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Central nervous system-specific alterations in the tryptophan metabolism in the 3-nitropropionic acid model of Huntington's disease

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ABSTRACT

Experiments on human samples and on genetic animal models of Huntington's disease (HD) suggest that a number of neuroactive metabolites in the kynurenine (KYN) pathway (KP) of the tryptophan (TRP) catabolism may play a role in the development of HD. Our goal in this study was to assess the concentrations of TRP, KYN, kynurenic acid and 3-hydroxykynurenine (3-OHK) in the serum and brain of 5-month-old C57Bl/6 mice in the widely used 3-nitropropionic acid (3-NP) toxin model of HD. We additionally investigated the behavioral changes through open-field, rotarod and Y-maze tests. Our findings revealed an increased TRP catabolism *via* the KP as reflected by elevated KYN/TRP ratios in the striatum, hippocampus, cerebellum and brainstem. As regards the other examined metabolites of KP, we found only a significant decrease in the 3-OHK level in the cerebellum of the 3-NP-treated mice. The open-field and rotarod tests demonstrated that treatment with 3-NP resulted in a reduced motor ability, though this had almost totally disappeared a week after the last injection, similarly as observed previously in most murine 3-NP studies. The relevance of the alterations observed in our biochemical and behavioral analyses is discussed. We propose that the identified biochemical alterations could serve as applicable therapeutic endpoints in studies of drug effects on delayed-type neurodegeneration in a relatively fast and cost-effective toxin model of HD.

1. Introduction

Huntington's disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder which results in cognitive, psychiatric and motor disturbances. HD is caused by an expansion of the cytosine–adenine–guanine (CAG) repeat in the gene coding for the *N*-terminal region of the huntingtin protein (Htt), which leads to the formation of a polyglutamine stretch. Above 39 CAGs, there is obligatory disease development (The Huntington's Disease Collaborative Research Group, 1993). Although the exact mechanisms through which mutant Htt (mHtt) leads to the characteristic neuropathology are not fully understood, the potential roles of excitotoxicity and a neuronal mitochondrial dysfunction are among the best-established concepts (Szalardy et al., 2012; Zádori et al., 2012).

Various evidence suggest that the involvement of striatal glutamatergic excitotoxicity in the development of HD is mediated predominantly by the overactivation of *N*-methyl-D-aspartate receptors (NMDARs), and most specifically through NR2B subunit-containing NMDARs at the extrasynaptic sites (Milnerwood et al., 2010). In line with this, the expression of mHtt has been shown to sensitize the NR2B subunit-containing NMDARs (Chen et al., 1999). There is evidence indicating that such excitotoxic injury is mediated at least in part by endogenous substances, including certain metabolites of the kynurenine pathway (KP) of the tryptophan (TRP) metabolism (Fig. 1; Zádori et al., 2011).

This pathway involves a number of neuroactive compounds. Among them, quinolinic acid (QUIN) is a weak NMDAR agonist (Stone and Darlington, 2002) which demonstrated an ability to induce excitotoxic injury, which led to QUIN toxicity being utilized as an early toxin model of HD (Beal et al., 1986). This toxic effect of QUIN was revealed to be augmented by another deleterious KP metabolite, 3-hydroxykynurenine (3-OHK), which can generate oxidative stress *via* the production of reactive oxygen species (ROS) (Guidetti et al., 2000). On the other hand, kynurenic acid (KYNA) is an NMDAR antagonist at the strychnine-insensitive glycine coagonist site (Perkins and Stone, 1982) and a weak antagonist on kainate- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-sensitive ionotropic glutamate receptors (Kessler et al., 1989). Furthermore, its inhibitory potential on presynaptic α7-nicotinic acetylcholine receptors is proposed to be of

