2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN-INDUCED ELEVATION OF INTRACELLULAR CALCIUM IONS IN CULTURED RAT HIPPOCAMPAL NEURONS AND ASTROGLIA

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ABSTRACT

The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 1,2,3,4-TCDD on the uptake of intracellular calcium in cultured rat hippocampal cells was investigated by interactive laser cytometry with microscopic image analysis. Incubation of neural cells with 10 to 100 nM concentrations of 2,3,7,8-TCDD showed a concentration-dependent increase in intracellular calcium levels as determined by laser cytometry in cells noninvasively labeled with Fluo-3. Maximal calcium uptake was observed within 3 min after addition of 2,3,7,8-TCDD whereas in cells treated with EDTA, no effects were observed. In contrast, 1,2,3,4-TCDD was inactive in this assay suggesting that the uptake of calcium into hippocampal neural cells was structure-dependent but may not be due to the classical aryl hydrocarbon (Ah) receptor-mediated signal transduction pathway.

INTRODUCTION

The Yu Cheng poisoning in Taiwan involved exposure of several thousand individuals to an industrial fluid which leaked into commercial rice oil [1]. The industrial fluid contained polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and quaterphenyls (PCQs) and there is evidence that the PCDFs may be the primary etiologic agents [2]. Infants exposed *in utero* to the Yu Cheng toxins exhibit many of the classical symptoms associated with exposure to 2,3,7,8-TCDD and related compounds. Moreover, the exposed children also display neurodevelopmental deficits such as poor cognitive development [3]. This study reports a tissue

culture model for studying the cellular and molecular neurotoxicity of Ah receptor agonists using rat hippocampal neural cells.

MATERIALS AND METHODS

Neural Cell Culture. Primary cell cultures of neurons and astroglia were prepared from hippocampal samples of 4-day old rat pups. Briefly, hippocampi were isolated and collected into 1 ml Ca^{2+} and Mg^{2+} free Hank's balanced salt solution buffered with 10 mM HEPES at 20°C. The hippocampal samples were transferred into a 10 ml culture tube containing 5 ml of trypsin (2 mg/ml) and incubated for 15 min. The samples were washed once with 5 ml of Hank's balanced salt solution plus 5 ml of fetal bovine serum (FBS) and incubated for 5 min to inhibit the trypsin. The Hank's/FBS was then replaced with Modified Eagle's Medium (MEM) containing the following: 25 mM HEPES, 1 mM sodium pyruvate, 20 mM KCl, 200 mM l-glutamine, 10 mM sodium bicarbonate, and 10% FBS. The cell suspension was then successively triturated through a 10 ml pipette, a pasture pipette, and then a fire polished pasture pipette. Five hundred μl of the resulting cell suspension were aliquotted into 8.5 ml of MEM. This cell suspension was then placed into a poly-L-lysine coated glass cover slip (Lab-Tek™) culture chamber. Cells were cultured in a humidified atmosphere of 5% CO_2 at 36.5 °C for 2 to 4 hr to allow for attachment. The culture medium was then replaced with 2.5 ml of fresh medium which was changed every other day thereafter.

Intracellular Ca^{2+} Fluorescence Analysis. Cells were noninvasively labeled with 3 μM Fluo-3/AM in phenol red-free medium for 1 hr. This membrane permeant, nonfluorescent acetoxymethylester (AM) is converted to fluorescent form by intracellular esterases and exhibits a 40-fold increase fluorescence intensity upon Ca^{2+} binding. Control experiments indicated that the probe concentration and dye loading were optimal and that no residual unconjugated dye was present when cells were analyzed.

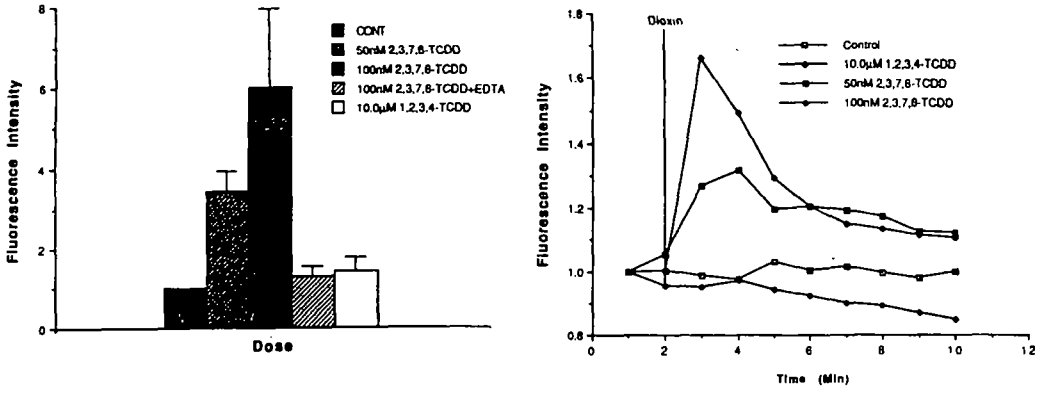
Selected cells in each chamber were scanned with the ACAS 570 at 488 nm and fluorescence emission was monitored without a barrier filter. Laser power was adjusted to optimize fluorescence detection without causing photobleaching of the dye or photodamage to cells. Excitation and detection parameters were kept constant in all experiments.

