). As shown in Fig. 1, A23187 at the concentration of 3 nM or higher shifted the di-BA-C_4 fluorescence profile to the lower intensity direction, indicating a hyperpolarization. Since the membrane of thymocytes possesses Ca^{2+}-activated K^+ conductance, the membrane potential of thymocytes is assumed to reach the equilibrium potential for K^+ in the presence of 30 nM A23187 (8, 9). The relation between the membrane potential versus the fluorescent intensity can be obtained from

![Fig. 1](image-url)
the Nernst equation at various external K\(^+\) concentrations ([K\(^+\)]\(_o\)) under the assumption that the intracellular K\(^+\) concentration ([K\(^+\)]\(_i\)) is 150 mM (Fig. 1). The fluorescence intensity corresponding to the membrane potential at the [K\(^+\)]\(_o\) of 5, 10, 25, and 50 mM is shown in the figure legend. In the case of the fluo-3 fluorescence, the tentative calibration was performed by the method using ionomycin and a heavy metal as previously reported (10). Thus, the [Ca\(^{2+}\)]\(_i\) was estimated by the following equation (10):

\[
[Ca^{2+}]_i = K_d(F - F_{\text{min}})/(F_{\text{max}} - F)
\]

where \(K_d\) is 400 nM at vertebrate ionic strength, \(F_{\text{max}}\) represents the maximum fluorescence intensity from the Ca\(^{2+}\)-saturated cellular dye, \(F_{\text{min}}\) represents the minimum fluorescence intensity predicted from the metal-free dye fluorescence and the background fluorescence (10), and \(F\) is the fluorescence intensity obtained during the experiment. The fluorescence intensity obtained during the experiment. The fluorescence intensity corresponding to the [Ca\(^{2+}\)]\(_i\) of 10 nM, 100 nM, and 1 \(\mu\)M is described in the respective figure legend.

A23187 (Sigma Chemical Co., U.S.A.) and the triorganotins were initially dissolved in dimethylsulfoxide and the final concentrations of solvent were less than 0.1%. Solvent at such concentrations did not affect any measurement of oxonol and fluo-3 fluorescence. The triorganotins used were triphenyltin chloride (TPT, Tokyo Kasei Co., Japan), diphenyltin dichloride (DPT, Aldrich Chemical Co., U.S.A.), and monophenyltin trichloride (MPT, Aldrich Chemical Co., U.S.A.). Other chemicals used here were purchased from Wako Pure Chemicals, Ltd., Japan.

RESULTS

Effects of TPT and A23187 on the fluorescence profile for membrane potential of thymocytes

We have previously described that the hyperpolarization in thymocytes occurred in a time-dependent manner and reached a steady state within 3 min after adding TPT at the concentration of 1 \(\mu\)M, and this hyperpolarization was sustained at the steady level for the next 2 min (5). In fact, as shown in Fig. 2, the di-BA-C\(_4\) fluorescence intensity was reduced during the 10-min period after adding 1 \(\mu\)M TPT, which produced the largest decrease in the fluorescence (hyperpolariza-

![Fig. 2. Time-dependent effect of 1 \(\mu\)M TPT on the di-BA-C\(_4\) fluorescence profile of 4000 thymocytes. Thin and thick (with arrow) line histograms were obtained respectively before and 1 to 20 min after adding TPT. The fluorescence intensity at 13, 29, 59 and 100 corresponds approximately to -90, -70, -50 and -30 mV, respectively, according to the method described in the text.](image)
(hyperpolarizing and depolarizing) effects on the membrane potential of thymocytes, depending on the exposure time of TPT (Fig. 3), while TPT at the concentration of 100 or 300 nM produced a persistent, but lesser degree, decrease in the fluorescence during the 30-min period following the addition of TPT (not shown). A23187 at concentrations ranging from 1 nM to 30 nM produced a dose-dependent decrease in the fluorescence. The maximum response (hyperpolarization) was obtained with A23187 in the concentration range of 10 to 30 nM. Therefore, in following experiments, the action of 1 μM TPT was compared with that of 30 nM A23187. The time course for reaching the minimum level of the fluorescence intensity produced by 1 μM TPT was much slower than that induced by 30 nM A23187 (Figs. 1, 2 and 3). In the case of A23187 at the concentration of 30 nM, the minimum level of di-BA-C4 fluorescence was obtained, and the fluorescence intensity after reaching the minimum level was almost constant during the next 20 min (Fig. 3). The mean intensity of the di-BA-C4 fluorescence decreased to 0.18 ± 0.11 (relative to the control intensity, mean ± S.D. in four experiments) in the presence of 30 nM A23187, while it was 0.52 ± 0.18 (mean ± S.D. in 4 experiments) in the case of 1 μM TPT. Thus, the result suggests that the hyperpolarization induced by 1 μM TPT did not reach the equilibrium potential for K⁺ (Fig. 3).

