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The Janus faces of 3-hydroxykynurenine: Dual redox modulatory activity and lack of neurotoxicity in the rat striatum

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ABSTRACT

3-Hydroxykynurenine (3-HK), an intermediate metabolite of the kynurenine pathway, has been largely hypothesized as a neurotoxic molecule contributing to neurodegeneration in several experimental and clinical conditions. Interestingly, the balance in literature points to a dual role of this molecule in the CNS: *in vitro* studies describe neurotoxic and/or antioxidant properties, whereas *in vivo* studies suggest a role of this metabolite as a weak neurotoxin. This work was designed to investigate, under different experimental conditions, whether or not 3-HK is toxic to cells, and if the redox activity exerted by this molecule modulates its actions in the rat striatum. In order to evaluate these effects, 3-HK was administered *in vitro* to isolated striatal slices, and *in vivo* to the striatum of rats. In striatal slices, 3-HK exerted a concentration- and time-dependent effect on lipid peroxidation, inducing both pro-oxidant actions at low (5–20) micromolar concentrations, and antioxidant activity at a higher concentration (100 μM). Interestingly, while 3-HK was unable to induce mitochondrial dysfunction in slices, at the same range of concentrations it prevented the deleterious effects exerted by the neurotoxin and related metabolite quinolinic acid (QUIN), the mitochondrial toxin 3-nitropropionic acid, and the pro-oxidant compound iron sulfate. These protective actions were related to the stimulation of glutathione S-transferase (GST) and superoxide dismutase (SOD) activities. In addition, 3-HK stimulated the protein content of the transcription factor and antioxidant regulator Nrf2, and some of its related proteins. Accordingly, 3-HK, but not QUIN, exhibited reductive properties at high concentrations. The striatal tissue of animals infused with 3-HK exhibited moderate levels of lipid and protein oxidation at short times post-lesion (h), but these endpoints were substantially decreased at longer times (days). These effects were

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correlated with an early increase in glutathione reductase (GR) and GST activities. However, these changes were likely to be merely compensatory as 3-HK-infused animals did not display behavioral (rotation) alterations or morphological changes in their injected striata. Altogether, these findings suggest that, despite 3-HK might exert pro-oxidant actions under certain conditions, these changes serve to evoke a redox modulatory activity that, in turn, could decrease the risk of cell damage. In light of this evidence, 3-HK seems to be more a redox modulatory molecule than a neurotoxic metabolite.

1. Introduction

Extensive research has demonstrated that neurodegenerative diseases share several deleterious mechanisms including excitotoxicity – a toxic process characterized by a sustained stimulation of excitatory amino acid receptors – , oxidative stress – which results of an impaired balance between the formation of reactive oxygen species (ROS) and antioxidant defenses – and inflammation – leading to a breakdown of the blood-brain barrier, cell infiltration and release of chemical mediators such as cytokines, chemokines and lipid mediators – , all of them ultimately leading to cell death (Halliwell, 2006; Dasuri et al., 2013; Niranjan, 2014).

The kynurenine pathway (KP) is a metabolic route in which the essential amino acid tryptophan is primarily metabolized, hence producing relevant intermediates for neuronal integrity and redox balance (Stone et al., 2007; Massudi et al., 2012; Schwarcz et al., 2012). Recent reports have shown that alterations of the KP metabolism and changes in the brain levels of its metabolites may play a key role in the pathogenesis of some neurodegenerative diseases, depressive disorders and schizophrenia (Schwarcz et al., 2012; Tan et al., 2012; Amaral et al., 2013). In addition, the KP has been associated with inflammatory responses in different neurological disorders, and this mechanism, which may be inherent to some KP metabolites, could contribute to the neurodegenerative pattern associated with these diseases (Chen et al., 2010; Tan et al., 2012). Some neuroactive metabolites are formed in the KP, including the well-known neurotoxin and glutamate agonist quinolinic acid (QUIN) (Schwarcz et al., 1984; Guidetti et al., 2006), the neuroprotectant and glutamate antagonist kynurenic acid (KYNA) (Schwarcz et al., 1983; Yu et al., 2004), and the redox active metabolites 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAAO) (Goldstein et al., 2000; Braidy et al., 2009; reviewed by Colín-González et al., 2013).

