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IGF-1 inhibits MPTP/MPP$^+$ -induced autophagy on dopaminergic neurons through the IGF-1R/PI3K-Akt-mTOR pathway and GPER

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INTRODUCTION

Autophagy dysfunctions are involved in the pathogenesis of Parkinson's disease (PD). In the present study, we aimed to evaluate the involvement of G protein-coupled estrogen receptor (GPER) in the inhibitory effect of insulin-like growth factor-1 (IGF-1) against excessive autophagy in PD animal and cellular models. 1-Methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment significantly induced mouse movement disorder and decreased the protein level of tyrosine hydroxylase (TH) in the substantia nigra (SN) and dopamine (DA) content in striatum. Along with the dopamine neuron injury, we observed significant upregulations of microtubule-associated light chain-3 II (LC3-II) and a-synuclein as well as a downregulation of P62 in MPTP-treated mice. These changes could be restored by IGF-1 pretreatment. Cotreatment with IGF-1R antagonist JB-1 or GPER antagonist G15 could block the neuroprotective effects of IGF-1. 1-Methy-4-phenylpyridinium (MPP$^+$) treatment could also excessively activate autophagy along with the reduction of cell viability in SH-SY5Y cells. IGF-1 could inhibit the neurotoxicity through promoting the phosphorylation of Akt and mammalian target of rapamycin (mTOR), which could also be antagonized by JB-1 or G15. These data suggest that IGF-1 inhibits MPTP/ MPP$^+$ -induced autophagy on dopaminergic neurons through the IGF-1R/PI3K-Akt-mTOR pathway and GPER.

RESULTS

1. Both IGF-1R and GPER are involved in the improvement of IGF-1 on the MPTP-induced movement disorder in mice

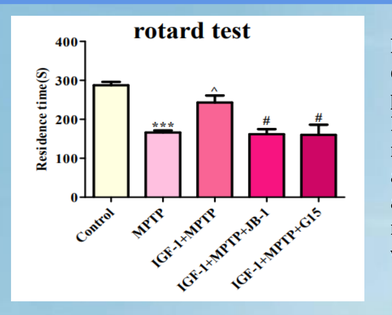

Fig. 1. Both insulin-like growth factor-1 receptor (IGF-1R) and G proteincoupled estrogen receptor (GPER) are involved in IGF-1 improvement on the 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced movement disorder in mice. IGF-1 was administrated in the presence or absence of JB-1 or G15 for 8 days in mice. After 3 days, MPTP injection (4 times with intervals of 2 h ip) was performed at 2 h for 1 day. On the 9th day, the motor skill was detected by rotarod equipment, and the residence time of mice on rotarod was recorded. Data are presented as means 6 SE (n = 6). ***P < 0.001 compared with control group; ^P < 0.05 compared with MPTP group; #P < 0.05 compared with IGF-1 + MPTP group.

2. IGF-1 rescues MPTP-induced decrease of the DA content in striatum and the blocking effect of JB-1 and G15.

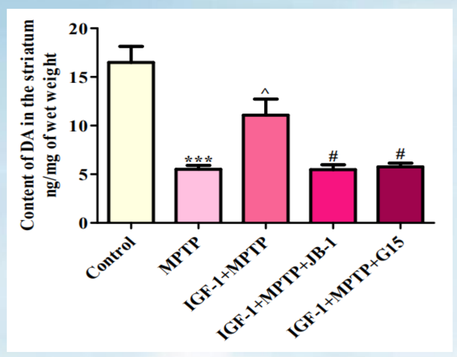

Fig. 2. Insulin-like growth factor-1 (IGF-1) rescues 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced decrease of the dopamine (DA) content in striatum. The blocking effect of JB-1 and G15 IGF-1 was administrated in the presence or absence of JB-1 or G15 for 8 days in mice. After 3 days, MPTP injection (4 times with intervals of 2 h ip) was performed at 2 h for 1 day. On the 9th day, after rotarod test, 2 sides of the striatum were collected, and the DA content was determined by for high-performance liquid chromatography (HPLC). Cotreatment with JB-1 or G15 could block the improvement of IGF-1 on the content of DA. Data are expressed as means 6 SE (n = 6). ***P < 0.001 compared with control group; ^P < 0.05 compared with MPTP group; #P < 0.05 compared with IGF-1 + MPTP group.

