

## **SENSITIZATION TO NEURONAL EXCITOTOXICITY BY FREE 3-NITROTYROSINE.**

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Oxidative nitration of free- and protein-bound tyrosine residues is increased in many neurodegenerative diseases. While nitrotyrosine is a useful marker of oxidative injury, we are interested in the potential biological and pathologic effects of free-nitrotyrosine (3NT) accumulation in neurons. An important mechanism of neuronal damage and death that is common to many neurodegenerative disorders is excitotoxicity due to over-excitation of glutamate receptors. Here, we examine whether 3NT exposure can influence glutamate-receptor mediated neuronal injury *in vitro*. Mouse forebrain neuronal cultures were treated with 3NT for 3 days, exposed to glutamate for 5 minutes, returned to media containing 3NT for 24 hours and cell death was measured by MAP2 immunoreactivity. 3NT (1 mM) treatment significantly increased glutamate-induced neurotoxicity (percent cell death with 30  $\mu$ M glutamate alone:  $20 \pm 9$  %; glutamate + 3NT:  $44 \pm 11$  %; mean  $\pm$  S.E.M,  $p < 0.05$ ). Because intraneuronal calcium ( $\text{Ca}^{2+}$ ) increases during glutamate exposure and increased  $[\text{Ca}^{2+}]$  contributes to neuron death during excitotoxicity, we measured intraneuronal  $[\text{Ca}^{2+}]$  with a Fluo-5F, a  $\text{Ca}^{2+}$ -sensitive fluorescent dye. Cultured neurons pre-treated with 3NT (1 mM) exhibited greater increases in  $\text{Ca}^{2+}$  after exposure to 10  $\mu$ M glutamate and impaired recovery to baseline  $[\text{Ca}^{2+}]$  following washout.

We have previously demonstrated that 3NT inhibits mitochondrial complex I and complex II of the electron transport chain. To determine whether 3NT affects mitochondrial function following glutamate exposure, we monitored mitochondrial membrane potential using TMRM, a mitochondrial membrane potential-sensitive fluorescent dye. Glutamate exposure causes mitochondrial depolarization and pre-treatment with 3NT (1 mM) alters the kinetics of this depolarization in a manner suggesting decreased latency to mitochondrial membrane potential collapse.

We also measured the effect of 3NT and NMDA exposure on dendritic morphology. Neurons were treated with 3NT (1 mM) for 4 days, exposed to NMDA (30  $\mu$ M) for 10 minutes, allowed to recover in buffer containing 10  $\mu$ M MK801 for 30 minutes, and then fixed and immunolabeled for MAP2 to examine dendritic morphology. NMDA treated neurons exhibited focal swellings along the dendrites which were reversible over the recovery period. Pretreatment with 3NT significantly impaired dendritic recovery (percent of neurons exhibiting damaged dendrites:  $24.7 \pm 3.5$ % control versus  $90.3 \pm 7.4$ % 3NT pre-treated; mean  $\pm$  SEM,  $p < 0.01$ ). 3NT treatment alone did not increase the incidence of focal swellings compared to untreated neurons.

Together, these results suggest that exposure to 3NT potentiates excitotoxicity by altering  $\text{Ca}^{2+}$  homeostasis, impairing mitochondrial function, and increasing acute damage to dendrites.

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