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Early and brain region-specific decrease of *de novo* cholesterol biosynthesis in Huntington's disease: A cross-validation study in Q175 knock-in mice

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ABSTRACT

Cholesterol precursors and cholesterol levels are reduced in brain regions of Huntington's disease (HD) mice. Here we quantified the rate of *in vivo de novo* cholesterol biosynthesis in the HD brain. Samples from different brain regions and blood of the heterozygous knock-in mouse model carrying 175 CAG repeats (Q175) at different phenotypic stages were processed independently by two research units to quantify cholesterol synthesis rate by ^2H_2O labeling and measure the concentrations of lathosterol, cholesterol and its brain-specific cholesterol catabolite 24-hydroxy-cholesterol (24OHC) by isotope dilution mass spectrometry. The daily synthesis rate of cholesterol and the corresponding concentration of lathosterol were significantly reduced in the striatum of heterozygous Q175 mice early in the disease course. We also report that the decrease in lathosterol was inversely correlated with CAG-size at symptomatic stage, as observed in striatal samples from an allelic series of HD mice. There was also a significant correlation between the fractional synthesis rates of total cholesterol and 24OHC in brain of wild-type (WT) and Q175 mice, supporting the evidence that plasma 24OHC may reflect cholesterol synthesis in the adult brain. This comprehensive analysis demonstrates consistent cholesterol biosynthesis defects in HD mouse models and suggests that plasma 24OHC may serve as a biomarker of brain cholesterol metabolism.

1. Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder clinically characterized by cognitive, psychiatric and motor disturbances (Bates et al., 2015). It is caused by a CAG repeat expansion in the huntingtin (htt) gene that encodes for polyglutamine. The number of CAG repeats in htt is inversely correlated with age at symptomatic onset in HD, with variable age-dependent penetrance between 36 and 39 CAG repeats, but full penetrance at 40 or more repeats (Ross et al., 2014).

Since the discovery of the HD mutation, findings from cell and animal models have highlighted mechanisms and pathways altered in HD and have provided insights into HD pathogenesis and progression. However, there is no single model that recapitulates the full spectrum of disease pathophysiology, requiring one to select the best model dependent on the biological question of interest. On the other hand, when specific alterations are consistently observed across different models, and meet the genetic relationship of polymorphic CAG extension *vs* phenotypic onset, then such alterations may have higher relevance and highlight mechanisms more proximal to the HD mutation.

There is a wealth of data showing that brain cholesterol metabolism is affected in HD models (Valenza et al., 2005; Valenza et al., 2007b; Valenza et al., 2007a; Valenza et al., 2010; Valenza et al., 2015a). Analytical approaches based on isotope dilution mass spectrometry, in combination with other biochemical and molecular analyses, have shown that cholesterol precursors are reduced in the brain of five HD rodent models before the onset of motor defects, and cholesterol content is decreased at later time points (Valenza et al., 2007a; Valenza et al., 2010).

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Cholesterol homeostasis may also be affected in HD patients early in the disease as the reduced levels of plasma 24-hydroxycholesterol (24OHC), the brain-specific catabolite of cholesterol, parallels the large decrease in caudate volume in gene-positive subjects from pre-manifest to HD stage (Leoni et al., 2008; Leoni et al., 2013). This suggests that cholesterol dysfunction may be linked to the HD mutation and might be relevant in the disease pathogenesis. First, a quarter of the cholesterol in humans is found in the brain and is produced locally because the blood-brain barrier (BBB) prevents its uptake from circulation (Dietschy and Turley, 2004). Secondly, although the rate of cholesterol synthesis decreases after myelination (Dietschy, 1984; Jurevics and Morell, 1995), the very small amount produced by everyday cholesterol synthesis in adulthood plays a role in neuronal function (Camargo et al., 2009). Third, the half-life of brain cholesterol is estimated to 5 years in humans (Bjorkhem et al., 1998; Saher and Simons, 2010), consistent with a late-onset disease. Fourth, to develop and maintain synapses, neurons have a high demand of cholesterol, which is supplied by astrocytes (Mauch et al., 2001; Valenza et al., 2007b; Nieweg et al., 2009; Valenza et al., 2015a). Finally, first attempts at delivering cholesterol (Valenza et al., 2015b) or at modulating cholesterol catabolism (Boussicault et al., 2016) in the mouse HD brain have been proven be beneficial. However, to date there are no direct measurements of cholesterol biosynthesis in the adult mouse HD brain and evidence of *CAG* length–dependence is missing.

Here we performed a cross-validation study aimed at measuring cholesterol biosynthesis *in vivo* in the adult mouse brain by independent methods. The tissue concentration of the cholesterol precursor, lathosterol, was compared to the fractional synthesis rate of cholesterol, and brain and plasma 24OHC measured by isotope labeling with deuterated water (Lee et al., 1994) the measures of cholesterol synthesis were then compared to the concentration of cholesterol and brain and plasma 24OHC, determined by isotope dilution mass spectrometry (Cohen et al., 1980). Control and heterozygous knock-in mice carrying 175 CAG repeats (herein referred as Q175; (Menalled et al., 2012) as well as mice carrying 20, 80, 111 CAG repeats were included in the study. The Q175 mouse model exhibits progressive and early-onset HD-related alterations, while the shorter repeat genotypes develop symptoms late in life or not at all; these mice enabled us to test whether any of the parameters measured were CAG dependent at different stages of disease. Additionally, we investigated whether the fraction of newly synthesized cholesterol, which is metabolized into 24OHC and can be measured in the blood, faithfully represents newly synthesized cholesterol in the brain, thus making this measurement potentially useful as a clinical biomarker for CNS cholesterol biosynthesis.