3. JB-1 and G15 antagonize the inhibitory effects of IGF-1 on MPTP/MPP$^+$-induced excessive autophagy both in vivo and in vitro.

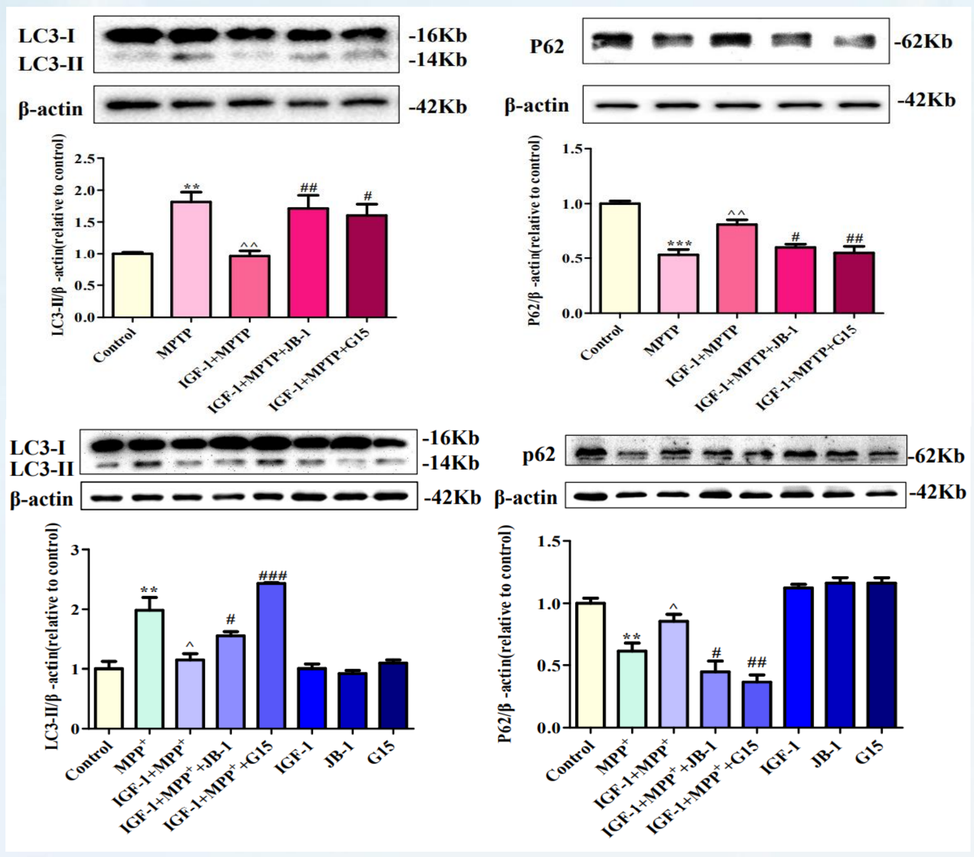

Fig. 3. JB-1 and G15 antagonize the inhibitory effects of insulin-like growth factor-1 (IGF-1) on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/1-methy-4- phenylpyridinium (MPP$^+$)-induced excessive autophagy both in vivo and in vitro. IGF-1 was administrated in the presence or absence of JB-1 or G15 for 8 days in mice. After 3 days, MPTP injection (4 times with intervals of 2 h ip) was performed at 2 h for 1 day. On the 9th day, after rotarod test, 2 sides of the substantia nigra (SN) were collected, and Western blot analysis was used to detect the protein expression of LC3-II and P62 (n = 6). Pretreatment with JB-1(1 μg/ mL) or G15 (1 μM) for 1 h and coincubation with IGF-1(50 ng/mL) for 24 h, followed by MPP$^+$ (1 mM) treatment for another 24 h in SH-SY5Y cells. Western blot was used to detect the protein expression of LC3-II and P62. Data were presented as means 6 SE (n = 3). **P < 0.01 and ***P < 0.001 compared with control group; ^P < 0.05 and ^^P < 0.01 compared with MPTP/MPP$^+$ -treated group; #P < 0.05, ##P< 0.01, and ###P< 0.001 compared with IGF-1 + MPTP/MPP$^+$ group.