Increased levels of 3-HK have been found in early stages of Huntington's disease (HD) patients (Schwarcz et al., 1984; Reynolds and Pearson, 1989; Pearson et al., 1995; Guidetti and Schwarcz, 2003), in Alzheimer's disease (AD) – in which 3-HK is assumed to be relevant for memory alterations (Savvateeva et al., 2000; Duleu et al., 2010) – and in the putamen of Parkinson's disease (PD) patients (Ogawa et al., 1992; reviewed by Colín-González et al., 2013). Also, transgenic mice models (R6/2 mice, YAC 128, and the chimeric HdhQ) have shown a substantial elevation of the 3-HK content (Guidetti et al., 2000; Guidetti et al., 2006; Sathyasaikumar et al., 2010). Moreover, genetic models of HD using invertebrates have shown an increased activity of kynurenine 3-monooxygenase, the enzyme responsible for 3-HK formation (Giorgini et al., 2005; Ramaswamy et al., 2007; Campesan et al., 2011).

Under physiological conditions, 3-HK undergoes auto-oxidation, forming an o-aminoquinone – a stronger oxidant agent that can be responsible for exacerbated oxidative damage – which can be responsible for the formation of different reactive oxygen species ($O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$) (Eastman and Guilarte, 1990; Ishii et al., 1992; Okuda et al., 1996; Hiraku et al., 1995; Vazquez et al., 2000). As a metabolic precursor, 3-HK might also account for QUIN formation, which in turn is responsible for cellular damage mainly mediated by excitotoxicity and oxidative damage (Schwarcz et al., 1984). In addition, 3-HK has also shown to be a generator of reactive species, acting as a potential endogenous neurotoxin in cerebellar granule, striatal and hippocampal neurons, as well as in neuronal hybrid cell lines, human neuroblastoma SH-SY5Y, PC-12 pheochromocytoma cells, and GT1-7 hypothalamic neurosecretory cells (Eastman and Guilarte, 1989; Okuda et al., 1998; Jeong et al., 2004; Smith et al., 2009). However, to our knowledge, there are only three studies using 3-HK in *in vivo* models in the CNS, but none of them has characterized in a detailed manner its mechanisms of toxicity. The intraventricular administration of 3-HK (634.21 mg/rat) was responsible for convulsive attacks in rats (Pinelli et al., 1984), whereas the intrastriatal injection of 3-HK (50 nmol) induced only tissue damage around the injected site, without any abnormal behavior in rats (Nakagami et al., 1996). Finally, it was suggested that 3-HK potentiates QUIN toxicity by means of a possible synergic interaction comprising a combination of direct NMDA receptor activation and free radical production (Guidetti and Schwarcz, 1999).

In contrast to these toxic features, under certain circumstances 3-HK is known to be a potent reductive agent that donates electrons, sometimes acting as an antioxidant agent. This behavior of 3-HK, which is opposite to its toxic character, has been described in several reports, some of which discuss a scavenging activity for this molecule, showing that 3-HK is able to scavenge $O_2^{\cdot-}$, $^{\cdot}OH$, peroxyl radicals, and $^{\cdot}NO$, also acting as an endogenous natural antioxidant (Goshima et al., 1986; Christen et al., 1990; Goda et al., 1999; Leipnitz et al., 2007; Backhaus et al., 2008). Still, its precise role in the CNS remains uncertain. Hence, 3-HK is an intriguing and puzzling compound found at increased levels in pathological conditions; consequently, characterizing and identifying the precise physiological and/or physiopathological roles exerted by