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Short Communication

The absence of indoleamine 2,3-dioxygenase expression protects against NMDA receptor-mediated excitotoxicity in mouse brain

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ABSTRACT

We previously showed that the expression and activity of indoleamine 2,3-dioxygenase (*Ido1*) are chronically elevated in the striatum of YAC128 mouse model of HD. This was followed by increased production of neurotoxic metabolite hydroxykynurenine (3-HK) in the striatum of symptomatic mice. We therefore hypothesized that the chronic Ido1 induction in the striatum of YAC128 mice leads to increased neurotoxicity in this mouse model; based on this hypothesis, we predicted that the absence of *Ido1* expression would result in decreased sensitivity to neurotoxicity in mice. The work described in this brief communication will include the characterization of $Ido^{-/-}$ striatum in terms of enzymatic expression and activity in the first step of the pathway. Additionally, we assessed the sensitivity of the striatum to excitotoxic insult in the absence of *Ido1* expression in the striatum of constitutive *Ido1* null mice ($Ido^{-/-}$) and demonstrated that $Ido^{-/-}$ mice are less sensitive to QA-induced striatal neurotoxicity. Finally, through measurement of kynurenine pathway (KP) metabolites in $Ido^{-/-}$ mice, we showed decreased levels of 3-HK in the striatum of these mice. This study suggests that the inhibition of the first step in the KP may be neuroprotective and should be considered as a potential therapeutic target in HD and other neurodegenerative diseases.

Introduction

We previously identified indoleamine 2,3-dioxygenase (*Ido1*) as a developmentally striatal-enriched transcript in a high-throughout gene expression profiling analysis of the mouse brain (Mazarei et al., 2010). Recently, we were able to show that Ido1 mRNA expression and enzymatic activity are chronically elevated in the striatum of the YAC128 mouse model of Huntington disease (HD) (Mazarei et al., 2013).

Ido1 is the first and the rate-limiting enzyme of the kynurenine pathway (KP) catalyzing the conversion of the essential amino acid L-tryptophan (trp) to L-kynurenine (kyn) (Higuchi and Hayaishi, 1967). In addition to our findings, induction of Ido1 has been implicated in the neuropathology of other neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Baran et al., 2000; Bonda et al., 2010; Chen et al., 2010; Clark et al., 2005; Darlington et al., 2007; Fu et al., 2011; Guillemin et al., 2005a; Huengsberg et al., 1998; Okamoto et al., 2009; Sardar et al., 1995; Szabó et al., 2011; Walker et al., 2006; Widner et al., 2002; Yamada et al., 2009); yet the precise role of Ido1 in the brain remains unknown. Two other enzymes, Ido1 and Tdo2, can also catalyze the conversion of trp to kyn and have not been extensively studied in brain.

N-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity is a well-established mechanism in HD. NMDA receptors (NMDARs) are ionotropic glutamate receptors involved in synaptic plasticity in brain. Under normal conditions, NMDARs in medium spiny neurons (MSNs) of the striatum receive input from the glutamatergic neurons of the cortex. Previous studies have demonstrated that over-activation of these receptors in the striatum plays an important role in the cellular processes involved in neuronal death in HD (Levine et al., 1999; Zeron et al., 2004). Enhanced excitotoxicity as a result of increased NMDAR-mediated currents has been demonstrated in several HD mouse models early in the pathogenesis (Cepeda et al., 2001; Graham et al., 2006; Levine et al., 1999; Zeron et al., 2004).

Involvement of the KP in HD was postulated when intrastriatal injection of the kynurenine pathway metabolite quinolinic acid (QA), into the mouse brain was found to cause selective degeneration of MSNs

Abbreviations: HD, Huntington's disease; *Ido1*, indoleamine 2,3-dioxygenase (gene); *Tdo2*, tryptophan 2,3-dioxygenase (gene); *Ido2*, indoleamine 2,3-dioxygenase 2 (gene); KP, kynurenine pathway; SP, serotonin pathway; Kyn, kynurenine; LC-MS/MS, liquid chromatography tandem mass spectrometry; Trp, tryptophan; QA, quinolinic acid; KA, kynurenic acid; MSN, medium spiny neurons; BBB, blood brain barrier; NMDA, N-methyl-D-aspartate.

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(but not interneurons) in the striatum. This selective degeneration is reminiscent of the striatal degeneration in HD and injections of exogenous QA (an NMDAR agonist) have been used for decades to generate chemical models of HD (Beal et al., 1986; Schwarcz et al., 1983). Additionally, the levels of endogenous QA are elevated in the brain and plasma of HD patients and mouse models (Guidetti et al., 2004, 2006). In the neurotoxic branch of KP, QA results from the breakdown of 3-hydroxykunurenine (3-HK) though a few steps in the pathway. However, the toxic effect of 3-HK is independent of NMDA receptor mediated toxicity and solely depends on its ability to generate free radicals (Szalardy et al., 2012).

The work in this study is based on the hypothesis that the observed elevation of Ido1 expression and activity in the striatum of YAC128 mice results in the increased sensitivity to NMDAR-mediated neurotoxicity observed in this mouse model (Graham et al., 2006). Based on this hypothesis, we predicted that absence of Ido1 expression should result in decreased sensitivity to NMDAR-mediated neurotoxicity in mice. To test this hypothesis, we sought to assess the sensitivity of brain (particularly the striatum) to NMDAR-mediated excitotoxic insult in the absence of Ido1 expression in constitutive *Ido1* null mice (*Ido*$^{-/-}$). These mice have mainly been used in the past in studies investigating the role of Ido1 in immunoregulation (Jung et al., 2009; Kiank et al., 2010; Ravishankar et al., 2012), tumor-induced tolerance (Hou et al., 2007), and immune-induced depression (Kim et al., 2012; O'Connor et al., 2009).

