NeuroImage 139 (2016) 53–64

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

In vivo imaging of brain glutamate defects in a knock-in mouse model of Huntington's disease

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ARTICLE INFO

Article history:
Received 3 February 2016
Revised 10 June 2016
Accepted 14 June 2016
Available online 16 June 2016

Keywords:
Chemical exchange saturation transfer
GluCEST
Huntington's disease
Glutamate
Mouse model
Neurodegenerative disease

ABSTRACT

Huntington's disease (HD) is an inherited neurodegenerative disease characterized by motor, cognitive and psychiatric symptoms. Atrophy of the striatum has been proposed for several years as a biomarker to assess disease progression in HD gene carriers. However, it does not provide any information about the biological mechanisms linked to HD pathogenesis. Changes in brain metabolites have been also consistently seen in HD patients and animal models using Magnetic Resonance Spectroscopy (MRS), but metabolite measurements are generally limited to a single voxel. In this study, we used Chemical Exchange Saturation Transfer imaging of glutamate (gluCEST) in order to map glutamate distribution in the brain of a knock-in mouse model (Ki140CAG) with a precise anatomical resolution. We demonstrated that both heterozygous and homozygous mice with pathological CAG repeat expansion in gene encoding huntingtin exhibited an atrophy of the striatum and a significant alteration of their metabolic profile in the striatum as compared to wild type littermate controls. The striatal decrease was then confirmed by gluCEST imaging. Surprisingly, CEST imaging also revealed that the corpus callosum was the most affected structure in both genotype groups, suggesting that this structure could be highly vulnerable in HD. We evaluated for the first time gluCEST imaging as a potential biomarker of HD and demonstrated its potential for characterizing metabolic defects in neurodegenerative diseases in specific regions.

Introduction

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by involuntary abnormal movements, as well as cognitive and psychiatric symptoms associated with early atrophy of the striatum and cerebral cortex (Harper, 1991; Walker, 2007). The mutation causing HD consists of an abnormal expansion of a CAG repeat in the gene coding the protein huntingtin (htt). There is no therapy to slow the progression of the disease which is fatal in 10–15 years after the onset of symptoms in young individuals. Despite active research in the past twenty years, the mechanisms underlying brain dysfunction and degeneration remain poorly understood. Mutation of huntingtin affects many cellular processes such as transcription (Sugars and Rubinsztein, 2003), neurotransmission, calcium homeostasis (Cowan and Raymond, 2006), autophagy and energy metabolism (Bossy et al., 2008; Damiano et al., 2010; Mochel et al., 2007; Mochel and Haller, 2011) in both neurons and astrocytes.

There is a limited number of methods allowing precise follow up of disease progression and understanding of physiopathological pathways. Clinical assessments along with neuroimaging techniques are likely the most robust criteria to characterize disease severity in HD patients. For instance, the atrophy of the striatum as measured by MRI is currently the best biomarker of disease progression in HD gene carriers (Aylward et al., 2004; Paulsen et al., 2008; Tabrizi et al., 2013). However, although anatomical alterations seen by MRI can be found many years before onset of symptoms in HD gene carriers, they do not provide any information about the biological mechanisms linked to HD pathogenesis. In the case of clinical trials, morphometric MRI might be crucial to determine whether the therapy modifies or slows down atrophy but does not give rapid indication about biological efficacy of the treatment. Thus, there is a need to develop novel "functional" imaging modalities. Defects in brain energy metabolism have been consistently found in HD patients and animal models. In particular, Positron Emission Tomography (PET) studies demonstrated large reduction in glucose consumption in the caudate/putamen in HD patients (Brouillet et al.,

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http://dx.doi.org/10.1016/j.neuroimage.2016.06.023

1999; Kuwert et al., 1989, 1990). Nonetheless, non-invasive in vivo methods to more broadly characterize energy metabolism in HD are rare. In vivo Magnetic Resonance Spectroscopy (MRS) can provide key insights of pathological molecular processes related to cell metabolism. Analysis of human HD cohorts or transgenic mouse models of HD using in vivo ^{1}H or ^{31}P MRS methods suggested early metabolic changes in the brain (Jenkins et al., 1998; Mochel et al., 2012; Sturrock et al., 2010; Zacharoff et al., 2012). Interestingly, several studies using ^{1}H MRS showed that the concentrations of glutamate and *N*-acetyl-aspartate, two metabolites mainly located in neurons, were decreased in HD patients and animal models (Jenkins et al., 1998, 2000, 2005). Glutamate is the major excitatory transmitter in the central nervous system and is involved in several aspects of normal brain functions including cognition, memory and learning. Its concentration in the brain is higher than all other amino acids (around 10 mmol/kg), and it plays a central role in brain metabolism (Sonnewald, 2014). Regulatory system of glutamate concentration in the brain is very complex (Magistretti and Allaman, 2015). In normal conditions, most of glutamate is located in cells, neurotransmission being governed by few micromolar of extracellular glutamate. Glutamate can become toxic if extracellular concentration is too high. In such case, glutamate overactivates ionotropic glutamate receptors, and producing major rise in Ca^{2+} concentration in the cytoplasm of neurons, triggers excitotoxicity (Beal, 2005; Brouillet et al., 1999; Cowan and Raymond, 2006). Thus, mechanisms to maintain low extracellular concentration are crucial for brain functions. In addition to its major role as an excitatory neurotransmitter, glutamate is central to several metabolic pathways related to energy metabolism and oxidative stress. For instance, it is involved in many crucial biological pathways such as the tricarboxylic acid (TCA) cycle (it is in exchange with TCA cycle intermediate alpha-ketoglutarate), regulation of glutathione synthesis and astrocyte–neuron interactions. Consequently, glutamate concentration has to be precisely regulated and a small defect can be very deleterious for the brain.