The hyperpolarization induced by both 1 μM TPT and 30 nM A23187 was completely blocked by 100 μM quinine, but not blocked by 10 mM tetraethylammonium and 5 mM 4-aminopyridine, suggesting an involvement of Ca²⁺-activated K⁺ conductance in the hyperpolarization (9). Such a depolarization was observed in Na⁺- and Ca²⁺-free Tyrode's solution where Na⁺ and Ca²⁺ were respectively replaced with equimolar choline and Mg²⁺ and 2 mM EGTA was added. It was unlikely that the depolarization induced by 1 μM TPT was mediated by Na⁺ and Ca²⁺.

Effects of TPT and A23187 on the fluo-3 fluorescence profile of thymocytes

Difference in the time course for reaching the minimum level of di-BA-C₄ fluorescence between TPT and A23187 suggested that the time course in increasing the [Ca²⁺]i by TPT was different from that by A23187. Therefore, the properties of TPT in increasing the [Ca²⁺]i was studied in the comparison with those of A23187 by using fluo-3 fluorescence. As shown in Fig. 4, 1 μM TPT augmented the fluo-3 fluorescence in a time-dependent manner. The saturation of fluorescence intensity was observed at 20 min after adding TPT. The increase of fluorescence intensity by A23187 followed a distinct time course. Thus, 30 nM A23187 produced a transient increase in the fluo-3 fluorescence intensity to maximum level within 1 min after adding A23187. Thereafter, the intensity of the fluorescence gradually, but slightly, decreased from the maximum level during the next 20 min (Fig. 5). In spite of the difference in time course between TPT and A23187, the maximal intensity of fluo-3 fluorescence produced by 1 μM TPT was similar to that by 30 nM A23187. The fluo-3 fluorescence increased to 11.1 ± 0.9 (relative to the control, mean ± S.D. in 4 experiments) in the case of 1 μM TPT and 12.8 ± 1.1 (mean ± S.D. in 4 experiments), for 30 nM A23187. Representative results are shown in Fig. 6.

Effects of the removal of external Ca²⁺ on TPT- and A23187-induced increase in the fluo-3 fluorescence

To determine the source of the increased [Ca²⁺], by TPT and A23187, the effects of these agents on the fluo-3 fluorescence of the thymocytes were examined in [Ca²⁺]o-free Tyrode's solution. TPT at the concentration of 1 μM increased the fluo-3 fluorescence in-

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**Fig. 3.** Time-dependent changes in the mean intensity of di-BA-C₄ fluorescence profile in the presence of 1 μM TPT (square) and 30 nM A23187 (circle). Ordinate indicates the relative value of the mean intensity of each fluorescence profile obtained from 4000 thymocytes. Because of the large number of the thymocytes examined, the standard error of the mean is overlapped by the symbol. Result shows a representative one in four experiments.
Fig. 4. Time-dependent change of the histogram for the fluo-3 fluorescence obtained from 4000 thymocytes in the presence of 1 μM TPT. Thin and thick (with arrow) line histograms were obtained before and 1 to 20 min after adding TPT, respectively. According to the calibration method described in the text, the fluorescence intensity of 20, 70 and 240 corresponds approximately to 10 nM, 100 nM and 1 μM [Ca$^{2+}$], respectively.

