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Effect of toluene diisocyanate on homeostasis of intracellular-free calcium in human neuroblastoma SH-SY5Y Cells

Pei-Shan Liu[a,*], Yin-Mei Chiung[b,c], Yi-Yun Kao[a]

[a]*Department of Microbiology, Soochow University, Shihlin, Taipei, Taiwan, R.O.C.*
[b]*Division of Occupational Medicine, Institute of Occupational Safety and Health, Taipei, Taiwan, R.O.C.*
[c]*Department of Microbiology and Immunology, National Defense Medical Center, Taipei, Taiwan, R.O.C.*

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Abstract

The mechanisms of TDI (2,4-toluene diisocyanate)-induced occupational asthma are not fully established. Previous studies have indicated that TDI induces non-specific bronchial hyperreactivity to methacholine and induces contraction of smooth muscle tissue by activating 'capsaicin-sensitive' nerves resulting asthma. Cytosolic-free calcium ion concentrations ($[Ca^{2+}]_c$) are elevated when either capsaicin acts at vanilloid receptors, or methacholine at muscarinic receptors. This study therefore investigated the effects of TDI on Ca^{2+} mobilization in human neuroblastoma SH-SY5Y cells. TDI was found to elevate $[Ca^{2+}]_c$ by releasing Ca^{2+} from the intracellular stores and extracellular Ca^{2+} influx. 500 μM TDI induced a net $[Ca^{2+}]_c$ increase of 112 ± 8 and 78 ± 6 nM in the presence and absence of extracellular Ca^{2+}, respectively. In Ca^{2+}-free buffer, TDI induced Ca^{2+} release from internal stores to reduce their Ca^{2+} content and this reduction was evidenced by a suppression occurring on the $[Ca^{2+}]_c$ rise induced by thapsigargin, ionomycin, and methacholine after TDI incubation. In the presence of extracellular Ca^{2+}, simultaneous exposure to TDI and methacholine led a higher level of $[Ca^{2+}]_c$ compared to single methacholine stimulation, that might explain that TDI induces bronchial hyperreactivity to methacholine. We conclude that TDI is capable of interfering the $[Ca^{2+}]_c$ homeostasis including releasing Ca^{2+} from internal stores and inducing extracellular Ca^{2+} influx. The interaction of this novel character and bronchial hyperreactivity need further investigation.

Keywords: Toluene diisocyanate; Calcium signaling; Muscarinic receptor; Human neuroblastoma SH-SY5Y cells

Introduction

2,4-Toluene diisocyanate (TDI) is widely used in the production of polyurethane foam and elastomers and in the manufacture of coating materials and adhesives. Acute and chronic exposure to TDI is toxic (Raulf-Heimsoth and Baur, 1998). In humans, acute exposure to high levels of TDI via inhalation results in severe irritation of the skin and eyes and affects the respiratory, gastrointestinal, and central nervous systems. Chronic inhalation exposure to TDI in humans results in significant decrease in lung function. At present, TDI is recognized as one of the main causes of occupational asthma induced by low molecular weight chemicals (Herd and Bernstein, 1994). Workers exposed to TDI may develop an asthma-like reaction characterized by wheezing, dyspnea, and bronchial constriction (Raulf-Heimsoth and Baur, 1998; Mapp et al., 1988; McKay and Brooks, 1984). The specific mechanisms of TDI-induced asthma are as yet unclear.

Asthma patients have an increased airway tone. Airway tone is mediated by the parasympathetic nerves, which release acetylcholine onto muscarinic receptors. M3 muscarinic receptors are present in human smooth muscle in both large and small airways (Whitsett and Hollinger, 1984; Mak and Barnes, 1990; Hislop et al., 1998; Chelala et al., 1998). Upon acetylcholine stimulation, calcium ion (Ca^{2+}) is released from internal stores via the IP3-sensitive Ca^{2+} release channels and is responsible for the rapid initiation of contraction (Whitsett and Hollinger, 1984; Baba et al., 1989;

* Corresponding author. Fax: +886 2 28831193.
E-mail address: psliu@mail.scu.edu.tw (P.-S. Liu).

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Berridge, 1993). In addition to internal Ca^{2+} release, extracellular Ca^{2+} entry into the cells of human smooth muscle contributes to the maintained contraction (Bourreau, 1993; Daniel et al., 1995). Methacholine is an agonist of muscarinic receptors and TDI has been shown to induce non-specific bronchial hyperreactivity to methacholine in humans (Gandevia, 1963; Butcher et al., 1977; O'Byrne et al., 1979; Burge, 1982; Cockroft, 1982) and in guinea pigs (Gandevia, 1963; Butcher et al., 1977; O'Byrne et al., 1979; Burge, 1982; Cockroft, 1982). TDI decreases the dosage of methacholine for bronchial contraction; however, to understand the mechanism of TDI-induced hyperreactivity, the role of TDI in M3-coupled cellular signaling needs to be investigated. The human neuroblastoma SH-SY5Y cells, possessing M3 muscarinic receptors (Lambert et al., 1989), are used in this study to investigate the effects of TDI on M3-coupled Ca^{2+} signaling.

