EVIDENCE TO IMPLICATE PHOSPHO-AKT (P-AKT) IN THE MECHANISMS UNDERLYING NEUROPROTECTION AFFORDED BY THE ESSENTIAL OIL OF BERGAMOT AGAINST EXCITOTOXICITY IN VITRO

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The essential oil of bergamot (BEO) comprises a volatile fraction (93-96% of total) and a non volatile fraction (4-7% of total) containing waxes, polymethoxylated flavones, coumarins and psoralens i.e. bergapten and bergamottine (1). Here we now report the original observation that neuroprotection afforded by BEO against NMDA-triggered SH-SY5Y cell death is associated with prevention of injury-induced decrease of p-Akt and p-GSK-3β levels. Fluorimetric assay revealed no activation of caspase-3 following exposure (2 min-20 h) of SH-SY5Y cells to NMDA (1 mM; n= 3 experiments per exposure time) whereas the Ca^{2+}-dependent neutral protease calpain I activity was detectable early after exposure to the excitotoxin. Accumulation of calpain-cleaved 145-150 kDa α-spectrin breakdown product reached statistical significance 5 min after NMDA addition (n= 3) and this was abrogated by a pretreatment with a neuroprotective concentration of BEO (0.01%; n= 3). Interestingly, exposure of SH-SY5Y cells to NMDA induces a fast and transient deactivation of Akt kinase. In fact, reduced phosphorylation of Akt is evident ($P<0.01$ versus control) at 2 and 5 min after NMDA exposure (n= 4), then it is followed by a trend towards an increase of phosphorylation peaking at 15-30 min and returning to control levels at 1-3 h after treatment (n= 4 experiment per exposure time). Preincubation with BEO (0.01%; n= 3) prevents deactivation of Akt and consequent reduction of Ser9 phosphorylation of downstream GSK-3β at 5 but not 2 min after NMDA exposure; the latter is accompanied by elevated GSK-3β activity that underlies cell death because incubation of SH-SY5Y cells with GSK-3 inhibitor IX (0.001-1 μM; n= 3 experiments per group) concentration-dependently reduced ($P<0.01$) cell death triggered by 1 mM NMDA. Interestingly, a pretreatment with LY294002 (0.002-20 μM; n= 4 experiments per concentration), a PI3K inhibitor, concentration-dependently reverted neuroprotection by BEO, whereas NMDA-induced cell death was not affected by this inhibitor supporting the conclusion that PI3K is involved in neuroprotection afforded by the phytocomplex.

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