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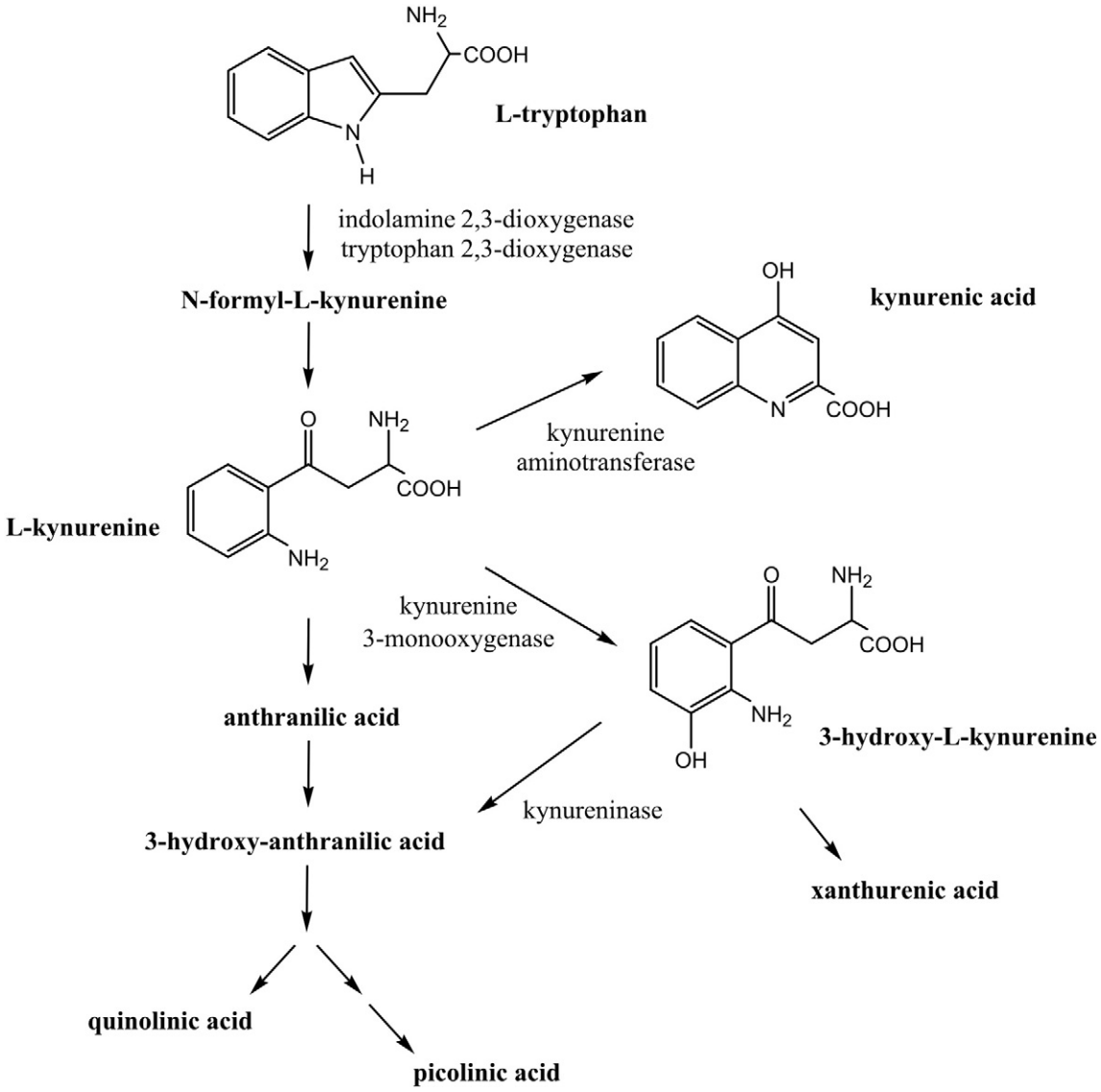

Fig. 1. Schematic depiction of the kynurenine pathway of the tryptophan metabolism.

high relevance at physiological KYNA levels, due to the suppression of presynaptic glutamate release (Hilmas et al., 2001). On this basis, KYNA was considered to be neuroprotective against NMDAR-modulated glutamatergic excitotoxicity, which has since been demonstrated by a variety of experimental evidence (Miranda et al., 1997; Sinor et al., 2000).

Besides the theoretical considerations, evidence of the presence of alterations in the KP of the TRP metabolism in tissues from HD patients and transgenic animals and in human post-mortem HD brains (Schwarcz et al., 1988; Beal et al., 1990, 1992; Heyes et al., 1992; Pearson and Reynolds, 1992; Jauch et al., 1995; Guidetti et al., 2004, 2006) is emerging. Briefly, these results indicate a relative decrease in KYNA concentrations relative to the levels of toxic neuroactive kynurenines (Zádori et al., 2011).

A decreased activity of the succinate dehydrogenase (SDH), complex II of the electron transport chain in post-mortem HD brains was one of the early findings suggestive of the role of a mitochondrial dysfunction in the development of HD (Stahl and Swanson, 1974). Furthermore, mHtt has been shown to be able to bind directly to the mitochondria, altering their normal function (Choo et al., 2004). In line with the decreased SDH activity, mitochondrial II complex inhibitors such as 3-nitropropionic acid (3-NP) or malonate have been found to be useful in the investigation of HD through their utilization in animal toxin models (Túnez et al., 2010). The 3-NP model is frequently applied as an easy and rapid way to study certain aspects of neurodegenerative processes in HD (Brouillet, 2014). Treatment with 3-NP evokes increases in the production of ROS and ·NO (La Fontaine et al., 2000) and the activation of apoptosis-related factors including caspase-3 and calpain (Duan et al., 2000; Bizat et al., 2003a), mechanisms through which 3-NP evokes striatal lesions in both rodents and primates in a rather selective manner. Several dosing regimens are applied to evoke characteristic neuropathological and behavioral alterations in rodents (Brouillet et al., 2005). Briefly, striatal neurodegeneration in rats occurs when the steady inhibition of SDH attains 50–60% (Alexi et al., 1998; Brouillet et al., 1998; Blum et al., 2002; Bizat et al., 2003b). In mice, there have been only a few studies of the dosage-related reduction in SDH activity. Lower cumulative doses (320 mg/kg) have been shown to induce an approximately 20% reduction of enzyme activity, which is not associated with marked striatal lesions (Fernagut et al., 2002a). However, higher cumulative doses (400–450 mg/kg) were widely used to induce measurable striatal lesions (Klivenyi et al., 2000, 2006). Accordingly, in a previous study we demonstrated a significant