Perfluorohexanesulfonate induces apoptosis of neural cell via NMDA receptor and subsequent PKC activation.

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Introduction

PFCs are ubiquitous environmental contaminants and accumulate in the human body via exposure from the food web. Perfluorohexanesulfonate (PFHxS) is one of the major PFCs detected in serum samples taken from the general population as well as in umbilical cord and breast milk. In particular, the serum level of PFHxS in children has been reported to be greater than adults [1]. This raised a concern over their health effects. It is reported that a single neonatal exposure to PFHxS caused behavioral and cognitive disturbance in adult mice [2] and that PFHxS induced neuronal cell apoptosis [3].

N-methyl-D-aspartic acid (NMDA) receptor, an ionotropic glutamate receptor (iGluR), mediates excitatory neural transmission. The activation of the NMDA receptor increases calcium influx into the postsynaptic cells, which plays a critical role in synaptic plasticity, memory formation and learning [4].

We have previously reported that PFHxS-induced neuronal apoptosis was inhibited by the NMDA receptor antagonist [5], suggesting that PFHxS induces excitotoxic neuronal cell death. The excess intracellular calcium loading disturbs ion homeostasis and leads to decreasing the level of intracellular ATP. AMP-activated protein kinase (AMPK) is activated in response to ATP depletion and is implicated in a series of catabolic pathways to restore cellular energy level, exerting its neuroprotective effect [6]. In addition to its involvement in metabolic processes, AMPK plays an important role in neuronal apoptosis [7]. In the present study, we examined the role of PKC and AMPK in PFHxS-induced neuronal apoptosis and the involvement of the NMDA receptor in AMPK activation, using the neuronal differentiated rat pheochromocytoma cell line, PC12 cells.

Materials and Methods

PC12 cell culture and neuronal differentiation. PC12 cells were purchased from the Korean cell line bank. 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 used for experiment.

Western blotting. Western blot analysis was performed as described previously [3]. The blots were incubated with anti-phospho-AMPK, anti-AMPK, anti-phospho-ACC, anti-ACC, anti-phospho-ERK, anti-ERK antibodies (Cell signaling, Beverly, MA), anti-GAP-43 antibody (Invitrogen, Carlsbad, CA) and anti-GAPDH antibody (Santa Cruz, Dallas,TX),

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 [3].

Results and Discussion

Protein kinase C (PKC) plays a pivotal role in neuronal function and development. PKC signaling pathway is known as an important factor in learning and memory processes [8]. Disruption of PKC signals in neuronal cells are associated with impaired motor dysfunction [9]. The present study shows that the selective translocations of PKC-α, βⅡ, and –δ from cytosol to membrane fractions was observed after PFHxS exposure as early as 15min and the translocation lasted up to 24hrs (Fig. 1). It is reported that alterations of PKC expression and translocation following PFOA and PFOS exposure paralleled behavioral deficits in an avian model [10]. This study suggests that activation of these particular PKC isoforms may perturb the normal signaling pathway and induce the dysregulation of neuronal cell proliferation, which is a hallmark of the neurological diseases. Blocking of PKC activation clearly inhibited casapase-3 activity (Fig.2B), indicating that PKC plays a significant role in PFHxS-induced apoptosis of neuronal cells. Caspase-3 activity induced by PFHxS exposure was weakened by NMDA blocker or calcium channel blockers (Fig. 3A) but increases of intracellular calcium were completely blocked by these blockers (Fig 3B). While all PKC isoforms were blocked by MK801, PKC-α was most affected by DTZ (Fig3C). It is suggested that PFHxS induces apoptosis of neuronal cells via NMDA receptor and subsequent PKC activation

AMPK in neuronal excitotoxic pathways is one of the downstream signal molecule of $[Ca^{2+}]_i$. and is involved in both cell survival and death. PFHxS is reported to increase the activation of AMPK which plays a pro-apoptotic role. The activation of AMPK by PFHxS is mediated by NMDA receptor activation and subsequent increase in the influx of $[Ca^{2+}]_i$ [5]. It is also reported that PFHxS-induced neuronal apoptosis was mediated by NMDA receptor-regulated ERK pathway. To test whether PKC plays a role on cross talk between AMPK and ERK pathways, we measured the phosphorylation of ERK and AMPK in the presence of an ERK inhibitor, PKC inhibitors, and PKC siRNA. While the activation of ERK induced by PFHxS was inhibited by PKC inhibitors, AMPK activation was only affected by PKC–δ inhibitor (Fig. 4A). siRNA analysis also showed that phosphorylations of ERK and AMPK were mediated by PKC-α and PKC–δ, respectively(Fig. 4B). The current study suggests that ERK and AMPK signaling pathways maybe activated via PKC-isoform selective mode of action.

