

Transference of Cytosolic Calcium to Nucleus in Neurons by Organotin Exposure

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Summary

In the previous study, we found that the exposure of tributyltin chloride (TBTC) made to disturb the balance of trace elements in brain tissues remarkably and to accumulate excessive calcium in olfactory bulb and olfactory epithelium¹⁻⁵⁾. Moreover, under the conditions, pathological cell death of neuron, especially granular neuron, was observed in olfactory bulb and olfactory epithelium¹⁻⁵⁾. Therefore, we have predicted that organotin-induced cell death of the olfactory neurons may be caused by the accumulation of excessive calcium. However, a mechanism on the cell death by the higher level concentration of calcium and the location of intracellular calcium have not been clear.

In this study, the olfactory neurons were isolated from olfactory bulb and the movement and location of intracellular calcium in the olfactory neurons after the exposure of TBTC were investigated by an *in vitro* experimental system. The movement and location of calcium in the olfactory neurons after the exposure of TBTC or the higher level concentration of calcium were observed by a confocal laser scanning microscopic system. As the results, transference of cytosolic calcium to nucleus in neurons after organotin-exposure was observed in the fluorescent images. This result suggests the transference of excessive calcium to the nucleus is strongly associated with the activation of endonuclease, DNase I in the nucleus, followed by the induction of apoptosis.

It is well-known that the exposure of tributyltin chloride (TBTC) induced olfactory lesions, which is also as an industrial injury¹⁻⁵⁾. However, the mechanism of the olfactory lesions remained to be unclear. In the previous study, we found that the exposure of TBTC made to disturb the balance of trace elements in brain tissues remarkably and to accumulate excessive calcium in olfactory bulb and olfactory epithelium¹⁻⁵⁾. Further, under the conditions, pathological cell death of neuron, especially granular neuron, was observed in olfactory bulb and olfactory epithelium¹⁻⁵⁾. Recently, we found DNase⁶⁾ activation by a single intraperitoneal injection of TBTC to rats¹⁾ and the DNase I activity change with the concentration of calcium in the olfactory bulb correlatively. Therefore, we have predicted that the movement of intracellular calcium might contribute to apoptosis in previous reports¹⁻⁵⁾.

In this study, to clarify the movement and location of intracellular calcium in the olfactory neurons after the exposure of TBTC (10^{-6} M) or the higher level concentration of calcium (10 mM), calcium imaging was carried out by a confocal laser scanning microscopic system, as compared with rat astrocyte-like cell line, RCR-1 cells which is high sensitive to calcium⁷⁾.

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Material and Method

1. Preparation of olfactory neurons for primary culture and TBTC treatment

The olfactory bulb was removed from the guillotined-brain from three-week-old Wistar-derived male rat by guillotine and carefully dissected in sucrose (0.25 M)⁸. The olfactory bulb was chemically digested with papain with 37°C for 40 min, followed by centrifugation (170 × g, 4 min, room temperature). The supernatant consisting of the merged solutions and debris was removed and cellular pellet was resuspended in Dulbecco's modified medium (DMEM) supplemented with 10 % heat-inactivated fetal calf serum (FCS). The cell suspension were seeded in 9 cm² culture dishes pre-coated overnight with polyethyleneimine in 5.0 % CO₂ at 37°C⁶. To inhibit the multiplication of glial cells, 1 μM cytosine β-D-arabinofuranoside was added to the culture dishes on the second day⁸⁻⁹ after the start. The olfactory neurons were cultured for 14 days. The olfactory neurons were exposed to TBTC (10⁻⁶ M) or the higher level concentration of calcium (10 mM) for calcium imaging.

