論 文

Oxytocin inhibits corticosterone-induced apoptosis in primary hippocampal neurons

（オキシトシンは初代海馬神経細胞におけるコルチコステロン誘導性アポトーシスを阻害する）

INTRODUCTION

Oxytocin (OT), a neuropeptide produced mainly in the paraventricular and the supraoptic nuclei of the hypothalamus, plays an important role in regulation of emotional, parental, and sexual behaviors. Previous studies showed that OT mediates antistress and antidepressant-like effects in mice and rats. Plasma OT levels increase during stress responses and decrease stress in humans. In addition, centrally administered OT inhibited stress-induced corticosterone (CORT) release in rats. OT receptors (OTRs) are strongly expressed in mouse hippocampus and amygdala. OT maintains hippocampal synaptic plasticity and memory during stress. OT also stimulates adult neurogenesis in rats subjected to glucocorticoid administration. OT may exert anti-stress effects by protecting hippocampal neurons from the damaging effects of glucocorticoids. In the present study, we demonstrated that OT inhibits CORT-induced apoptosis in primary hippocampal neurons.

MATERIALS AND METHODS

Primary hippocampal and glial cultures

Primary cultures of hippocampal neurons and glial cells were prepared from newborn wild type and OTR knockout (OTR KO) C57BL6 mouse pups (postnatal day 0-2). The cells were cultured for a total of 7 days before proceeding to further experiments; i.e. 7 DIV (days in vitro).

Drug Application

The OT stock 100 μM solution was prepared with deionized water and the CORT stock 100 mM solution was prepared with dimethyl sulfoxide (DMSO) before use. Cultures were exposed to CORT with or without OT for 24 h. Control cultures were treated with DMSO, at a final concentration of less than 0.5%.

Immunoblot analysis

Hippocampal neurons were lysed with N-PER neuronal protein extraction reagent containing protease inhibitor and phosphatase inhibitor to extract proteins. HeLa cells were prepared by sonication in boiled 1% sodium dodecyl sulfate buffer. Proteins were transferred onto polyvinylidene difluoride membranes, and were incubated overnight at 4°C with the appropriate antibodies: anti-OTR at 1:2000 and anti-β-actin at 1:4000. Western blot bands were detected by enhanced chemiluminescence technique using ECL prime detection kit.

Immunocytochemistry

Primary hippocampal neuronal cultures were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 10% goat serum. Rabbit monoclonal antibody against OTR (anti-OTR) and mouse polyclonal antibody against microtubule associated protein 2 (anti-MAP2) were used as primary antibodies.

In situ detection and measurement of apoptotic cells by TdT-mediated dUTP nick end labeling assay (TUNEL)

The assay makes use of the enzymatic action of TdT, which adds dUTP labeled with TMR red to the ends of DNA fragments. The cultured cells were fixed with 4% paraformaldehyde and permeabilized using freshly prepared 0.1% Triton X-100 in 0.1% sodium citrate. Then the fixed cells were incubated with TUNEL reaction mixture in a humidified atmosphere at 37°C for 1 h in the dark. Hoechst (1:1000) was added to the wells for nuclear staining. TUNEL positive cells were counted manually, and the percentage of positive cells was calculated for each sample.

Statistical analysis

To test the effects of different concentrations of CORT on primary hippocampal neurons and glial cells, a one-way ANOVA followed by Tukey–Kramer post hoc test or Bonferroni test was used to compare multiple conditions. To test the protective effects of OT against CORT on primary neurons, Welch's ANOVA followed by Games-Howell test was used.

RESULTS

Expression of OTR in primary mouse hippocampal neuronal cultures

In 7 DIV primary mouse hippocampal neurons, OTR was strongly expressed and localized in the soma of mature cultured hippocampal neurons. Both primary hippocampal neurons and HeLa cells expressed OTR at similar levels.

CORT induced apoptosis in primary hippocampal neurons but not in glial cells

Neurons and glial cells were treated with vehicle or 10, 50, 100, or 500 μM CORT for 24 h. CORT induced apoptosis in primary hippocampal neurons in a dose dependent manner; compared to the control group, a significant increase in TUNEL-positive cells started at a dose of 50 μM CORT, and the number became higher with 100 μM CORT and the highest with 500 μM CORT. In glial cultures, a significant number of apoptotic cells were observed only at a very high CORT concentration (500 μM).

OT attenuated CORT-induced apoptosis in primary hippocampal neurons

Primary hippocampal neurons were incubated for 24 h in 100 μM CORT with or without 1 μM OT. The number of TUNEL-positive cells was significantly higher in CORT-treated neurons than in those treated with vehicle, whereas co-treatment with OT caused a dramatic decrease in the number of apoptotic cells.

OT failed to rescue primary mouse hippocampal neurons prepared from OTR-KO mice from CORT-induced apoptosis

OTR-KO hippocampal neurons were treated with vehicle, 100 μM CORT (CORT) or 100 μM CORT + 1 μM OT (CORT+OT) for 24 h. 100 μM CORT induced significant apoptosis in the OTR-KO neurons while cotreatment with OT failed to protect primary neurons from CORT-induced apoptosis in the absence of OTR.

DISCUSSION

The present study attempted to explore the possible neuroprotective effect of OT against CORT-induced neuronal damage in hippocampal neurons. Regarding the cytotoxic effects of CORT, our findings highlighted two salient points. Firstly, high concentrations of CORT (50 μM) were required to induce significant neuronal death in mouse hippocampal neurons. High resistance of hippocampal neurons to CORT might be partly related to difference in expression of glucocorticoid receptors either in terms of the number or the isotype, as well as

in glucocorticoid metabolism. Secondly, glial cells in cultures were refractory to CORT-induced apoptosis. It might be related to lesser production of reactive oxygen species as well as a greater capacity to buffer their cytotoxic actions in glial cells. In our study, OT counteracted the action of CORT and protects the hippocampal neurons from apoptosis. As OT failed to rescue hippocampal neurons from CORT-induced apoptosis in the absence of OTR, it was concluded that OT acts via OTR to protect them. Our findings suggest that this might reduce the exposure of developing neurons from the toxic effects of glucocorticoids.

CONCLUSION

OT has inhibitory effects on CORT-induced neuronal death in primary cultured hippocampal neurons, and these effects are mediated via acting on OTR. The findings suggest that OT could have a physiological role in the development of brain as well as a pharmacological value in treating stress-related disorders.