References

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Fig1. Time-related PKC activation by PFHxS

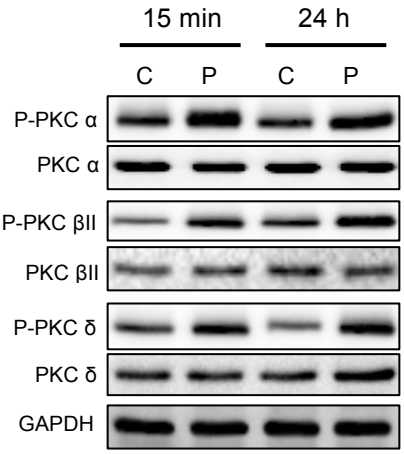

Fig2. Role of PKC in PFHxS induced neuronal cell death

(A) (B)

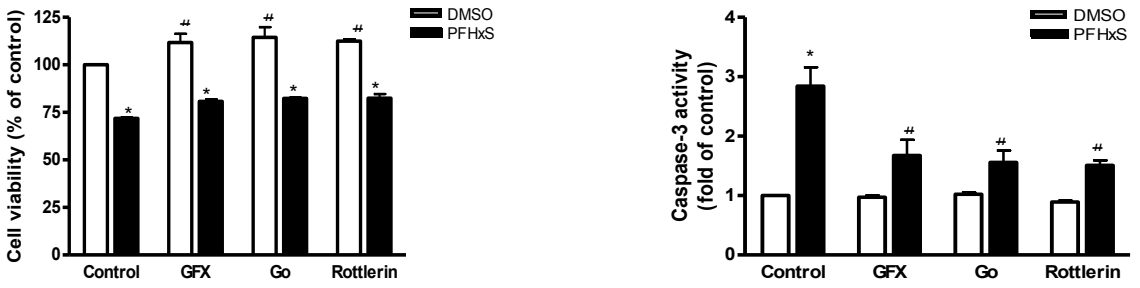

Fig3. The regulation of apoptosis and PKC activation by NMDA receptor and calcium channel

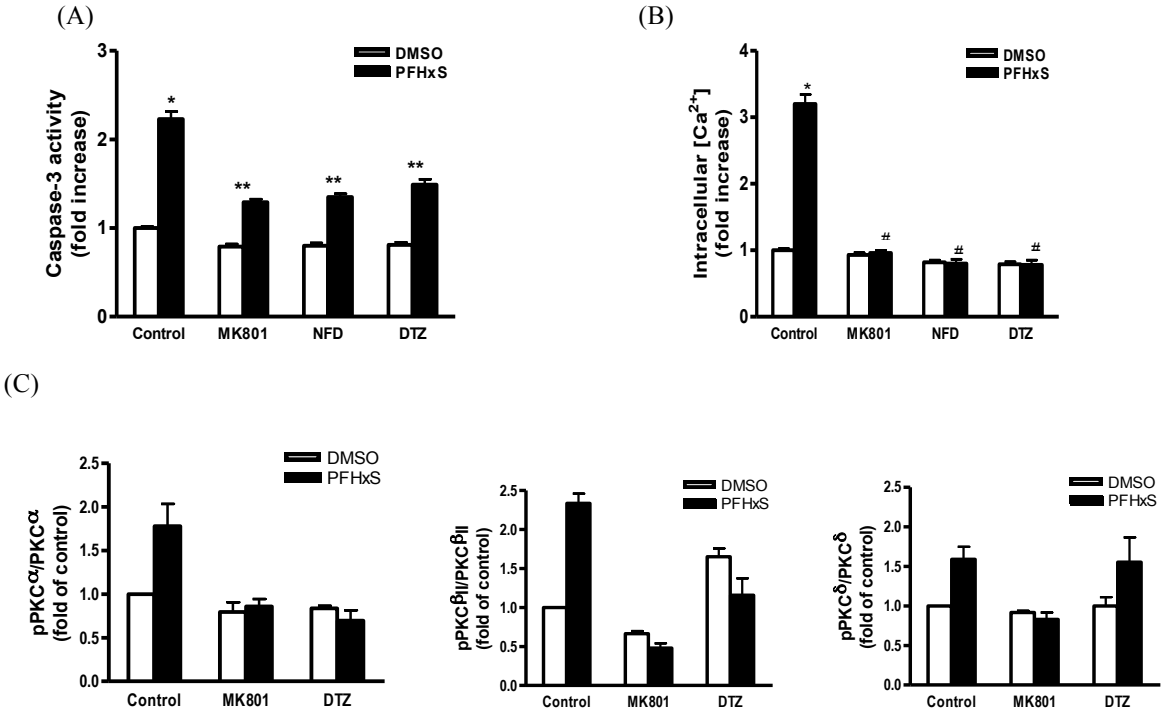

Fig4. The involvement of PKC in ERK and AMPK pathways