2. RCR-1 cells culture and TBTC treatment

Rat astrocyte-like cell line RCR-1 cells were obtained from Health Science Research Resources Bank. RCR-1 cells were cultured in a culture medium; DMEM supplemented with 10 % FCS in 25 cm² culture flasks in a humidified atmosphere of 5.0 % CO₂ at 37°C. RCR-1 cells were detached by exposure to 0.05 % trypsin and washed with DMEM supplemented with 10 % FCS. The detached RCR-1 cells were seeded in 9 cm² culture dishes at 3.0 × 10⁵ cells per dish for calcium imaging. RCR-1 cells were exposed to TBTC (10⁻⁶ M) or the higher level concentration of calcium (10 mM) for calcium imaging.

3. Calcium imaging

For intracellular calcium imaging, olfactory neurons and RCR-1 cells were loaded with 5.1 μM fluo-3/AM¹⁰ in Hepes ringer buffer (118 mM NaCl, 5.5 mM D-glucose, 2 mM L-glutamic acid, 2 % MEM, 10 mM Hepes, 1 mM NaH₂PO₄, 4.7 mM KCl, 1.13 mM MgCl₂, 1.25 mM CaCl₂, 0.1 mM EGTA, pH7.4). Olfactory neurons and RCR-1 cells were scanned for 1 h after the treatment with TBTC (10⁻⁶ M) or the higher level concentration of calcium (10 mM) by a confocal laser scanning microscopic system LSM510 META (Carl Zeiss), equipped with the inverted microscope (Axiovert 200 M, Carl Zeiss). After the plateau was observed, 535 nm emission fluorescent images after excitation at 488 nm were obtained using the confocal laser scanning microscopic system. A 40 × oil-immersion objective was used.

Results and Discussion

To clarify the movement and location of intracellular calcium ([Ca²⁺]_i) in the olfactory neurons after the exposure of TBTC (10⁻⁶ M) or the higher level concentration of calcium (10 mM) ([Ca²⁺]_{ex}), calcium imaging was carried out by a confocal laser scanning microscopic system using Fluo3/AM. In normal olfactory neuron, [Ca²⁺]_i was distributed spatially in the cytosol as shown in Fig. 1(A). However, within 5 min after the exposure of TBTC (10⁻⁶ M), [Ca²⁺]_i of the cytosol was remarkably transferred to nucleus as shown in Fig. 1(B). Further, under the exposure of the higher level concentration of calcium (10 mM), [Ca²⁺]_{ex} was intensely distributed in the cytosol within five minutes as shown in Fig. 1(C)(D). Moreover, in RCR-1 cells, the movement and location of calcium were all similar to the results of the olfactory neurons as shown in Fig. 2(A)(B)(C)(D) and the transference of cytosolic calcium to the nucleus was observed by TBTC exposure. Further, in our another experiment on DNA fragmentation, typical DNA ladder patterns were observed within 12 h after the exposure of TBTC (10⁻⁶ M). From these results, an important action of TBTC in the olfactory bulb appeared to make to transfer [Ca²⁺]_i from the cytosol to the nucleus. That is to say, TBTC-induced apoptosis appeared to depend on the transference of cytosolic calcium to nucleus.

