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Mechanism of taurine protection against endoplasmic reticulum stress induced by glutamate in primary cortical neurons

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Our recent studies showed that taurine, a free amino acid present in high concentrations in a variety of organs of mammalians, can provide protection against oxidative stress-induced cell injury in PC-12 cells through preserving the integrity of ER[1] and taurine in neuronal systems can exert a protective function against toxicity induced by glutamate[2]. Here we present a study to investigate the potential protective benefits of taurine against endoplasmic reticulum stress induced by glutamate in primary cortical neuronal cultures. We found that taurine suppresses the up-regulation of GRP78, caspase12, GADD153/CHOP and Bim induced by glutamate, suggesting that taurine may exert a protective function against glutamate by reducing the ER stress and taurine can down-regulate the ratio of cleaved ATF6 and full long ATF6, and p-IRE1 expression, manifesting that taurine inhibits the ER stress induced by glutamate through suppressing ATF6 and IRE1 pathway.

[1]. Wu JY, Wu H, Jin Y, Wei J, Sha D, Prentice H, Lee HH, Lin CH, Lee YH, Yang LL: Mechanism of Neuroprotective Function of Taurine, *Adv. Exp. Med.*, 2009, 643:, 169; [2]. Pan C, Prentice H, Wu JY: Taurine Protection against Endoplasmic reticulum stress induced by oxidative stress, *J. Biomed. Sci.*, 2010, 17

Mechanism of taurine protection against endoplasmic reticulum stress induced by glutamate in primary cortical neurons

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Abstract

Our recent studies showed that taurine, a free amino acid present in high concentrations in a variety of organs of mammalians, can provide protection against oxidative stress-induced cell injury in PC-12 cells through preserving the integrity of ER[1] and taurine in neuronal systems can exert a protective function against toxicity induced by glutamate[2]. Here we present a study to investigate the potential protective benefits of taurine against endoplasmic reticulum stress induced by glutamate in primary cortical neuronal cultures. We found that taurine suppresses the up-regulation of GRP78, caspase12, GADD153/CHOP and Bim induced by glutamate, suggesting that taurine may exert a protective function against glutamate by reducing the ER stress and taurine can down-regulate the ratio of cleaved ATF6 and full long ATF6, and p-IRE1 expression, manifesting that taurine inhibits the ER stress induced by glutamate through suppressing ATF6 and IRE1 pathway. [1]. Wu JY, Wu H, Jin Y, Wei J, Sha D, Prentice H, Lee HH, Lin CH, Lee YH, Yang LL: Mechanism of Neuroprotective Function of Taurine, *Adv. Exp. Med.*, 2009, 643:, 169; [2]. Pan C, Prentice H, Wu JY: Taurine Protection against Endoplasmic reticulum stress induced by oxidative stress, *J. Biomed. Sci.*, 2010, 17

Materials and Methods

Cell culture

Primary cortical neuronal cell cultures were prepared using a similar protocol as described previously. Briefly, rat embryos at 17-18 days were removed and brains were isolated from the fetuses and kept in basal media Eagle supplemented with 2 mM glutamine, 26.8 mM glucose, and 20% heat-inactivated fetal bovine serum. This medium is referred to as growth media Eagle (GME). The cortices then were dissociated by passing the tissue through a 14-G cannula and the cells were centrifuged at 200g/min for 5 min at 25°C. The obtained pellet was re-suspended in GME and plated on appropriate tissue culture plates pre-coated with 5 ug/ml of poly-D-lysine. Cells were maintained for 1 hour in a humidified incubator (37°C, 99% humidity and 5% CO2) before the incubation medium was replaced with serum-free neural basal medium supplemented with B27 and 500 uM glutamine. The cultures were maintained in an incubator for 14 -18 days.

Measurement of Cell Viability

Cell viability was measured by ATP assay and cell death was measured by LDH assay. Cultured neurons at 14 days in vitro were pre-incubated with taurine, sulindac, DETC-MESO and G-CSF for 1 hour. Then the neurons were treated with 100 uM glutamate for 1 hours or hypoxia for 24 hours followed by reoxygenation for 24 hours. ATP solution was added to each well and cells were incubated for 10 minutes. The amount of ATP was quantified using a luciferase reaction assay. The luminescent intensity was detected by a luminometer (SpectraMax, Molecular Devices) after transferring the lysate to a standard opaque walled multi-well plate.

Statistical Analysis

All data showed in figures were expressed as the mean SEM. The Student's t-test or one-way ANOVA was used to compare means between groups. Differences of $P<0.05$ were considered statistically significant.

Key results

Effect of glutamate on ER stress in cultured neurons

It was found that the ER stress markers such as GRP78, CHOP and caspase -12 are all increased under glutamate stimulation.