2. Material and methods

2.1. Deuterated water labeling and tissue collection in c57bl/6 mice

For deuterated water labeling, 4-months old animals received a priming intraperitoneal (i.p.) bolus of 49 mL/kg 0.9% NaCl in 99.9% ^2H_2O and were maintained on 8% ^2H_2O in drinking water for two weeks until sacrifice. This regimen results in steady state values of about 5% excess ^2H enrichment of body water. At the end of the labeling period, mice were euthanized and blood drawn by cardiac puncture. Samples from 5 mice were pooled and centrifuged to separate plasma and stored at −80 °C. Brain was removed and dissected into striatum, cortex and cerebellum and frozen on dry ice. Samples from 5 mice were pooled by brain region and stored at −80 °C.

2.2. Deuterated water labeling and tissue collection in WT and Q175 mice

Given that homozygosity is very rare in humans, all the analyses were performed in the heterozygous Q175 mice. Striatum, cortex, cerebellum, and blood from all animals have been provided by PsychoGenics (USA). 5 weeks old animals were labeled with ^2H_2O for one week whereas 6-months and 12-months old mice were labeled for two weeks with ^2H_2O as described above. Animals were food deprived for 4 h and perfused with saline prior to tissue collection. The number of hemizygous and age-matched WT littermates used for the experiments were indicated in the figure legends.

2.3. Myelin isolation

Individual brain region from 20 mice (tissue from 5 mice were pooled per sample for n = 4 samples per group) was weighed and homogenized with tissue homogenizer (Fastprep®24-MP Biomedical) in 10 mL of 30% sucrose solution. 10 mL of 10% sucrose solution was layered over 10 mL of brain homogenate in polycarbonate ultracentrifuge tube and centrifuged at 25000RPM for 30 min in SW-17 rotor. The myelin layer at the interface of the two sucrose solutions was collected with a Pasteur pipette and the pellet constituted the 'myelin-depleted fraction'. The procedure was repeated twice to further deplete the pellet of myelin by resolubilizing the pellet with 2.5 mL of 30% sucrose and layering with 1.9 mL of 10% sucrose solution in 4 mL tubes. The tubes were centrifuged at 2500RPM for 30 min in Ti 50.4 and the myelin at the interface was extracted with 2 mL pasture pipette. For further purification of isolated myelin, the combined myelin extract washed with water (1:1, v/v), resuspended in 2.5 mL of 30% sucrose solution and layered with 1.9 mL of 10% sucrose. The tubes were centrifuged at 25000RPM for 30 min in Ti50.4 rotor, the myelin at the interface of the two solutions removed and washed with water. The purified myelin and the myelin-depleted fraction were reconstituted in 500ul of water and stored at −20 °C.

2.4. Brain sterols (lathosterol, desmosterol cholesterol and 24OHC) synthesis rates

Lipid was extracted in 2:1 chloroform methanol from myelin-enriched and myelin-depleted fractions of each brain region. 125 μg of butylated hydroxytoluene (BHT) was added to 75 μL lipid extract and alkaline hydrolysis was performed with 2 mL of NaOH in 90% ethanol for 2 h at 50 °C. The sterols were extracted three times with 4 mL of cyclohexane and evaporated under stream of nitrogen. The dried sample was acetylated for subsequent analysis by GC/MS as described previously (Lee et al., 1994) on an Agilent Technologies GC6890N equipped with DB-17MS column (30 m × 0.25 mm × 0.25 μm) and 5973 N mass detector operated in selected ion-monitoring mode. The selected ions corresponding to M0 & M1 mass isotopomers for cholesterol were *m/z* 368 and 369, for 24OHC were *m/z* 426 and 427, for lathosterol were *m/z* 428 and 429, and for desmosterol were *m/z* 366 and 367. For each analyte, fractional synthesis was calculated as described below.

2.5. Calculation of fractional synthesis

Cholesterol and 24OHC synthesis rate are expressed as fractional synthesis (%) over the duration of label. The fractional synthesis (f) was calculated on the basis of the precursor-product, or rise-to-plateau, approach, $f = EM1/A^{\infty}$, where EM1 represents the mass + 1-labeled species in excess of natural abundance, and A^{∞} represents the theoretical plateau or asymptotic value for fully labeled moiety. The theoretical plateau or asymptotic value A^{∞} during ^2H_2O labeling was determined by mass isotomer distribution analysis (MIDA) of the combinatorial labeling pattern based on precursor 2H–enrichment (Hellerstein and Neese, 1992). When the duration of label was different for different age-groups, data for cholesterol and 24OHC are represented as synthesis rate per day, calculated as $-\ln(1-f)/t$, where (f) is fractional synthesis and (t) the duration in days of label.