AMPK-mediated Neuroprotection on Cellular Models of Parkinson's Disease

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ABSTRACT

Objective: To test the neuroprotective effects of AMP-activated protein kinase (AMPK) in Parkinson's disease (PD) cellular models.

Background: Regulation of dysfunctional mitochondria, one of the pathogenic mechanisms proposed for PD, may be an effective therapeutic approach in this neurodegenerative disorder. AMPK is a critical regulator of mitochondria in response to energy deprivation.

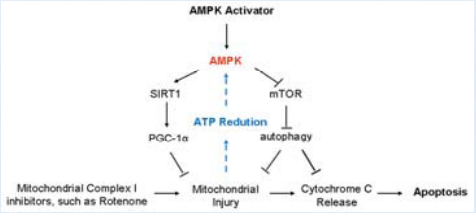
Design/Method: SH-SY5Y cells or SH-SY5Y cells transfected with wild-type AMPK, AMPK-T172D mutant (constitutively active AMPK termed as "CA-AMPK"), or AMPK-K157A mutant (domain negative AMPK termed as "DN-AMPK") constructs were treated with mitochondrial complex I inhibitors rotenone or MPP^+ followed by cell viability and apoptosis assay.

Results: Rotenone and MPP^+-induced apoptosis is accompanied by an increase of AMPK activity. The apoptosis is enhanced by AMPK inhibitor compound C, indicating a neuroprotective role of AMPK. We also showed that CA-AMPK attenuated rotenone or MPP^+-induced apoptosis as compared to control cells or cells transfected with wild-type AMPK or DN-AMPK, further supporting that AMPK activation may have neuroprotective effects on mitochondrial dysfunction-related cell injury.

Conclusion: We conclude that activation of AMPK exerts neuroprotective effects on PD cellular models.

BACKGROUND

Mitochondrial dysfunction has been implicated in Parkinson's disease (PD) (1). AMP-activated protein kinase (AMPK) is a major metabolic energy sensor and master regulator of metabolic homeostasis (2). Activation of AMPK maintains energy balance by switching on catabolic pathways and/or by switching off anabolic pathways that consume ATP (3). AMPK is expressed throughout the central nervous system (4,5). Possible roles of AMPK in the functional maintenance and even survival of neurons has only recently gained attention (6). The role of AMPK in PD has not been fully explored.



Flow chart: Proposed role of AMPK in PD cellular model. Mitochondrial complex I inhibitor causes mitochondrial injury, ATP reduction and cytochrome c release, leading to apoptosis. ATP deficiency activates AMPK as an auto-regulatory mechanism and facilitates mitochondrial energy metabolism via SIRT1/PGC-1α signaling pathway and autophagy induction.

METHODS

Wild-type AMPK, AMPK-T172D mutant (constitutively active AMPK termed as "CA-AMPK"), or AMPK-K157A mutant (domain negative AMPK termed as "DN-AMPK") constructs were kind gifts from Dr. Joohun Ha, Department of Biochemistry and Molecular Biology, School of Medicine Kyung Hee University. SH-SY5Y cells were transiently transfected with theses plasmids using lipofectamine™ 2000 followed by exposure to mitochondrial complex I inhibitors rotenone or MPP^+. Cellular ATP levels were measured with ATP Colorimetric Assay kit. Cell viability was determined by MTT assay and apoptosis was determined by Western blot assay.

RESULTS

Mitochondrial complex I inhibitors caused reduction of ATP and cell injury in SH-SY5Y cells

Mitochondrial inhibitor rotenone or MPP^+ significantly caused reduction of cellular ATP levels (Fig. 1A) and decrease of cell viability (Fig. 1B) in a time-dependent manner, which is similar to that in cells cultured under the condition of GD (Fig. 1A, 1B). Rotenone or MPP^+ also increased apoptosis of cells (Fig.2A, 2B) accompanied by the increased protein level of p-AMPK and p-ACC, a downstream protein of p-AMPK (Fig. 2B).

AMPK inhibition enhanced susceptibility of cells to mitochondrial inhibitors-induced injury of cells

Compound C inhibited activation of AMPK and enhanced rotenone/MPP^+-induced apoptosis (Fig. 3A, 3B) and loss of cell viability (Fig. 3C). indicating that compound C enhanced the susceptibility of cells to rotenone/MPP^+-induced injury via AMPK inhibition.

