THE ROLES OF AMPK IN PERFLUOROHEXANESULFONATE (PFHxS) INDUCED NEUROTOXIC PATHWAY

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Introduction

Perfluoroalkyl compounds (PFCs) have been used in a variety of industrial and consumer applications and detected in relatively high levels in humans compared to other environmental contaminants[1]. Due to its extreme stability, PFCs are accumulated in the environment and biomagnified via food web[2]. Perfluorohexanesulfonate (PFHxS), one of the major PFCs, has been found in serum from general population as well as in umbilical cord and breast milk[3-5]. This raised a great concern on its possible detrimental health effects, especially neurotoxic effect. It has been shown that the serum levels of PFHxS are positively related with the occurrence of attention deficiency/hyperactivity disorder (ADHD) and increased impulsivity in children[6,7]. Recent animal study has shown that a single neonatal exposure to PFHxS caused behavioral and cognitive disturbance in adult mice[8]. However, studies on its neuronal effects are limited and not much is known about the underlying mechanisms.

The apoptosis of neuronal cells, a prominent feature of various neurodegenerative diseases is regulated by diverse signaling pathways. NMDA receptor has been extensively studied and is known to be involved in both cell protection and apoptosis of neuronal cells. The activation of NMDA receptor increases Ca^{2+} influx into cells, which is responsible for multiple cellular consequences including memory formation and learning[9]. However, overstimulation of NMDA receptor resulted in exctitotoxic cell death[10]. Among downstream signaling pathways of NMDA receptor, the roles of MAPKs are widely studied. Recently, AMP-activated protein kinase (AMPK) known as a sensor of cellular energy status has been emerged as an important signaling molecule involved in diverse neuronal cell function including cell death[11,12].

In previous studies, we have shown that PFHxS induced neuronal cell apoptosis which involved the activations of NMDA-receptor and ERK[13]. To date, the role of AMPK in the action of PFHxS in neuron has not been studied. Here, we examined the involvement of AMPK in PFHxS-induced apoptosis of neuronal cells. The upstream regulation of AMPK was also investigated to understand the underlying mechanism responsible for PFHxS induced neurotoxicity.

Materials and Methods

PC12 cell culture and exposure. Cells were grown in RPMI 1640 Medium supplemented with 10% horse serum (HS), 5% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin and differentiated by

treatment with NGF (100 ng/ml) for 4 days, and then used for experiment.

Western blotting. Western blot analysis was performed as described previously[13].

MTT assay. Cell viability was measured using tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT).

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) as described previously[13].

TUNEL assay. DNA fragmentation was detected with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) assay kit (Promega, Madison, WI).

Results and Discussion

AMPK has been shown to be involved in both cell survival and death in various cell types including neuron[14,15]. We first examined whether PFHxS has an effect on AMPK activity. AMPK activation was measured by western blotting. The phosphorylation of AMPK increased about 2 fold at 6 h, which sustained up to 24 h (Fig 1A). This shows that PFHxS induced gradual and sustained activation of AMPK. The phosphorylation of AMPK was inhibited by AMPK inhibitor, compound C (CC) (Fig 1B). To evaluate the role of AMPK in PFHxS-induced neuronal apoptosis, caspase-3 activity was measured. The increased casapse-3 activity by PFHxS was significantly inhibited by CC, suggesting the pro-apoptotic role of AMPK (Fig 1C). TUNEL assay shows similar results, where the increased number of TUNEL positive cells by PFHxS was reduced by CC (Fig 1D).

Previously, we have shown that PFHxS-induced neuronal apoptosis was significantly reduced by NMDA receptor antagonist, MK801 and ERK inhibitor, PD98059. Moreover, the ERK activation by PFHxS was efficiently reduced by MK801[13]. Since the increased intracellular calcium concentration is one of the mechanisms responsible for neuronal cell death induced by overstimulation of NMDA receptor, the effects of Ca^{2+} channel blockers, diltiazem (DTZ) and nifedipine (NFP) on PFHxS-induced ERK activation were examined. Similar to MK801, DTZ and NFP substantially reduced ERK activation (Fig 2A) and casapse-3 activity (Fig 2B), indicating that NMDA-receptor mediated increase in $[Ca^{2+}]_i$ plays an important role in PFHxS-induced neuronal cell apoptosis.

Recent studies have shown the regulation of AMPK activation by NMDA receptor-mediated pathway[16,17]. Therefore, the role of NMDA receptor/$[Ca^{2+}]_i$ in PFHxS-increased AMPK activation was examined. The pretreatments of MK801, NFP and DTZ blocked AMPK activation, indicating the role of NMDA receptor/$[Ca2+]_i$ as upstream regulators of AMPK activation (Fig 2C).These results suggest that NMDA receptor coupled Ca^{2+} channel stimulation play a key role in the process of apoptosis, where AMPK and ERK are downstream signal molecules. In future study, the identification of cross talk between AMPK and ERK pathways as well as downstream pathways of AMPK and ERK activation responsible for PFHxS-induced apoptosis of neuronal cells is warranted.

