

# Nicotinamide and 1-methylnicotinamide reduce homocysteine neurotoxicity in primary cultures of rat cerebellar granule cells

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Nicotinamide is an important cofactor in many metabolic pathways and a known neuroprotective substance, while its methylated product, 1-methylnicotinamide, is a suspected neurotoxin. Homocysteine is a risk factor in Alzheimer's disease and neurodegeneration, causing inhibition of methylation processes and inducing excitotoxicity. In this study, using primary cultures of rat cerebellar granule cells and propidium iodide staining, we investigated the neurotoxicity of nicotinamide and 1-methylnicotinamide, and their neuroprotective potential in acute and sub-acute homocysteine neurotoxicity. Our results demonstrated that nicotinamide and 1-methylnicotinamide applied for 24 h to cultures at concentrations of up to 25 mM had no effect on neuronal viability. Moreover, nicotinamide at concentrations of 5–20 mM and 1-methylnicotinamide at 1–10 mM applied to cells 24 h before, and for 24 h after an acute 30 min application of 25 mM D,L homocysteine, reduced neuronal damage. 1-Methylnicotinamide at concentrations of 250 and 500  $\mu$ M showed neuroprotective activity during a sub-acute 24-h exposure to 2.5 mM D,L-homocysteine, while 5 and 25 mM nicotinamide also evoked neuroprotection. These findings do not support suggestions that 1-methylnicotinamide may act as an endogenous neurotoxic agent; rather, they indicate the neuroprotective ability of nicotinamide and 1-methylnicotinamide in homocysteine neurotoxicity. The exact mechanisms of this neuroprotection are unclear and require further investigation.

Key words: cerebellar granule cells, excitotoxicity, homocysteine, 1-methylnicotinamide, nicotinamide, neuroprotection

## INTRODUCTION

Nicotinamide (NAM), the amide form of niacin (vitamin B<sub>3</sub>), is an essential precursor of NAD<sup>+</sup> required for cellular energy metabolism. A number of recent studies have demonstrated neuroprotective effects of NAM, not only in *in vitro* models of cytotoxicity, but also *in vivo* in different forms of brain ischemia (Mokudai et al. 2000, Chong et al. 2002, 2004, Bedalov and Simon 2004, Tam et al. 2005, Feng et al. 2006, Lee et al. 2006). Proposed mechanisms of NAM-evoked neuroprotection include stabilization of the mitochondrial membrane potential, reduction of caspase activation and inhibition of PARP (for review see Maiese and Chong 2003).

1-Methylnicotinamide (MNA) is the endogenous product of NAM methylation in many tissues includ-

ing brain: a reaction catalyzed by nicotinamide N-methyl transferase (NNMT, EC2.1.1.1) using S-adenosyl methionine as the methyl donor (Fukushima et al. 2002, Parsons et al. 2002). MNA is a recognized anti-inflammatory agent (Gebicki et al. 2003); however, it has been suggested that MNA may be neurotoxic and might play a role in the pathogenesis of Parkinson's disease (Fukushima et al. 1995, 2002, Ogata et al. 2000). There are epidemiological data indirectly supporting this idea (Williams and Ramsden 2005, Williams et al. 2005). In addition, an MPP<sup>+</sup>-mimicking effect of infrastratial MNA injections has been described (Fukushima et al. 2002). It is not clear whether MNA might induce neurodegeneration in non-dopaminergic neurons, like glutamatergic cerebellar granule cells in culture, or if this compound shares the neuroprotective properties of its precursor NAM.

Given the availability of an adequate *in vitro* model for studying the putative neuroprotective effects of NAM and MNA, the impact of homocysteine (Hcy)

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