Fig. 5. Time-dependent change of the histogram for the fluo-3 fluorescence obtained from 4000 thymocytes in the presence of 30 nM A23187. Thin and thick (with arrow) line histograms were obtained before and 1 to 20 min after adding A23187, respectively. The fluorescence intensity of 20, 70 and 240 corresponds approximately to 10 nM, 100 nM and 1 μM [Ca$^{2+}$], respectively.
tensity during the period of 5 min after adding the agent (Fig. 7). However, a further increase in the fluorescence intensity was not observed at 10 to 20 min after adding TPT (not shown). As shown in Fig. 8, the degree of the increased intensity of fluo-3 fluorescence by TPT under the nominally \([Ca^{2+}]_{o}\)-free condition was less than those by TPT at various \([Ca^{2+}]_{i}\), which were calculated with the following equation (11):

\[
\text{added Ca} = \frac{1 + K'(\text{EGTA} + [Ca])}{1 + [Ca]K'} [Ca]
\]

where \([Ca]\) represents the free calcium concentration, \([\text{EGTA}]\) represents the concentration of EGTA, and \(K'\) is an apparent association constant for \(Ca^{2+}\)-EGTA of \(10^{7.1}\). The fluo-3 fluorescence was augmented by 1 \(\mu\)M TPT in a \([Ca^{2+}]_{i}\)-dependent manner (Fig. 8). The mean intensity of fluo-3 fluorescence obtained from 4000 thymocytes increased to 3.0 ± 0.3 (relative to the control, mean ± S.D. in 3 experiments) under the nominally \([Ca^{2+}]_{o}\)-free condition, 3.9 ± 0.6 at the calculated \([Ca^{2+}]_{o}\) of 27 nM, 5.2 ± 0.6 at 139 nM \([Ca^{2+}]_{o}\), and 10.2 ± 0.9 at 125 \(\mu\)M \([Ca^{2+}]_{o}\). On the other hand, A23187 at the concentration of \(1 \times 10^{-8}\) M initially augmented the fluo-3 fluorescence. However,
the fluorescence intensity returned to the control level or less within 3 min after adding A23187 (Fig. 7). Reintroduction of Ca\(^{2+}\) increased the fluorescence intensity (not shown).

*Comparison of potency of TPT in increasing intracellular Ca\(^{2+}\) with those of DPT and MPT*

The toxicity of organotin compounds to mammals generally increases from mono- to triorganotins (5). In this study, the potency of TPT in increasing [Ca\(^{2+}\)]\(_i\), was compared with those of DPT and MPT at the same concentration (1 \(\mu\)M). MTP did affect the fluo-3 fluorescence of thymocytes even if the cells were exposed to MPT for 20 min (Fig. 9). DPT was found to slightly augment the fluorescence, although the potency of DPT was much less than that of TPT.

![Figure 8](image1.png)

*Fig. 8.* The [Ca\(^{2+}\)]\(_i\)-dependent increase in the intensity of the fluo-3 fluorescence by 1 \(\mu\)M TPT. The [Ca\(^{2+}\)]\(_i\) was increased by adding the respective concentration of CaCl\(_2\) to the nominally [Ca\(^{2+}\)]\(_i\)-free solution containing 2 mM EGTA. Respective [Ca\(^{2+}\)]\(_i\)'s calculated by the equation described in the text were 27 nM for adding 0.1 mM CaCl\(_2\) into the [Ca\(^{2+}\)]\(_i\)-free solution, 139 nM for 0.3 mM and 12.5 \(\mu\)M for 2 mM. Effect of TPT was examined 20 min after adding TPT.

