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Lambda-cyhalothrin disrupts the up-regulation effect of 17β-estradiol on post-synaptic density 95 protein expression *via* estrogen receptor α-dependent Akt pathway

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ABSTRACT

Lambda-cyhalothrin (LCT), one of the type II pyrethroids, has been widely used throughout the world. The estrogenic effect of LCT to increase cell proliferation has been well established. However, whether the estrogenic effect of LCT will influence neuro-development has not been investigated. In addition, 17β-Estradiol (E2) plays a crucial role in neurodevelopment and induces an increase in synaptic proteins. The post-synaptic density 95 (PSD95) protein, which is involved in the development of the structure and function of new spines and localized with estrogen receptor α (ERα) at the post-synaptic density (PSD), was detected in our study by using hippocampal neuron cell line HT22. We found that LCT up-regulated PSD95 and ERα expression, estrogen receptor (ER) antagonist ICI182,780 and phosphatidylinositol-4; 5-bisphosphate 3-kinase (PI3K) inhibitor LY294,002 blocked this effect. In addition, LCT disrupted the promotion effect of E2 on PSD95. To investigate whether the observed changes are caused by ERα-dependent signaling activation, we next detected the effects of LCT on the ERα-mediated PI3K-Protein kinase B (PKB/Akt)-eukaryotic initiation factor (eIF) 4E-binding protein 1 (4E-BP1) pathway. There existed an activation of Akt and the downstream factor 4E-BP1 after LCT treatment. In addition, LCT could disrupt the activation effect of E2 on the Akt pathway. However, no changes in cAMP response element-binding protein (CREB) activation and PSD95 messenger ribonucleic acid (mRNA) were observed. Our findings demonstrated that LCT could increase the PSD95 protein level *via* the ERα-dependent Akt pathway, and LCT might disrupt the up-regulation effect of E2 on PSD95 protein expression *via* this signaling pathway.

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Introduction

Pyrethroids have been widely used in the last five decades in agriculture, household insect control and public health all over the world, due to their broad spectrum and high-efficiency insecticidal effect (Butenhoff et al., 2006; Elliott et al., 1978; Hirano, 1989). This extensive usage has led to an increased exposure of the general population to pyrethroids and caused damage to human health (Alavanja et al., 2004). The main effect of pyrethroids is to disrupt the voltage-gated sodium channels and voltage-gated calcium channels, causing the depolarization of nerve membranes and the release of neurotoxin (Kadala et al., 2014; Shafer & Meyer, 2004; Soderlund, 2012; Wang & Wang, 2003). Other mechanisms of pyrethroids, including their actions on voltage-gate chloride channels and $GABA_A$ receptors, have also been proposed (Soderlund, 2012). Massive evidence has demonstrated that the nervous system of mammals could also be affected by pyrethroids (Casida & Durkin, 2013; Casida et al., 1983). Impairments in memory and cognition with a loss of hippocampal neurons were observed when treating prenatal and early postnatal rats with pyrethroids (Sinha et al., 2006). In an *in vivo* study, pyrethroids also induced spatial learning and memory deficits with a decrease in presynaptic proteins, including N-methyl-D-aspartate receptor 1 (NMDAR1), synaptophysin, and 6synapsin I (Chen et al., 2012). However, contemporaneous studies demonstrated an opposite effect of pyrethroids on neurodevelopment. Neurotrophic effects of pyrethroids on neurodevelopment, especially on the increase of spine density and length, have also been reported (Ihara et al., 2012; Ihara et al., 2009; Matsuya et al., 2012; Takasaki et al., 2013).

The formation and function of new spines require the synthesis of new proteins at the postsynaptic density (PSD) (Steward & Schuman, 2001). One fundamental structural protein is the postsynaptic density 95 (PSD95) protein, which is involved in the maturation of excitatory synapses and localized with the estrogen receptor α (ERα) at PSD of the hippocampal neurons (McEwen et al., 2001). After exposure to pyrethroids, the PSD95 expression increased unexpectedly (Chen, et al., 2012; El-Husseini et al., 2000). However, the promoting mechanisms of these pyrethroids on neurodevelopment have not been clearly explored.