TDI is thought to mimic the actions of capsaicin in isolated human airways (Chitano et al., 1994) and induces smooth muscle contraction by activating capsaicin-sensitive nerves to cause asthma (Mapp et al., 1988, 1990a, 1990b). Capsaicin acts on the vanilloid receptor 1 to activate a Ca^{2+}-permeable ion channel that triggers Ca^{2+} influx and causes Ca^{2+}-induced Ca^{2+} release (Gunthorpe et al., 2002). In the present study we set out to investigate the role of TDI on cellular Ca^{2+} mobilization because TDI is known to act on capsaicin-sensitive nerves by mimicking capsaicin.

TDI-induced asthma leads to a neutrophil influx and cell-mediated immune responses (Bentley et al., 1992; Finotto et al., 1991; Mapp et al., 1994). After exposure to TDI, inflammatory cells including eosinophils and mast cells are degranulated in the airway mucosa (Saetta et al., 1992). Degranulation is the phenomenon of secretion of the granules of eosinophils and mast cells which is triggered by an elevation of cytosolic Ca^{2+}. Although these reports suggest that TDI may act similarly to a Ca^{2+} stimulator, the specific role of TDI on Ca^{2+} mobilization is unknown. In this study we investigate the role of TDI in Ca^{2} mobilization and M3-coupled Ca^{2} signaling in human neuroblastoma SH-SY5Y cells.

Materials and methods

Chemicals. Methacholine, epibatidine, hexamethonium, atropine, verapamil, digitonin, thapsigargin, ionomycin, EGTA, and TDI (crystal form, purity 97%) were all obtained from Sigma. NaCl, KCl, and other salts were obtained from Merck Co. Fura-2 acetoxymethyl ester was obtained from Molecular Probes Co. TDI (solution, purity 97%) was purchased from the Tokyo Kasei Kogyo company.

Human neuroblastoma SH-SY5Y cell culture. The human neuroblastoma cell line, SH-SY5Y, was cultured in a minimally essential medium supplemented with 10% fetal bovine serum and 100 U penicillin/streptomycin and grown in a 5% CO_2 humidified incubator at 37 °C (Lambert et al., 1989; Lu et al., 2004). The medium was changed every 3–4 days and subcultured every 7 days. The confluent cells were collected to process the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) measurements.

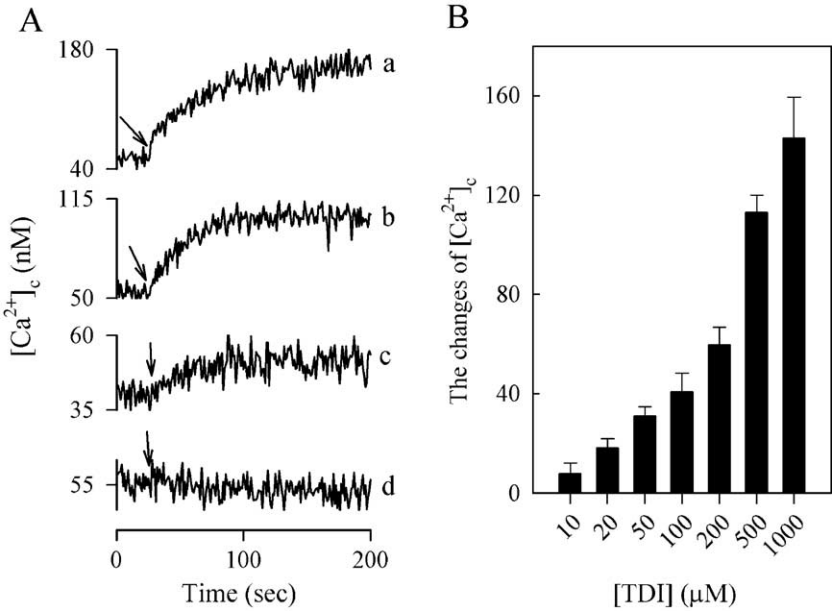

Fig. 1. TDI-induced increase in $[Ca^{2+}]_c$ in the presence of extracellular Ca^{2+}. (A) The $[Ca^{2+}]_c$ levels in fura-2-loaded cells, stimulated with TDI at concentrations of 500, 100, 20 and 0 μM represented in curves a, b, c, and d, respectively. (B) The concentration-dependent increase in $[Ca^{2+}]_c$ induced by TDI. The $[Ca^{2+}]_c$ were calculated as the difference between its maximal increase and the basal level. The data obtained from three batches cells to carry individual experiments, each experiment had triplicate observation in each dosage.