![Figure 9](image2.png)

*Fig. 9.* Comparison of potency of 1 \(\mu\)M TPT in augmenting the fluo-3 fluorescence with those of 1 \(\mu\)M diphenyltin (DPT) and 1 \(\mu\)M monophenyltin (MPT). Effect of organotin was studied 20 min after adding the respective agent. The fluorescence intensity of 20, 70 and 240 corresponds approximately to 10 nM, 100 nM and 1 \(\mu\)M [Ca\(^{2+}\)]\(_i\), respectively.
DISCUSSION

TPT at the concentration of 1 \( \mu \)M exerted dual effects on the membrane potential (di-BA-C4 fluorescence) of the thymocytes as shown in Fig. 2. Hyperpolarization induced by TPT was suppressed by quinine, the blocker for \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) conductance, but not by tetraethylammonium and 4-aminopyridine, the blockers for voltage-dependent \( \text{K}^{+} \) conductance. Therefore, it was pharmacologically suggested that the \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) conductance was involved in the hyperpolarization induced by TPT. The membrane of T-lymphocytes including thymocytes has been reported to possess \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) conductance (9). TPT was proven to increase the \([\text{Ca}^{2+}]_{i}\) of the thymocytes (Fig. 4). Therefore, it was likely that the hyperpolarization occurring in the presence of TPT was mediated by \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) conductance activated by the \([\text{Ca}^{2+}]_{o}\), increased by TPT. Even in the sustained increase of \([\text{Ca}^{2+}]_{i}\) (Fig. 6), the membrane potential of the thymocytes became depolarized in the presence of TPT, although persistent hyperpolarization was observed in the case of 30 nM A23187 (Fig. 3). Since TPT exerts potent inhibitory action on the voltage-dependent \( \text{K}^{+} \) channels of mammalian brain neurons (12), it may be plausible to suggest that TPT also inhibits the \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) channel. However, the depolarization beyond the control membrane potential can not be elicited by the blockade of \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) channels. Since the depolarization of thymocytes was observed in the \( \text{Na}^{+} \) - and \( \text{Ca}^{2+} \)-free Tyrode’s solution containing TPT, TPT may exert a direct action on the membrane \( \text{K}^{+} \) permeability. However, electrophysiological experiments must be performed to elucidate the mechanism for the depolarization of thymocytes.

The properties of TPT in increasing the \([\text{Ca}^{2+}]_{i}\) are different from those of A23187 as evidenced by the following observations. First, TPT augmented the fluo-3 fluorescence in a time-dependent manner during the 20-min period after its application in the presence of \([\text{Ca}^{2+}]_{o}\), while the maximum intensity of fluorescence was obtained within 1 min in the case of A23187 (Fig. 6). Yet the maximum level of fluo-3 fluorescence increased by 1 \( \mu \)M TPT was similar to that by 30 nM A23187. Secondly, the difference of the time course in affecting \([\text{Ca}^{2+}]_{i}\) between TPT and A23187 became more obvious under the nominally \([\text{Ca}^{2+}]_{o}\)-free condition, although the maximum intensity of fluo-3 fluorescence induced by both agents was greatly reduced. TPT slightly, but persistently, augmented the fluo-3 fluorescence, while A23187 induced only a transient change (an increase) in the fluorescence. Therefore, the source of the increased \([\text{Ca}^{2+}]_{i}\), by TPT and A23187 is key to revealing the difference between them.