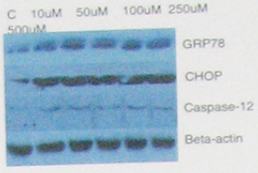

Fig. 1 Western blot analysis of expression of GRP78, CHOP and Caspase-12 in cultured neuronal preparations. Neurons were treated with various concentrations of glutamate ranging from 10-500uM as indicated for 1 hour. C is the control. Beta-actin is used as a control.

Effect of taurine on glutamate-induced neuronal injury in cultured neurons.

Taurine was found to protect against neuronal injury at 1 mM and resulted in greatest protection of 80.1 +/-9.8 % and 87.4+/-0.6% cell viablity at 5 mM and 10 mM respectively, compared to control levels of 48.6% cell viability with glutamate treatment alone, as shown in Fig.2.

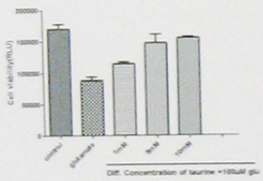

Fig. 2 Neuroprotective effect of taurine against glutamate-induced excitotoxicity measured by ATP assay. Primary cortical neurons were treated with 1, 5, 10mM taurine for 1 hour before exposure to 100uM glutamate for 1 hours.

Effect of taurine on hypoxia /reoxygenation- induced neuronal injury

Taurine was found to protect against hypoxia and re-oxygenation induced neuronal injury. In cells without taurine treatment hypoxia+reoxygenation resulted in cell viability of 59.2+/-1.1%. By contrast treatment with taurine was found to be protective with 74 +/-1.6% cell viabilty at 1 mM taurine and 80.7+/-4.6% viability at 5mM taurine, as shown in Fig.3.

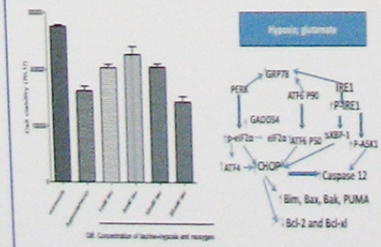

Fig. 3 Neuroprotective effect of taurine against hypoxia /reoxygenation measured by ATP assay. Primary cortical neurons were treated with 1, 5, 10 and 25mM taurine for 1 hour before hypoxia. Neurons were treated for 24 hours in hypoxia chamber following by reoxygenation for 24 hours; Fig. 4 Mechanism of glutamate or hypoxia induced ER stress pathways.

Effect of taurine on glutamate-induced up-regulation of ATF4 and cleaved ATF6 in cultured neurons.

Taurine was found to reduce glutamate-induced up-regulation of ATF4, a ER stress marker. In addition, glutamate-induced up-regulation of the ratio of cleaved ATF6 : ATF6 was also greatly reduced by taurine suggesting that taurine greatly reduces glutamate-induced ER stress as shown in Fig.5

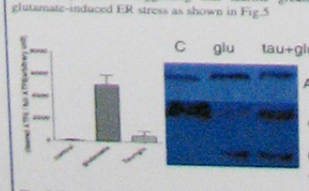

Fig. 5 Western blot analysis of expression of ATF4 and ATF6. Primary cortical neurons were incubated with taurine for 1 hour followed by 100uM glutmate treatment.

Effect of taurine on glutamate-induced up-regulation of caspase-12 and Grp78 in cultured neurons.

Taurine was found to reduce glutamate-induced up-regulation of Caspase-12 and Grp78, ER stress markers, suggesting that taurine reduces glutamate-induced ER stress as shown in Fig.6

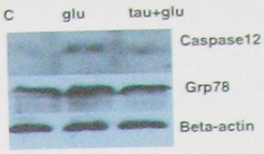

Fig. 6 Western blot analysis of expression of Caspase-12 and Grp78. Primary cortical neurons were incubated with taurine for 1 hour followed by exposure to 100uM glutmate for 1 hour.

Effect of sulindac on glutamate-induced neuronal injury in cultured neurons.

In the absence of sulindac glutamate treatment resulted in 57.2+/-6.3% cell viability. Treatment with 20 micromolar and 50 micromolar sulindac resulted in 70.5+/-6.7% and 79+/-4.2% cell viability respectively, as shown in Fig. 7.

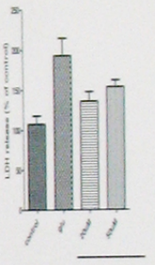

Fig. 7 Neuroprotective effect of sulindac against glutamate-induced excitotoxicity measured by LDH assay. Primary cortical neurons were treated with 20 and 50uM sulindac for 1 hour before exposure to 100uM glutamate for 1 hours.