4. IGF-1 restores autophagy in MPP$^+$-treated SH-SY5Y cells and the blocking effect of JB-1 and G15.

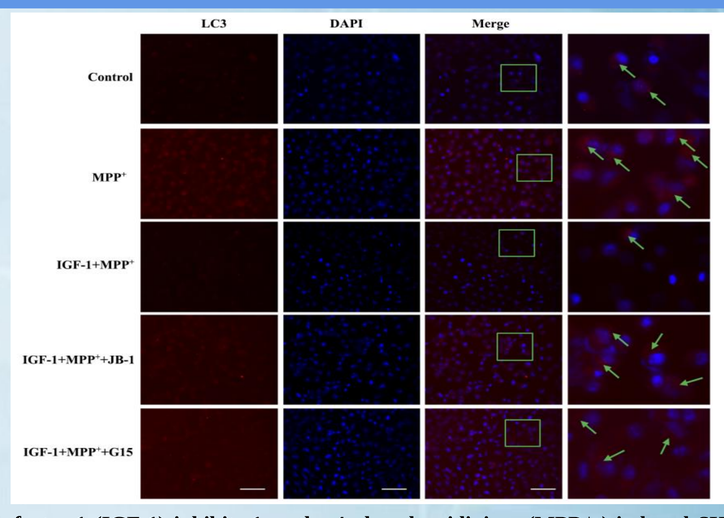

Fig. 4. Insulin-like growth factor-1 (IGF-1) inhibits 1-methy-4-phenylpyridinium (MPP$^+$)-induced SH-SY5Y cell autophagy, and the blocking effect of JB-1 or G15 SH-SY5Y cells was pretreated with IGF-1 in the presence or absence or JB-1 or G15 for 24 h, then cotreatment with MPP$^+$ for 24 h. A punctuated distribution of LC3 was detected by immunofluorescence staining (red fluorescence), and DAPI (blue fluorescence) was used to stain nuclei (scale bar, 20 mm).