As to the source of the \([\text{Ca}^{2+}]_{i}\) increased by TPT, the removal of \([\text{Ca}^{2+}]_{o}\) greatly reduced the availability of TPT in augmenting the fluo-3 fluorescence, suggesting the involvement of \([\text{Ca}^{2+}]_{o}\) (Fig. 7). Furthermore, the effect of TPT on the \([\text{Ca}^{2+}]_{i}\) became more profound when the \([\text{Ca}^{2+}]_{o}\) increased from nominally \([\text{Ca}^{2+}]_{o}\)-free to the calculated \([\text{Ca}^{2+}]_{o}\), as shown in Fig. 8. Therefore, it is concluded that a part of the increased \([\text{Ca}^{2+}]_{i}\) by TPT is dependent on external \( \text{Ca}^{2+}\). TPT may promote \( \text{Ca}^{2+} \)-influx to thymocytes. The potency of 1 \( \mu \)M TPT in increasing the \([\text{Ca}^{2+}]_{i}\) was greater than that of DPT at the same concentration, while MPT exerted little action on the \([\text{Ca}^{2+}]_{i}\). Therefore, it suggests that the lipophilic property of organotin is partly responsible for the action, although the mechanism for promoting \( \text{Ca}^{2+} \)-influx to thymocytes could not be elucidated by the present data. Our results support the general concept that the toxicity of organotin compounds to mammals increases from mono- to triorganotins (5). An increase in the \([\text{Ca}^{2+}]_{i}\), by TPT was also observed under the nominally \([\text{Ca}^{2+}]_{o}\)-free condition (Fig. 7). The cytosolic \( \text{Ca}^{2+} \) concentration of the resting cells is usually maintained below 100 nM by active \( \text{Ca}^{2+} \) extrusion through the plasma membrane and by the coordinated activity of \( \text{Ca}^{2+} \)-sequestering systems located in the mitochondrial, endoplasmic reticular, and nuclear membranes. Therefore, it is plausible to suggest the involvement of the effect of TPT on such active transport mechanisms for maintaining intracellular \( \text{Ca}^{2+} \) homeostasis. The triorganotins were found to bind to a component of the ATP synthase complex, leading to a direct inhibition of ATP production (13). Incubation of rat thymocytes with trialkyltin resulted in a reduction in the intracellular ATP levels (14, 15). If it is the case for TPT, TPT would produce a malfunction of active intracellular \( \text{Ca}^{2+} \)-sequestering systems, resulting in an increased \([\text{Ca}^{2+}]_{i}\). With regard to the source of the increased \([\text{Ca}^{2+}]_{i}\); in the presence of A23187, it is likely that the fluorescence intensity increased by A23187 is profoundly dependent on the presence of \([\text{Ca}^{2+}]_{o}\), since under the nominally \([\text{Ca}^{2+}]_{o}\)-free condition, A23187 caused only a transient increase in the fluo-3 fluorescence (Fig. 7). One may raise a question about the source for the initial increased \([\text{Ca}^{2+}]_{i}\) by A23187 in the \([\text{Ca}^{2+}]_{o}\)-free solution. Ionomycin also exerted a similar transient effect on the \([\text{Ca}^{2+}]_{i}\); of thymocytes under the \([\text{Ca}^{2+}]_{o}\)-free condition (L. Chikahisa and Y. Oyama, unpublished observation). Ionomycin was reported to affect the endoplasmic reticulum, resulting in an increased \([\text{Ca}^{2+}]_{i}\); under the \([\text{Ca}^{2+}]_{o}\)-free condition without changing the cellular ability of \( \text{Ca}^{2+} \)-extrusion in the cell membrane (16). If
it is also the case for A23187, the \([\text{Ca}^{2+}]_i\) increased by A23187 would be extruded by the membrane \(\text{Ca}^{2+}\) pump, resulting in the transient change in the \([\text{Ca}^{2+}]_i\).

TPT-induced increase in the \([\text{Ca}^{2+}]_i\) may have an important implication on the cytotoxicity since an abnormal increase in the \([\text{Ca}^{2+}]_i\) would generally initiate pathological states and disturb the homeostasis unless an increased \([\text{Ca}^{2+}]_i\) is sequestered by the intracellular organelles and/or is extruded by the membrane (17).

Since TPT increased the \([\text{Ca}^{2+}]_i\) in the \([\text{Ca}^{2+}]_o\)-free solution, TPT is possibly suggested to exert actions on several biological systems to control \([\text{Ca}^{2+}]_i\). If it is a common feature of TPT cytotoxicity, the toxicity of TPT on organs and/or organ systems may be based on the TPT-induced increase in \([\text{Ca}^{2+}]_i\).

Acknowledgments
This study was partly supported by the Research Grant awarded to Y. Oyama from the Nippon Life Insurance Foundation and by the Grant-in-Aids (No. 03607409 to Y. Oyama) from the Minister of Education, Science and Culture, Japan.