Effect of sulindac on hypoxia /reoxygenation-induced neuronal injury

Sulindac was found to protect hypoxia /reoxygenation -induced neuronal injury at 25uM as shown in Fig.8. In the absence of sulindac exposure to hypoxia+reoxygenation resulted in a decrease in cell viability to 45.4+/-2.7%. Treatment with 25 uM and 50 uM sulindac substantially prevented cell death and resulted in 91.4+/-0.8% and 67.4+/-1.3% viability respectively.

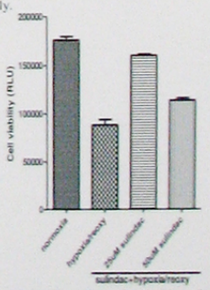

Fig. 8 Neuroprotective effect of sulindac under hypoxia condition measured by ATP assay. Primary cortical neurons were preincubated with 25 and 50uM sulindac for 1 hour before treatment with hypoxia for 24 hours following by reoxygentation 24 hours.

Effect of sulindac on glutamate-induced up-regulation of ATF4 and PUMA in cultured neurons.

Sulindac was found to reduce glutamate-induced up-regulation of ATF4 and PUMA, two ER stress markers suggesting that sulindac greatly reduces glutamate-induced ER stress as shown in Fig.9

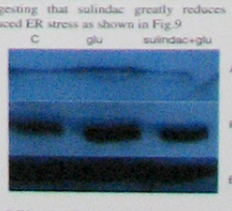

Fig. 9 Western blot analysis of expression of ATF4 and PUMA. Primary cortical neurons were incubated with sulindac for 1 hour followed by exposure to 100uM glutmate for 1 hour.

Effect of taurine on glutamate-induced PERK pathway in ER stress in cultured neurons.

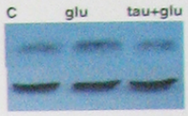

Fig. 10 Western blot analysis of expression of P-eIFalpha and eIF2alpha. Primary cortical neurons were incubated with taurine for 1 hour followed by glutamate for 1hour..

Effect of DETC-MeSO on glutamate-induced neuronal injury in cultured neurons.

DETC-MeSO was found to protect glutamate-induced neuronal injury at as low as 25uM as shown in Fig.11. Exposure to glutamate resulted in a decrease to 50.1+/-9.3% in cell viability. Treatment with DETC-MeSO was protective with 25 uM and 50 uM DETC-MeSO resulting in levels of cell viability of 87.0+/-0.4% and 85.2+/- 2.1% respectively.

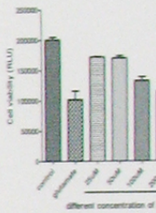

Fig. 11 Neuroprotective effect of DETC-MESO under hypoxia condition measured by ATP assay. Primary cortical neurons were preincubated with 25, 50, 100 and 200uM DETC-MeSO for 1 hour before exposure to 100uM glutamate for 1 hour.

Effect of taurine on glutamate-induced up-regulation of p-IRE1 in cultured neurons.

Taurine was found to reduce glutamate-induced up-regulation of p-IRE1, suggesting that taurine greatly reduces glutamate-induced IRE1 pathway in ER stress as shown in Fig.12

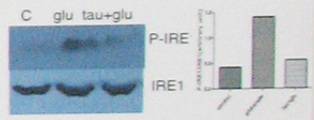

Fig. 12 Western blot analysis of expression of P-IRE1 and IRE1. Primary cortical neurons were incubated with taurine for 1 hour followed by exposure to 100uM glutmate for 1 hour.

Effect of taurine on glutamate-induced up-regulation of p-IRE1 in cultured neurons.

DETC-MeSO was found to reduce hypoxia /reoxygenation -induced up-regulation of GRP78, an ER stress marker suggesting that DETC-MeSO greatly reduces hypoxia /reoxygenation -induced ER stress as shown in Fig.13

Fig. 13 Western blot analysis of expression of GRP78. Primary cortical neurons were incubated with DETC-MESO for 1 hour followed by hypoxia for 24 hours and reoxygentation for 24 hours.

Effect of G-CSF on hypoxia /reoxygenation -induced neuronal injury in cultured neurons.

G-CSF was found to protect hypoxia /reoxygenation -induced neuronal injury at as low as 12.5ng/ml as shown in Fig.14. Exposure to hypoxia+reoxygenation resulted in a decrease in cell viability to 53.3+/-7.4% of control levels. Treatment with GCSF with 12.5 ng/ml or 25 ng/ml was protective against hypoxia+reoxygenation and resulted in levels of cell viability of 75+/-8.1% and 85.1+/-8.3% respectively.

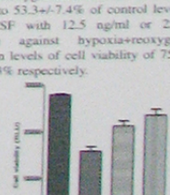

Fig. 14 Neuroprotective effect of G-CSF under hypoxia condition measured by ATP assay. Primary cortical neurons were preincubated with 12.5 and 25ng/ml G-CSF for 1 hour before treatment with hypoxia for 24 hours following by reoxygentation 24 hours.