5. IGF-1 ameliorates the protein expressions of TH and a-synuclein in PD model and the blocking effect of JB-1 and G15

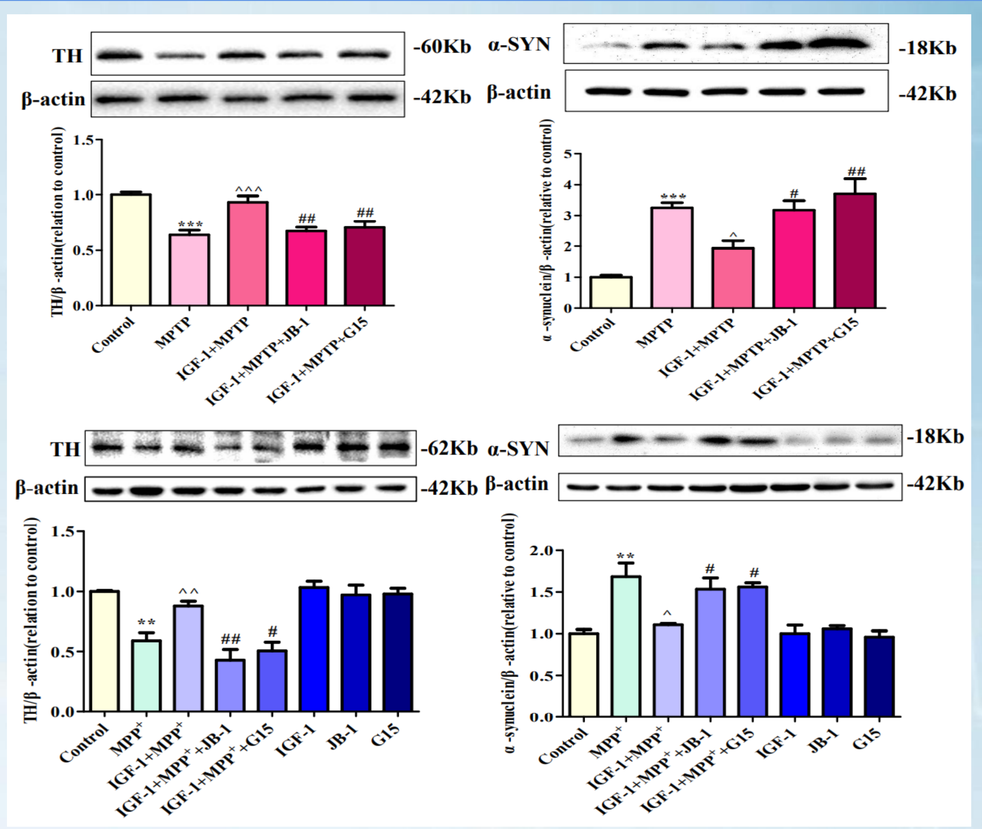

Fig. 5. Insulin-like growth factor-1 (IGF-1) ameliorates the protein expressions of tyrosine hydroxylase (TH) and a-synuclein (a-syn) in the Parkinson's disease (PD) model. The blocking effect of JB-1 and G15 IGF-1 was administrated in the presence or absence of JB-1 or G15 for 8 days in mice. After 3 days, 1-methyl-4-phenyl1,2,3,6-tetrahydropyridine (MPTP) injection (4 times with intervals of 2 h ip) was performed at 2 h for 1 day. On the 9th day, after rotarod test, 2 sides of the substantia nigra (SN) were collected, and Western blot analysis was used to detect the protein expression of TH and a-syn. SH-SY5Y cells were pretreated with IGF-1 in the presence or absence or JB-1 or G15 for 24 h, then cotreatment with 1-methy-4-phenylpyridinium (MPP$^+$) for 24 h. Protein expressions of TH and a-syn as well as b-actin were detected by Western blot. Data are expressed as means 6 SE (n = 3). **P < 0.01 and ***P < 0.001 vs. control group; ^P < 0.05, ^^P < 0.01, and ^^^P < 0.001 vs. MPTP/MPP$^+$ group; #P < 0.05 and ##P < 0.01 vs. IGF-1 + MPTP/MPP$^+$ group.

6. PI3k/Akt/mTOR pathway is involved in the neuroprotective effect of IGF-1 and the antagonizing effects of JB-1 and G15 in SH-SY5Y cells.

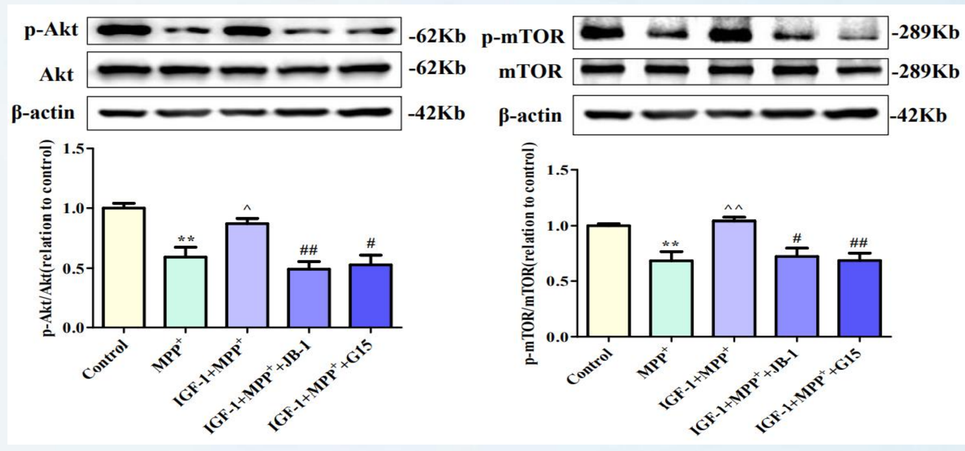

Fig. 6. Phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is involved in the neuroprotective effect of insulin-like growth factor-1 (IGF-1) and the antagonizing effects of JB-1 and G15 in SH-SY5Y cells. Cells were seeded in 6 wells that were pretreated with JB-1 or G15 for 1 h, followed by treatment with IGF-1 for 24 h and then cotreatment with 1-methy-4-phenylpyridinium (MPP$^+$) for another 24 h. the phosphorylation of Akt and mTOR was measured by Western blot. Data are presented as means 6 SE (n = 3). **P < 0.01 vs. control group; ^P < 0.05 and ^^P < 0.01 vs. MPP$^+$ group; #P < 0.05 and ##P < 0.01 vs. IGF-1 + MPP$^+$ group.

CONCLUSIONS

IGF-1 exerts a neuroprotective effect against MPTP/MPP$^+$ -induced neurotoxicity through inhibiting aberrant activation of autophagy. The potential mechanism might be related to the IGF-1R/PI3k/Akt-mTOR signaling pathway and GPER activation. Clarification of this new mechanism may further extend the signaling pathway of the neuroprotective effects of IGF-1 in the central nervous system.

ACKNOWLEDGEMENT

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