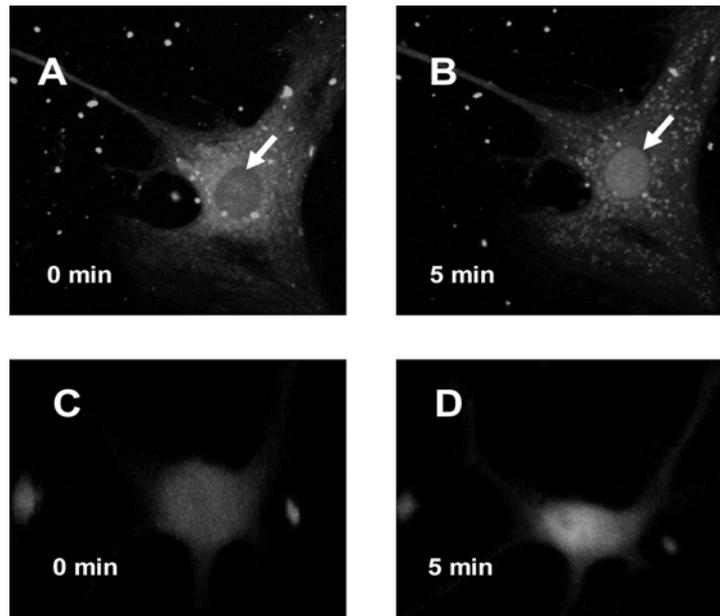


Fig. 1 Fluorescent images of $[Ca^{2+}]_i$ in olfactory neurons. Olfactory neurons were scanned for 1 hour after treatment with TBTC (10^{-6} M) or the higher level concentration of calcium (10 mM). (A) Normal primary culture of olfactory neuron, (B) Primary culture of olfactory neuron treated with TBTC (10^{-6} M) for 5 minutes, $[Ca^{2+}]_i$ was transferred from cytosol to nucleus (white arrow). (C) Normal primary culture of olfactory neuron, (D) Primary culture of olfactory neuron treated with the higher level concentration of calcium (10 mM) for 5 minutes, $[Ca^{2+}]_{ex}$ was promptly flowed into cytosol, but not sectionally transferred to nucleus.

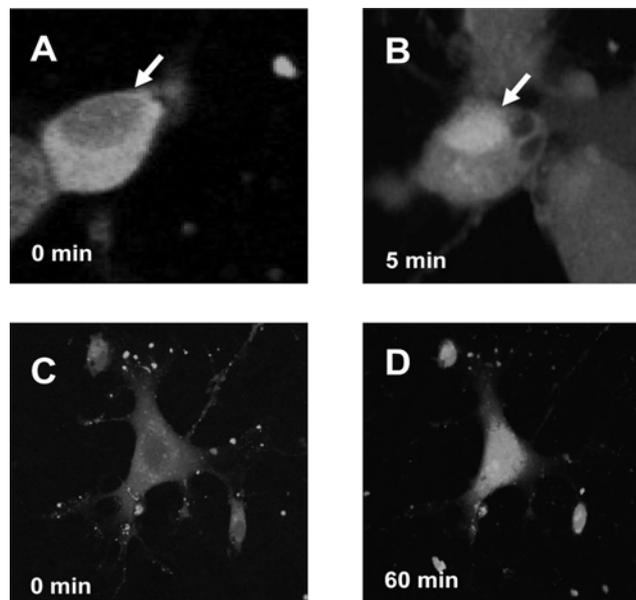


Fig. 2 Fluorescent images of $[Ca^{2+}]_i$ in RCR-1 cells. RCR-1 cells were scanned for 1 hour after treatment with TBTC (10^{-6} M) or the higher level concentration of calcium (10 mM). (A) Normal RCR-1 cells, (B) RCR-1 cells treated with TBTC (10^{-6} M) for 5 minutes, $[Ca^{2+}]_i$ was transferred from cytosol to nucleus (white arrow), (C) Normal RCR-1 cells, (D) RCR-1 cells treated with the higher level concentration of calcium (10 mM) for 60 minutes, $[Ca^{2+}]_{ex}$ was promptly flowed into cytosol, but not sectionally transferred to nucleus.

In our previous study, we revealed that TBTC was transported to and accumulated in a region of the Golgi apparatus and ER around nucleus, but not to or in the plasma membrane or the nucleus because of their hydrophobicity, and also destroyed the specific stratified structure of the Golgi apparatus and the characteristic reticular structure of the ER, in human skin fibroblasts (SF-TY), after the exposure of TBTC³⁾. Therefore, we predict that these phenomenon probably occur in the olfactory neurons, too.

In addition, endonuclease, DNase I is localized in the ER, and is released and transported to the nucleus by ER stress which is induced through an impairing of the structure and functions of the ER. Therefore, organotin-exposure appears to induce a release of DNase I from the ER and the transport to the nucleus by ER stress¹⁾.

From these results, the transference of excessive calcium to the nucleus appears to be strongly associated with the activation of endonuclease, DNase I at the nucleus, followed by the induction of apoptosis.

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