Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue3abst113P.pdf

Effects Of Nitric Oxide Signalling On Growth And Health Of Cortical Cultures

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The free radical Nitric Oxide (NO) has been found to play significant roles in neurotransmission as well as neurodevelopment in the Central Nervous System (CNS) (1).The neuronal isoform of the enzyme Nitric Oxide Synthase (NOS) responsible for the production of the majority of the NO in the brain is expressed during embryonic and post embryonic development of the cerebral cortex (2, 3). This study hypothesises that neuritic growth and synapse formation can be influenced by NO signalling.

Cortical neurons were cultured from either neonate Wistar or Sprague Dawley rat pups on postnatal day 0-1. The NO donor DETA/NONOate (100µM) or nNOS inhibitor 7NI (1µM) were added every 48hours after day three of cell culture to observe the effects of exogenous NO application and endogenous NO production on the health of cells, number of neurons and neurite lengths. Stock solutions of DETA/NONOate and 7NI were prepared in NaOH and DMSO respectively and treatments with NaOH and DMSO at equivalent concentrations served as controls. After 14 days of cell culture, Immunocytochemistry methods were used to stain the neurons for the dendritic marker microtubule-associated-protein-2 (MAP-2) and the neurite lengths measured from images obtained by fluorescence microscopy. Cell toxicity assay using Hoechst and Sytox Red dyes for live and dead cells were used to determine the health of cells after 14 days in culture. Data was analysed using One-way ANOVA followed by Tukey post-hoc tests for pairwise multiple comparison.

The average results of 20 samples each obtained from three independent cell cultures showed that the health of cells (60-70% live cells at time of assay) and average neurite length per neuron (700-900µm) were not significantly different in all groups studied. However, the chronic application of exogenous NO led to a decrease in overall neuron number to 11.0 ± 8.8 neurons/mm^2 and total cell count to 79.7± 39.1/ mm^2, compared to 60-95 neurons/mm^2 and 570-700 cells/mm^2 under control conditions (One-way ANOVA: p=0.008 for total cell count). In contrast, chronic nNOS inhibition did not have effects on the parameters studied giving corresponding values of 72.1 ± 53.7 neurons/mm^2 and 678.2 ± 179.9 cells/ mm^2. Despite the changes in total cell numbers caused, the ratio of neuronal to non – neuronal cells remained unchanged in all groups. This study has shown that there is a decrease in overall cell and neuron number upon the addition of exogenous NO to cortical cultures without apparent effects on neuritic growth in surviving neurons. This is markedly different to previous work performed in this laboratory using the nervous system of the pond snail *Lymnaea stagnalis* (4) which showed that neurite growth was increased by endogenous NO production suggesting significant species specific differences in the effects of NO on neuronal growth.

(1) Steinert JR *et al*. (2010) *The Neuroscientist*, **16**: 435

(2) Knowles RG and Moncada S (1994). *Biochem J*. **298** (2): 249-258

(3) Stern M *et al*. (2010) *J Comp Neurol* , **518**: 1157-1175

(4) Straub VA *et al*. (2013) *The Journal of Neuroscience*, **33** (13): 5626-5637