Materials and methods

Animals of both genotypes (WT and *Ido*$^{-/-}$) used in this study were 3-month-old adult mice. *Ido*$^{-/-}$ mice have been generated by Andrew Mellor (Baban et al., 2004) and purchased from the Jackson laboratories. Four to five mice of similar gender per genotype and tissue were used for transcription experiment. Five mice of similar gender were used of each genotype for QA injection experiment. Three to four mice of similar gender per genotype were used.

Quantitative real-time PCR

The ABI 7500 real-time PCR system (Applied Biosystems) and the Fast SYBR® Green Master mix (Applied Biosystems) were used for all qPCR experiments.

Absolute quantity of the targets in each sample was calculated based on the standard curve method. Standard curves were created using 10-fold serial dilutions of either mouse striatum, or cerebellum cDNA. The relative amount of mRNA in each well was calculated as the ratio between the target mRNA and a normalization factor (NF) described before (Vandesompele et al., 2002). Primer sequences are listed in Supplementary Table 1.

Intra-striatal injection of quinolinic acid

Quinolinic acid (QA; Sigma) was dissolved into 0.1 M PBS. Three-month-old mice of mixed sex were anesthetized by inhalation of 4% isoflurane with 1% oxygen and positioned in a stereotaxic frame. Mice received unilateral intrastriatal injections of 6 nmol QA dissolved in 0.5 ml PBS (7–11 mice/genotype). Coordinates of the injection site are as follows: +0.8 mm anterior to Bregma, 1.8 mm mediolateral, and 3.5 mm dorsoventral to the neocortex.

Quantitative analysis

Fluoro-jade B staining for lesion analysis

Fluoro-jade B (FJB; Millipore, Temecula, CA) staining was performed in serial 25 μm coronal sections to evaluate cell death after QA. For QA, every 8th section through the entire striatum (Bregma +1.10 mm to Bregma −0.94 mm, total of 15 sections) was mounted on a glass slide and processed for FJB staining.

Lesion quantification and neuronal cell counts

FJB-positive lesions in QA-injected sections were quantified using Stereo Investigator (MicroBrightfield). All stereology was performed blinded to genotype and treatment.

In a set of adjacent sections, the number of NeuN-positive neurons in the striatum was estimated using the Optical Fractionator method (400 × 400 mm grid, 25 × 25 mm probe). Because FJB staining in adjacent sections showed lesions only in the anterior part of the striatum, counting was performed on every 6th section in a total of 5 sections from Bregma 0.98 mm to Bregma −0.10 mm.

LC–MS/MS

The LC–MS/MS was performed on mouse striatum samples following a protocol previously described by us (Mazarei et al., 2013).

Statistics

Expression data were analyzed 2-way ANOVA followed by post hoc comparisons using the Bonferroni tests for Figs. 1a, b, and c and two-tailed Student's *t* test in Figs. 1d, e, f, and g (GraphPad Prism version 5.0).

Results

Characterization of Ido$^{-/-}$ *mice*

Given the potential of Ido2 and Tdo2 to facilitate trp to kyn conversion in the absence of Ido1, we sought to assess the expression patterns of the transcripts corresponding to these enzymes in the brain of YAC128 mice and *Ido*$^{-/-}$ adult mice. We showed that *Ido2* expression pattern is similar to that of *Ido1* in that it is highly enriched in the striatum compared with the cerebellum (Figs. 1a and b). Similar to *Ido1*, and unlike our expectations, *Ido2* mRNA was not expressed the striatum of *Ido*$^{-/-}$ mice (Figs. 1a and b). Based on this, we propose that these animals are null for both *Ido1* and *Ido2*. In addition, *Ido2* expression did not change significantly in the striatum of YAC128 mice (Fig. 1b). Similar to *Ido2*, expression pattern of *Tdo2* mRNA was investigated in the striatum and cerebellum of WT and *Ido*$^{-/-}$ mice. As reported previously, *Tdo2* was predominantly expressed in the cerebellum (Mazarei et al., 2013), where its expression did not differ in WT and *Ido*$^{-/-}$ mice (Fig. 1c). Overall, these data demonstrate that the lack of Ido1 expression in the brain of *Ido*$^{-/-}$ animals was not compensated for by increased mRNA expression of other known enzymes of tryptophan-degradation.

Ido$^{-/-}$ *mice are less sensitive to QA-induced striatal neurotoxicity*

To test our hypothesis that the absence of Ido1 expression would render mice less sensitivity to neurotoxicity, we assessed the sensitivity of the striatum to NMDAR-mediated excitotoxic insult in constitutive *Ido1* null mice (*Ido*$^{-/-}$). QA-induced striatal neurotoxicity in *Ido*$^{-/-}$ mice was compared to WT mice. Fluoro-jade (fj) was used to stain QA-induced degenerating neurons in the striatum of WT and *Ido*$^{-/-}$ mice sacrificed 7 days following intrastriatal injections of QA. This revealed a trend toward smaller QA-induced lesion volume in the striatum of *Ido*$^{-/-}$ mice, which approached statistical significance ($p = 0.07$) (Fig. 1d). We also used the neuronal marker NeuN to count the remaining neurons in the striatum of these mice (Fig. 1e). A significantly greater number of striatal neurons remained in *Ido*$^{-/-}$ mice ($p = 0.003$) compared to WT mice (Fig. 1e) following intrastriatal QA injection. In order to rule out the potential baseline genotypic difference as an explanation for the observed neuronal cell count difference in