In this context, ^{1}H MRS has been widely used to precisely monitor alterations of metabolic profiles and particularly glutamate concentration. However, one important limitation of MRS is that glutamate measurements are confined to relatively large voxels, due to limited sensitivity of the method. Thus, it would be of major interest to find functional biomarkers related to energy metabolism that could be monitored in the entire brain and with good anatomical resolution in HD animal models or gene carriers. Recently, CEST (Chemical Exchange Saturation Transfer) has been proposed to indirectly detect dilute molecules with labile protons (Ward et al., 2000; Ward and Balaban, 2000; Wolff and Balaban, 1990). Exchangeable protons, for example amine ($-NH_2$) or amide ($-NH$) groups, exhibit a resonance frequency that is shifted relative to bulk proton frequency. Likewise, exchangeable protons can be selectively saturated using radiofrequency (RF) pulse, leading to a decrease of water signal due to magnetization exchange. The potential of CEST imaging to map glutamate level has already been demonstrated in both rodent and human brains at high magnetic fields (≥7 T) (Cai et al., 2012, 2013; Carrillo-de Sauvage et al., 2015). It is thus possible to map local alteration in glutamate concentrations, which may be a potential indicator of regional energy defects in vivo.

Here, we investigated whether gluCEST could represent a relevant biomarker of HD. To do so, we studied a knock-in (Ki) mouse model of HD. Several Ki mouse models have already been described in the literature expressing various length of CAG triplet, from 50 to 175 (Lin et al., 2001; Menalled, 2005; Wheeler et al., 2002). These Ki mice are usually characterized by the slowly progressive appearance of the symptoms, which mimics more closely human HD pathology than severe and rapid mouse models (Lin et al., 2001; Menalled and Chesselet, 2002; Menalled et al., 2003; Wheeler et al., 2000). In the present study, we developed and applied gluCEST imaging to examine brain glutamate concentrations in a slowly progressing mouse model of HD, the Ki mice expressing chimeric mouse/human exon 1 containing 140 CAG repeats inserted in the murine Htt gene (Ki140CAG) (Menalled et al., 2003).

Materials and methods

Ki140CAG and wild type littermate mice

Mice were housed in a temperature-controlled room maintained on a 12 h light/dark cycle. Food and water were available ad libitum. All animal studies were conducted according to the French regulation (EU Directive 2010/63/EU — French Act Rural Code R 214-87 to 131). The animal facility was approved by veterinarian inspectors (authorization n° A 92-032-02) and complies with Standards for Humane Care and Use of Laboratory Animals of the Office of Laboratory Animal Welfare (OLAW — n°#A5826-01). All procedures received approval from the ethical committee (authorization n°2015060417243726vl (APAFIS#770).

In the present study, we used knock-in mice expressing chimeric mouse/human exon 1 containing 140 CAG repeats inserted in the murine Htt gene (Ki140CAG). Ki140CAG mice colony was maintained by breeding heterozygotes Ki140CAG males and females (Menalled et al., 2003). Mice were N3 (B6) on a 129 Sv × C57BL/6J background. Genotype was determined from PCR of tail snips taken at 10–15 days of age for Ki140CAG mice. The resulting mice of our colony were divided in 2 groups depending on their genotype; heterozygous and homozygous for the Htt gene. Ki140CAG mice were compared to their wild type (WT) littermate controls.

Preliminary behavioral testing of the Ki140CAG mouse colony

Open-field test

We used groups of WT ($n = 3$, 3 females), heterozygous ($n = 5$, 3 females, 2 males), and homozygous ($n = 8$, 5 females, 3 males) mice. The three groups of 7–15 month-old mice were matched for age (mean ± SEM in months: WT, 9.33 ± 1.86; heterozygous, 11.10 ± 2.68; homozygous, 11.81 ± 0.72) and were not statistically different in body weight (mean ± SEM in grams: WT, 27.30 ± 0.51; heterozygous, 29.05 ± 2.9; homozygous 26.47 ± 1.21). We assessed the spontaneous locomotor activity of Ki140CAG mice in an automated Ethovision XT/open-field apparatus (Noldus). Mice were transferred to the testing room and allowed to acclimate for at least 30 min before testing. The open-field consisted of a square arena (50 × 50 cm, 40 cm high). The floor was divided into two virtual concentric parts, with an inner zone in the middle of the arena (25 × 25 cm) and an outer zone extending from the outer edge of the inner zone to the walls of the structure. Animals were placed in the center of the arena at the start of the test. Locomotor activity was monitored for 10 min with a video camera mounted on the ceiling directly above the center of the arena. The floor of the arena was washed with 70% ethanol between tests, to remove the odor of the previous subject. A computerized tracking system (Ethovision XT, Noldus IT) calculated total locomotion, speed, and time spent immobile by image analysis. We also recorded duration of rearing and grooming behavior during the 10 min trial by visual inspection of the behavior of each animal using time encoded video recording (the Observer, Noldus).

Rotarod test

We used groups of WT ($n = 5$, 4 females, 1 male), heterozygous ($n = 9$, 1 female, 8 males), and homozygous ($n = 10$, 4 females, 6 males) mice. Mice were different from those used for the open-field test. The three groups of 10–13 month-old mice were matched for age (mean ± SEM in months: WT, 10.60 ± 1.03; heterozygous, 11.10 ± 0.33; homozygous, 11.50 ± 0.52) and were not statistically different in body weight (mean ± SEM in grams: WT, 28.22 ± 0.79; heterozygous, 28.03 ± 0.74; homozygous 26.27 ± 0.83). The rotarod test was used to evaluate the motor coordination and strength of the mice.