It has been well documented that 17β-Estradiol (E2) promotes neurodevelopment and enhances synaptic protein expression (Inagaki et al., 2012; Tange et al., 2014). E2 binds to estrogen receptor (ER) to activate nuclear- or membrane-initiated signaling to regulate synaptogenesis (Akama & McEwen, 2003; Roepke et al., 2009; Yang et al., 2010). E2 binding to estrogen nuclear receptors and inducing messenger ribonucleic acid (mRNA) transcription is the classical estrogen signaling (Jin et al., 2010), which promotes neurodevelopment (Mamounis et al., 2014; Sawyer et al., 2006); and in ER-mediated rapid signaling, the phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K)-Protein kinase B (PKB/Akt) signaling pathway plays an important role in promoting PSD95 expression, which could be inhibited by ICI 182,780, an ER antagonist (Akama & McEwen, 2003; Vasudevan & Pfaff, 2007). Akt is one of the E2-responsive signaling intermediates which can regulate protein translation *via* mammalian targeting of the rapamycin (mTOR) signaling pathway (LoPiccolo et al., 2008), and phosphorylated Akt results in the activation of eukaryotic initiation factor (eIF) 4E-binding protein 1 (4E-BP1), which relieves the translational repression (She et al., 2010).

Lambda-cyhalothrin (LCT), a type II synthetic pyrethroid, has been extensively used in public health. Learning and memory impairments have been detected when rats are exposed to LCT (Ansari et al., 2012). In addition, LCT also displayed an estrogenic effect and promoted MCF-7 cell proliferation, but ICI182,780 could block this effect (H. Chen et al., 2002; Zhao et al., 2008). Furthermore, the reporter gene assays in CHO cells illustrated that LCT affected ERα-dependent signaling (Du et al., 2010). However, the effects of LCT on neurodevelopment have not been explored and the mechanisms have not been demonstrated. We hypothesized that LCT influences the cytoskeleton protein PSD95 expression *via* ERα dependent signaling in HT22 cells.

1. Materials and methods

1.1. Materials

17β-Estradiol (E2), ICI182,780 and LY294,002 were purchased from TOCRICS Bioscience (Ballwin, MO, USA). LCT was obtained from Sigma-Aldrich (St. Louis, MO, USA). ECL and loading buffer were purchased from Tanon Science & Technology (Shanghai, China). Anti-phosphorylated Akt antibody, anti-Akt antibody, anti-phosphorylated 4E-BP1 antibody and anti-4E-BP1 antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-phosphorylated CREB antibody and anti-CREB antibody were purchased from Millipore (Billerica, MA, USA). Anti-ERα antibody, anti-PSD95 antibody and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). The CCK-8 Cell Counting Kit was obtained from Beyotime Biotechnology (Jiangsu, China).

1.2. Cell culture and treatment

The HT22 cell line is a subclone of HT4 (Morimoto & Koshland, 1990), which is derived from the mouse hippocampus (Li et al., 1997). The HT22 cell line (Cat No. 10122101, Jennio Biotech, Guangzhou, China) was obtained as a gift from Prof. Zhai (Department of Occupational and Environmental Health, Anhui Medical University, Anhui, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA) with 10% fetal bovine serum (FBS, Hyclone, USA) and 1% penicillin/streptomycin (Beyotime, China) in the presence of 5% CO_2 at 37°C to allow cells to be 80% confluent. To assay E2 and LCT effects, cells were cultured in phenol red-free Neurobasal media (GIBCO, USA) containing 2% B-27 supplement (GIBCO, USA), 1% L-Glutamine (Sigma-Aldrich, USA) and 1% penicillin/streptomycin (Beyotime Biotechnology, China) for 24 hr before adding E2 or/and LCT. When detecting the effect of ER inhibiters, ICI 182,780 was added 30 min before E2 or/and LCT incubation. After being incubated for 24 hr, the cells were harvested for subsequent studies. LCT, E2, ICI182,780 and LY294,002 were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA).