Signaling pathways associated with PFC-induced apoptosis of neuronal cells–

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Introduction

A number of studies suggest that neuronal exposure to environmental toxicants in early life increases the risk of neurodegenerative diseases [1] Perfluoroalkyl compounds (PFCs) have been ubiquitous in environment. Due to their extreme stability, PFCs are known to accumulate in human via food web [2]. Exposure to PFCs has been associated with developmental neurotoxicity in laboratory animals. High dose exposure leads to neonatal mortality and neurologic delays. Low dose exposure induces behavioral defects in acetylcholine system, which persists into adulthood life [3]. Accordingly, potential of this compound to elicit developmental neurotoxicity draws public concern over the long-term health effects. Perfluorohexanesulfonate (PFHxS) is one of the most found PFCs in human blood. This compound is now detected in serums from general population [4]. It is recently reported that a single neonatal exposure to PFHxS caused behavioral and cognitive disturbance in adult mice [5]. We reported that PFHxS induced neuronal cell apoptosis, indicating the potential neurotoxic effect of PFHxS [6]. Apoptosis is a key element in determining neuronal toxicity. However, the mechanism of PFC-induced neurotoxicity is still in its infancy. The present study attempted to analyze the potential signaling pathways associated with PFHxS-induced apoptosis in a rat pheochromocytoma cell line PC12 cells.

Materials and Methods

PC12 cell culture and neuronal differentiation. Cells were grown in RPMI 1640 Medium supplemented with 10% horse serum (HS), 5% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin. For neuronal differentiation, cell culture medium was changed to RPMI 1640 containing 1 % HS, 5 % FBS and 1 % penicillin/streptomycin. Then, cells were treated with NGF (100 ng/ml) for 5~6 days, and then used for experiment.

Western blotting. Western blot analysis was performed as described previously [6].

Calcium detection. Cells were lysed by homogenization and intracellular calcium concentration was measured by using commercially available assay kits (Abcam, Cambridge, UK).

Caspase-3 activity assay. The caspase-3 activity was measured with colorimetrically labeled substrate, Ac-DEVD-pNA by using commercially available assay kits (Chemicon, Billerica, MA, USA) as described previously [6].

Results and Discussion

One of the most pivotal second messenger molecules involved in neuronal function and development is protein kinase C (PKC). PKC signaling pathways have been implicated as an important factor in learning and memory processes [7]. Alteration of PKC in neuronal cells is suggested to be associated with impaired motor dysfunction[8]. Immunoblot analysis revealed the translocations of PKC-α, βⅡ, and –δ from cytosol to memebrane fractions following PFHxS exposure (Fig. 1). It is reported that alterations of PKC expression and translocation following PFOA and PFOS exposure paralleled behavioral deficits in an avian model [9]. Our results suggest that alteration of these particular isoforms may perturb the normal signaling pathway and induce the dysregulation of neuronal cell proliferation, which may result in the neurological diseases. Blocking of PKC activation led to the inhibition of cell viability and casapase-3 activity (Fig.2), indicating that PKC plays a pivotal role in PFHxS-induced apoptosis of neuronal cells. Caspase-3 activity induced by PFHxS exposure was dampened by NMDA blocker or calcium channel blockers (Fig. 3A) and increases of intracellular calcium were completely blocked by MK801, nifedipine, or DTZ (Fig 3B). While phosphorylation of PKC- βII was selectively blocked by MK801 (Fig3C), all PKC isoforms were by DTZ (fig3D). It is suggested that PFHxS-induced apoptosis may be mediated by NMDA receptor and subsequent PKC activation.

AMPK is known as one of the downstream signal molecule of $[Ca^{2+}]_i$ in neuronal excitotoxic pathways. It is involved in both cell survival and death. We previously reported that PFHxS increased the activation of AMPK which plays a pro-apoptotic role and that the activation of AMPK is regulated by NMDA receptor activation and subsequent increase in the influx of $[Ca^{2+}]_i$. [10] It is also reported that PFHxS-induced neuronal apoptosis was mediated by NMDA receptor-regulated ERK pathway. To examine role of PKC on cross talk between AMPK and ERK pathways, the phosphorylation of ERK and AMPK was measured in the presence of compound C and PD98059 (PD), an ERK inhibitor and PKC inhibitors. The activation of ERK induced by PFHxS was inhibited by MK801, DTZ and NFD but not by compound C. Inhibitors of PKCs altered phosphorylation of ERK but not AMPK (Fig 4 A and B). These results suggest that ERK and AMPK are regulated by NMDA receptor in different signaling pathways and ERK is downstream molecule of NMDA receptor and PKC signaling pathways.

References

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Fig1. PKC activation by PFHxS

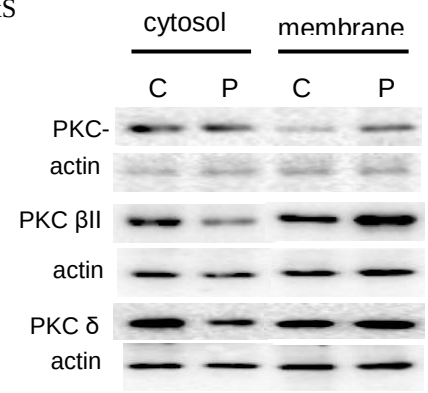

Fig2. Role of PKC in PFHxS induced neuronal cell death

(A) (B)

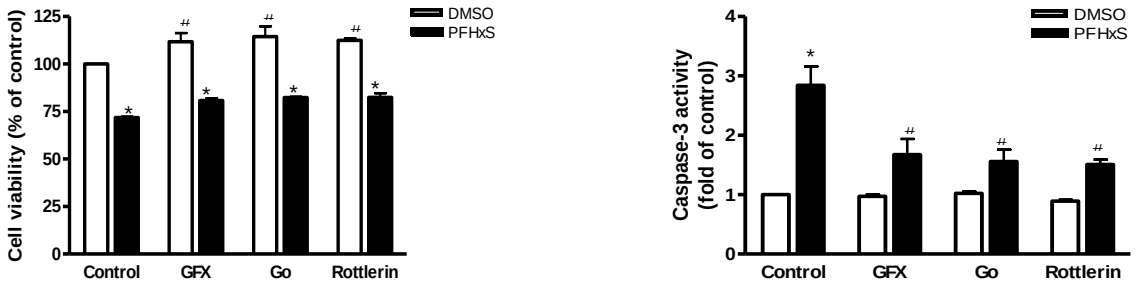

Fig3. The regulation of PKC activation by NMDA receptor and calcium channel

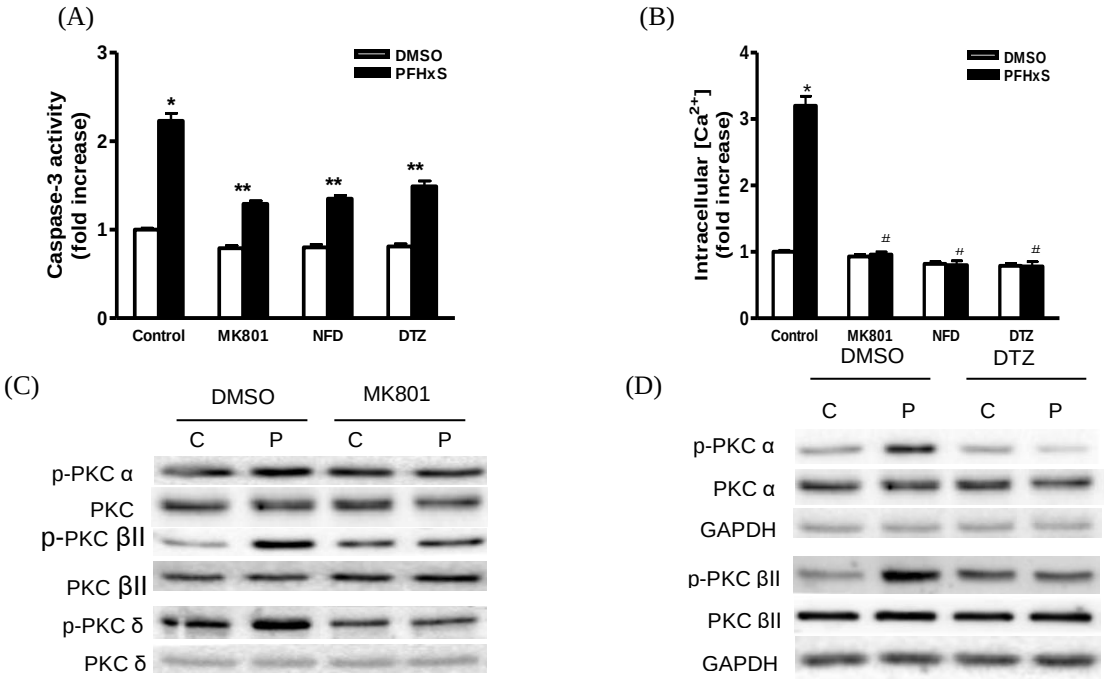

Fig4. The involvement of PKC in ERK and AMPK pathways