AMPK activation reduced rotenone/MPP^+-induced loss of cell viability and increase of apoptosis

Rotenone/MPP^+-induced loss of cell viability (Fig. 4A, 4B) and the increase of apoptosis (Fig. 4C, 4D) were attenuated in cells transfected with CA-AMPK plasmid, which were enhanced in cells transfected with DN-AMPK plasmid as compared to that in other groups.

FIGURES

Figure 1

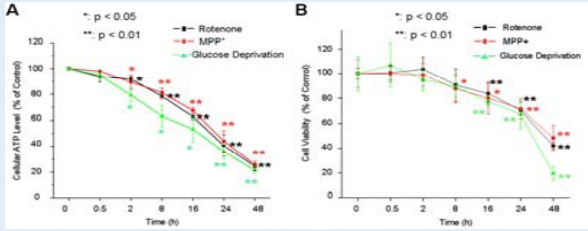


Figure 2

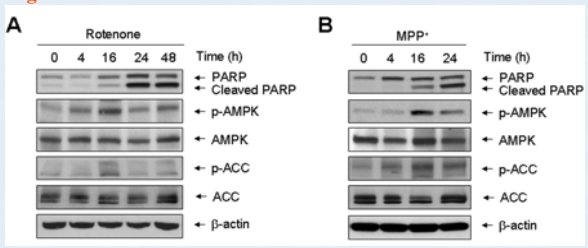


Figure 3

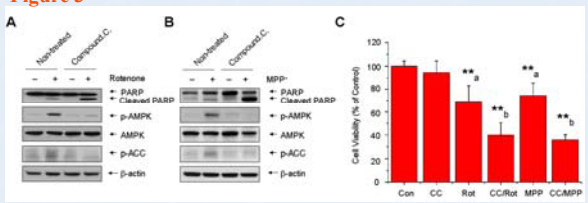
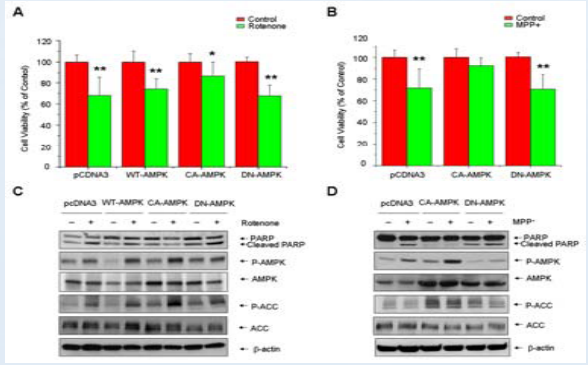


Figure 4



LEGENDS

Figure 1 Cellular ATP levels and cell viability

SH-SY5Y cells were treated with rotenone (5μM), MPP^+ (0.5mM) or cultured in the medium without glucose, a condition termed as "glucose deprivation (GD)", for different time durations. Cellular ATP levels (A) and cell viability (B) were determined and data were expressed as percentage of control and shown as means ± SD.

Figure 2 Apoptosis and AMPK activation

SH-SY5Y cells were treated with (A) rotenone (5 μM) or (B) MPP^+ (0.5mM) for different time durations. The apoptosis and the activation of AMPK were determined by Western blot assay.

Figure 3 AMPK inhibition and cell injury

SH-SY5Y cells were pretreated with compound C (5 μM) followed by addition of rotenone or MPP^+ for 24 h. The apoptosis (A, B) and cell viability (C) were determined by Westernblot and MTT assay (B).

Figure 4 AMPK activation and cell survival

SH-SY5Y cells were transfected with wt-AMPK, CA-AMPK, DN-AMPK plasmid or its vector control followed by exposure to rotenone (A, C) or MPP^+ (B, D) for 24 h. Cell viability (A, B), apoptosis and AMPK activation (C, D) were determined by MTT and Western blot assay.

CONCLUSIONS

Activation of AMPK exerts neuroprotective effects on PD cellular models. Further studies are required to demonstrate various roles of AMPK in animal models of PD.

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