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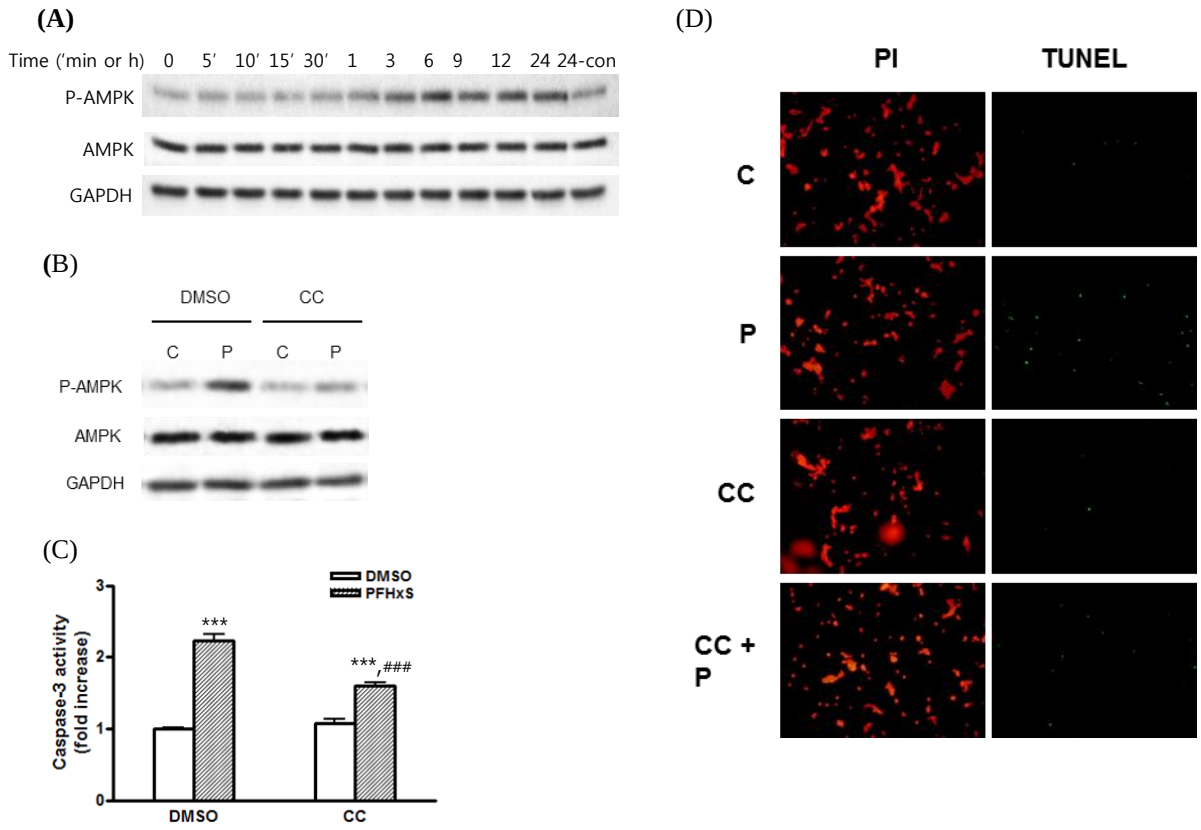

Figure 1. The effects of PFHxS on AMPK activation (A, B) and role of AMPK in PFHxS-induced apoptosis of neuron (C,D) (C, control; P, PFHxS; CC, compound C).

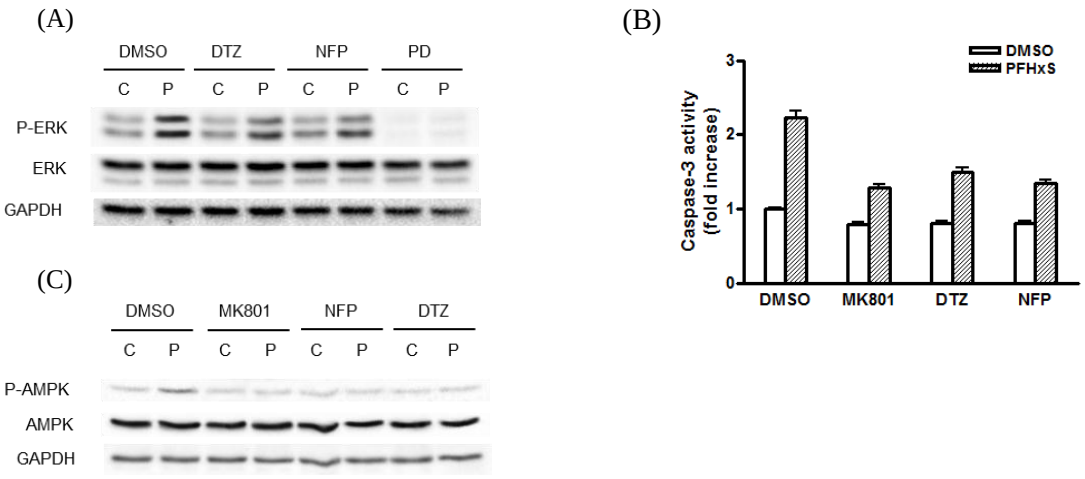

Figure 2. The effects of NMDA receptor and Ca2+ channel blockers on AMPK activation (A) and apoptosis (B) induced by PFHxS (C, control; P, PFHxS; NFP, nifedipine; DTZ, diltiazem; PD, PD98059).