REFERENCES
1 Luijten, J.G.: Applications and biological effects of organotin compounds. In Organotin Compounds, Edited by Sawyer, A.K., p. 931 – 974, Decker, New York (1971)
2 Van der Kerk, G.J.M.: The organic chemistry of tin. Chem. Tech. 8, 356 – 365 (1978)
3 Wilkinson, R.R.: Technoeconomic and environmental assessment of industrial organotin compounds. Neurotoxicology 5, 141 – 158 (1984)
4 Snoeij, N.J., Pennink, A.H. and Seinen, W.: Biological activity of organotin compounds — an overview. Environ. Res. 44, 335 – 353 (1987)
5 Oyama, Y., Chikahisa, L., Tomiyoshi, F. and Hayashi, H.: Cytotoxic action of triphenyltin on mouse thymocytes: A flow-cytometric study using fluorescent dyes for membrane potential and intracellular \(\text{Ca}^{2+}\). Japan. J. Pharmacol. 57, 419 – 424 (1991)
6 Oyama, Y., Chikahisa, L., Hayashi, A., Ueha, T., Sato, M. and Matoba, H.: Triphenyltin-induced increase in the intracellular \(\text{Ca}^{2+}\) of dissociated mammalian CNS neuron: Its independent from voltage-dependent \(\text{Ca}^{2+}\) channels. Japan. J. Pharmacol. 58, 467 – 471 (1992)
7 Rink, T.J., Montecucco, C., Hesketh, T.R. and Tsien, R.Y.: Lymphocyte membrane potential assessed with fluorescent probes. Biochim. Biophys. Acta 595, 15 – 30 (1980)
8 Mintia, A., Kao, J.P.Y. and Tsien, R.Y.: Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. J. Biol. Chem. 264, 8171 – 8178 (1989)
9 Wilson, H.A. and Chused, T.M.: Lymphocytes membrane potential and \(\text{Ca}^{2+}\)-sensitive potassium channels described by oxonol dye fluorescence measurements. J. Cell. Physiol. 125, 72 – 81 (1985)
10 Kao, J.P.Y., Haroutunian, A.T. and Tsien, R.Y.: Photocchemically generated cytosolic calcium pulses and their detection by fluo-3. J. Biol. Chem. 264, 8179 – 8184 (1989)
11 Portzehl, H., Caldwell, P.C. and Ruegg, J.C.: The dependence of contraction of muscle fibres from the crab \(\text{Maia squinado}\) on the internal concentration of free calcium ions. Biochim. Biophys. Acta 79, 581 – 591 (1964)
12 Oyama, Y. and Akaike, N.: Triphenyltin: a potent excitatory neurotoxicant. Its reciprocal effects on the voltage-dependent Na and K currents of mammalian brain neuron. Neurosci. Lett. 10, 193 – 205 (1990)
13 Aldridge, W.N.: The influence of organotin compounds on mitochondrial functions. Adv. Chem. Ser. 157, 186 – 196 (1976)
14 Snoeij, N.J., Van Iersel, A.A.J., Pennink, A.H. and Seinen, W.: Toxicity of triorganotin compounds: comparative in vivo studies with a series of trialkyltin compounds and triphenyltin chloride in male rats. Toxicol. Appl. Pharmacol. 81, 274 – 286 (1985)
15 Snoeij, N.J., Punt, P.M., Pennink, A.H. and Seinen, W.: Effects of tri-n-butyltin chloride on energy metabolism, macromolecular synthesis, precursor uptake and cyclic AMP production in isolated rat thymocytes. Biochim. Biophys. Acta 852, 234 – 243 (1986)
16 Furukawa, K., Tawada-Iwata, Y. and Shigekawa, M.: Modulation of plasma membrane \(\text{Ca}^{2+}\) pump by membrane potential in cultured vascular muscle cells. J. Biochem. 106, 1068 – 1073 (1989)
17 Nicotera, P., Bellomo, G. and Orrenius, S.: The role of \(\text{Ca}^{2+}\) in cell killing. Chem. Res. Toxicol. 3, 484 – 494 (1990)