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Possible mediation of quinolinic acid-induced hippocampal damage by reactive oxygen species

Review Article

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Summary. Several differences exist between quinolinic acid and N-methyl-D-aspartate (NMDA) in the potency and pharmacology of their neurotoxic actions in the brain, suggesting that quinolinic acid may act by mechanisms additional to the activation of NMDA receptors, possibly involving lipid peroxidation. In the present review, studies are considered which have attempted to determine whether free radicals might contribute to the neuronal damage induced by quinolinic acid. Following Injections into the hippocampus of anaesthetised rats, quinolinic acid induced damage is prevented by melatonin, by an action not blocked by the melatonin receptor blocker luzindole. Deprenyl, but not the non-selective monoamine oxidase inhibitor nialamide, also prevent quinolinic acid-induced damage. In vitro, seversl groups have shown that quinolinic acid can induce lipid peroxidation of brain tissue The results suggest that free radical formation contributes significantly to quinolinic acid-induced damage *in vivo*.

Keywords: Amino acids – Quinolinic acid – Kynurenines – Melatonin – Deprenyl – Antioxidants – Free radicals – Reactive oxygen species – Neuroprotection – Neurotoxicity – Excitotoxicity

Introduction

Quinolinic acid, an endogenous agonist at N-methyl-D-aspartate (NMDA) receptors (Stone and Perkins, 1981; Perkins et al., 1983; Stone, 1993), produces neuronal damage after local injection into the brain (Schwarcz et al., 1983). It is also of interest because of its possible role in the neuronal damage accompanying a range of pathological states (Stone, 1993). For example, the levels of quinolinic acid in the brain increase following infection with HIV or

the simian equivalent, SIV, when the concentration in cerebrospinal fluid can rise up to 400-fold (Heyes et al., 1992). These levels are higher than those able to produce neuronal damage. Following treatment, the levels of quinolinic acid decline in parallel with improvements in disease activity.

The neurotoxicity of quinolinic acid is more pronounced than can be accounted for solely by the activation of NMDA receptors since quinolinic acid is less active in producing neuronal excitation whereas they are equiactive in producing neuronal damage. There are also qualitative differences (Foster and Schwarcz, 1989) as well as pharmacological differences (Perkins and Stone, 1983; Winn et al., 1991) between the neurotoxic effects of NMDA and quinolinic acid. Some of the these differences could be explained if quinolinic acid-induced damage were mediated partly by free radicals, and this possibility has been explored by several groups working *in vivo* and *in vitro*.

Studies of lipid peroxidation

A major consequence of the effects of reactive oxygen species on living tissue is the formation of oxidised products of cellular membrane lipids. These include malondialdehyde and 4-hydroxynonenal, both of which can be detected in vitro (Zaleska and Floyd, 1985) and *in vivo* (Kogure et al., 1982) following cerebral insults of various kinds. These lipid peroxidation products are in turn toxic, causing disruption of cellular enzymes, membrane receptors and transport processes (Braughler, 1985; Picklo et al., 1999; Brown-Galatola and Hall, 1992).

Reactive oxygen species may also damage nucleic acid structure, compromising cell survival directly and potentially modifying gene expression, leading to disorders of cell proliferation (Morel and Baroiki, 1998). The oxidation of thiols and the formation of carbonyl groups on proteins can lead to widespread deterioration in cell viability, with loss of receptor, enzyme and transporter functions (Brown-Galatola and Hall, 1992).

Rios and Santamaria (1991) were the first to demonstrate that quinolinic acid could elevate the formation of thiobarbituric acid reactive substances, a measure of lipid peroxidation, after incubating with brain homogenates for 30 minutes. Concentrations of 20–80 μM were effective in raising peroxidation up to 50% above control values.

In a subsequent, *in vivo*, study, the same authors demonstrated that the intrastriatal injection of quinolinic acid also increased lipid peroxidation. In addition, however, they found that the effect, along with the neuronal damage produced, was prevented by the non-competitive NMDA antagonist dizocilpine (Santamaria and Rios, 1993).

It is difficult to reconcile this ability of quinolinic acid to produce peroxidation via a receptor mechanism, with the result of a more recent study showing that the oxidising activity of quinolinic acid may not be exerted directly on neural tissue, but that it requires the obligatory presence of iron (Stipek et al., 1997). It may be that the proposed complex formation

between quinolinic acid and iron has a greater effect on NMDA receptors than virgin quinolinic acid, and this possibility would seem to merit further exploration.