Following incubation, the cells were washed twice with medium. In experiments involving both neuronal and astroglial Ca^{2+} measurements, 2,3,7,8-

TCDD (with and without 2 mM EDTA) and 1,2,3,4-TCDD were micropipetted into the cell culture chamber. Treatment with the calcium ionophore ionomycin (final concentration of 1 μM in 0.5% DMSO) was performed over the last five scans of each experiment in order to assess endpoint fluxes of internal Ca^{2+} stores in all cells analyzed.

RESULTS AND DISCUSSION

Neurodevelopmental deficits have been observed in the offspring of mothers poisoned by PCB-contaminated rice in the Yu Cheng poisoning incident in Taiwan [1-3]. These results suggest that the highly toxic PCDF and/or PCB contaminants in the rice oil are responsible for the observed neurotoxicity. Previous *in vivo* studies have shown that Ah receptor agonists are neurotoxicants in rodents [4]; however, cellular and molecular models have not been developed for studying the neurotoxicity of these compounds. Recently it has been suggested that the encoding process for learning and memory is a result of stable changes within discrete populations of hippocampal neurons [5]. Therefore, this study investigates the effects of 2,3,7,8-TCDD and related compounds on cultured hippocampal cells. The results summarized in Figure 1 demonstrate that 2,3,7,8-TCDD caused a concentration-dependent elevation of intracellular calcium levels as determined by interactive laser cytometry. Moreover, EDTA blocks the 2,3,7,8-TCDD-induced response. The 2,3,7,8-TCDD-mediated uptake of cellular calcium has previously been reported in thymocytes [6] and is associated with various indices of cell injury. The results also showed that 1,2,3,4-TCDD, a weak Ah receptor agonist, does not affect intracellular calcium uptake. The structure-dependent response by 2,3,7,8- and 1,2,3,4-TCDD suggests a role for the Ah receptor in mediating this response and this is consistent with the identification of the 9S cytosolic Ah receptor in the hippocampal neural cells (data not shown). However, since the effects of 2,3,7,8-TCDD in these cells are observed within 3 min after exposure to the toxin, it is unlikely that the mechanism of toxicity involves direct modulation of gene transcription. Current studies are focused on characterizing the neurotoxic effects of 2,3,7,8-TCDD on various brain cell populations and delineating the mechanism associated with 2,3,7,8-TCDD-induced neurotoxicity.



Astroglia Neurons

Figures 1 and 2. Effects of 2,3,7,8-TCDD and 1,2,3,4-TCDD on calcium uptake in hippocampal astroglia and neurons.

REFERENCES

1. Kuratsune M, Shapiro RE. *PCB Poisoning in Japan and Taiwan*. New York: Alan R. Liss, 1984.
2. Kunita N, Kashimoto T, Miyata H, Fukushima S, Hori S, Obana H. Causal agents of Yusho. *Amer J Ind Med* 1984;5:45-58.
3. Rogan WJ, Gladen BC, Hung K, Koong S, Shih L, Taylor JS, Wu Y, Yang D, Ragan NB, Hsu C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science* 1988;241:334-336.
4. Tilson HA, Davis GJ, Mclachlan JA, Lucier GW. The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. *Environ Res* 1979;18:466-474.
5. Alkondon M, Costa ACS, Radhakrishnan V, Aronstam RS, Albuquerque EX. Selective blockade of NMDA-activated channel currents may be implicated in learning deficits caused by lead. *FEBS* 1990;261:124-130.
6. McConkey DJ, Hartzell P, Duddy SK, Hakansson H, Orrenius S. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin kills immature thymocytes by Ca^{2+}-mediated endonuclease activation. *Science* 1988;242:256-258.