Neuronal protection by anti-oxidants

One of the few *in vivo* studies of neuroprotection has revealed that both the histopathological and the neurological consequences of the intrastriatal administration of quinolinic acid can be prevented by α-phenyl-t-butyl nitrone (Nakao and Brundin, 1997). This compound is a spin-trap reagent, able to interact with high selectivity with free radical molecules to form stable complexes, preventing their having extensive injurious effects on cell membranes. This result is, therefore, strongly suggestive of a role for free radicals in the damaging effects of quinolinic acid.

In our own work, we have found that quinolinic acid (120 nmols) produced a dose-dependent loss of neurones in areas CA1, CA3 and CA4 and resulted in the infiltration of the pyramidal cell layers and surrounding tissue by microglial cells. NMDA also produced damage in the CA3 region, an area which we chose to use for quantitative comparisons. The quinolinate damage was prevented by the co-administration of melatonin applied both by intrahippocampal injection simultaneously with quinolinic acid, and twenty-four hours later as an intraperitoneal injection. Neither the administration of melatonin alone at this dose, nor its ethanolic vehicle, affected the pyramidal neurone population.

Luzindole is an antagonist of at least one of the melatonin receptors, and it was used in order to discriminate the possibility that melatonin was acting as an anti-oxidant as opposed to via a receptor mechanism. In fact, luzindole had no effect on neuronal survival alone, and did not prevent the protection afforded by melatonin.

We have also found that deprenyl produced a significant level of protection against quinolinic acid, whereas the non-selective monoamine oxidase inhibitor nialamide did not affect the amount of neuronal damage produced.

Mechanisms of protection

Activation of neuronal NMDA receptors is associated with an influx of calcium ions leading to the generation of reactive oxygen species (Choi, 1987; Hartley et al., 1993; Velazquez et al., 1997; Atlante et al., 1997). This action may underlie the receptor-mediated component of lipid peroxidation encountered by Rios and Santamaria (1991) and Santamaria and Rios (1993). The reports of damage limitation by spin trap reagents such as α-phenyl-t-butylnitrone (Nakao and Brundin, 1997) are clearly consistent this view.

Melatonin is a highly efficient scavenger of free radicals (Hardeland et al., 1993; Hardeland and Rodriguez, 1995; Reiter et al., 1995), while deprenyl can also protect neurones partly by scavenging free radicals and partly by

increasing the activity of antioxidant enzymes (Mytilineou et al., 1997; Wu et al., 1993; Koutsilieri et al., 1994). Melatonin has been shown to protect neurones against a variety of toxic insults (Giusti et al., 1995; Cagnoli et al., 1995; Lezoualch et al., 1996; Uz et al., 1996; Cho et al., 1997) and can prevent lipid peroxidation induced in vitro by kainate, nitric oxide or hydrogen peroxide (Melchiorri et al., 1995; Sewerynek et al., 1995; Reiter et al., 1995; Escames et al., 1997). The failure of luzindole to prevent the protection produced by melatonin argues against a receptor-mediated effect. (Dubocovich 1995) of at least a significant proportion of the quinolinic acid induced damage and free radical generation.

Overall, therefore, existing data indicate that part of the neuronal damage produced by quinolinic acid may be due to the enhanced formation of free radicals, and that this is likely to be independent of NMDA receptor activation (Giusti et al., 1995; Cazevieille et al., 1997). This is supported by other data from our laboratory indicating that melatonin does not prevent the excitotoxicity produced by NMDA itself. The mechanism of quinolinic acid's toxicity may, therefore, involve at least partly a direct interaction with cell constituents. Damage produced by quinolinic acid may, alternatively, be the result of the gliosis which follows excitotoxic challenge, since activated microglia are known to produce free radicals.

Deprenyl protects cultured dopamine neurones against damage mediated by NMDA receptors (Mytilineou et al., 1997), and reduces hippocampal damage produced by cerebral ischaemia (Paterson et al., 1997). The mechanism of protection does not seem to involve monoamine oxidase inhibition (Wu et al., 1993; Gerlach et al., 1994; Tatton et al., 1996), since protection was not mimicked by nialamide. Deprenyl can promote neuronal regeneration and neuritogenesis (Iwasaki et al., 1994; Koutsilieri et al., 1994). Chronic administration of deprenyl increases the activities of two of the antioxidant enzymes, superoxide dismutase and catalase (Carrillo et al., 1994a,b,c). It also scavenges hydroxyl and peroxyl radicals (Thomas et al., 1997).

In conclusion, the neurodegeneration produced by quinolinic acid may be partly dependent upon the generation of reactive oxygen species in addition to its stimulant effect on NMDA receptors.

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