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A failure in energy metabolism and antioxidant uptake precede symptoms of Huntington's disease in mice

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Huntington's disease has been associated with a failure in energy metabolism and oxidative damage. Ascorbic acid is a powerful antioxidant highly concentrated in the brain where it acts as a messenger, modulating neuronal metabolism. Using an electrophysiological approach in R6/2 HD slices, we observe an abnormal ascorbic acid flux from astrocytes to neurons, which is responsible for alterations in neuronal metabolic substrate preferences. Here using striatal neurons derived from knock-in mice expressing mutant huntingtin (STHdhQ cells), we study ascorbic acid transport. When extracellular ascorbic acid concentration increases, as occurs during synaptic activity, ascorbic acid transporter 2 (SVCT2) translocates to the plasma membrane, ensuring optimal ascorbic acid uptake for neurons. In contrast, SVCT2 from cells that mimic HD symptoms (dubbed HD cells) fails to reach the plasma membrane under the same conditions. We reason that an early impairment of ascorbic acid uptake in HD neurons could lead to early metabolic failure promoting neuronal death.

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Huntington's disease (HD) is a progressive, autosomal dominant, neurodegenerative disorder[1]. The disease is caused by an expanded polyglutamine (polyQ) stretch in the IT15 gene, resulting in major cell loss in the striatum[2]. The *IT15* gene codes for a protein called huntingtin (Htt). Wild-type (WT) Htt has an important role in the intracellular transport of vesicles, organelles and trafficking of proteins to the cell surface[3,4]. Expansion of a glutamine stretch within the Htt protein to >40 repeats (mHtt) appears to confer a dominant toxic property that is deleterious to neurons and detrimental to normal Htt biological activities.

Defects in energy metabolism have been observed in presymptomatic and symptomatic subjects[5–9]. This deregulation in brain energy metabolism makes it reasonable to suspect the presence of increased amounts of oxidative species. Indeed, oxidative damage is evident, as well as impaired superoxide dismutase (SOD) activity[8] and a decrease in ascorbic acid levels[10,11]. Ascorbic acid is an important antioxidant that is concentrated in the brain[12]. It plays a fundamental role in the modulation of neuronal metabolism (for reviews, refer to the studies by Castro *et al.*[13] and Beltrán *et al.*[14]). During synaptic activity, ascorbic acid is released into the extracellular space[15–18] and is taken up by neuronal cells[19]. This uptake of ascorbic acid occurs via a specific neuronal ascorbic acid transporter, the sodium-dependent vitamin C transporter, SVCT2[20]. Intracellular ascorbic acid is able to sustain an adequate energy supply that induces uptake of monocarboxylates such as pyruvate and lactate[21]. It is also used to maintain redox balance in the cell[12].

R6/2 is a transgenic mouse model that expresses exon 1 protein of human Htt, which contains ~150 CAG repeats. These mice develop a neurological phenotype that mimics the disease characteristics including many of the motor-control deficits that are characteristic of HD[22,23] and show a pronounced and sustained loss of ascorbic acid in the extracellular fluid. This only occurs during periods of behavioural activation. Rather than demonstrating a deficit in brain ascorbic acid, these mice seem unable to maintain adequate levels of ascorbic acid during behavioural activation. Thus, a failure in ascorbic acid uptake may be closely linked with metabolic failure and redox imbalance in HD.

In this study, we demonstrate that a failure in ascorbic acid flux from astrocytes to neuronal cells occurs and that this phenomenon precedes the onset of HD-like symptoms in mice. The failure is accompanied by an alteration in neuronal metabolic energy substrate preferences. We also show a failure in neuronal ascorbic acid uptake in an HD mouse and cellular models. In cells that express mutant Htt (mHtt), Huntingtin-associated protein 1-SVCT2 (HAP1-SVCT2) colocalization is decreased and the neuronal ascorbic acid transporter, SVCT2, fails to reach the plasma membrane. Ascorbic acid is an essential protector against oxidative damage in brain neurons and modulates neuronal metabolism during synaptic activity. Therefore, early failure in ascorbic acid release could lead to metabolic failure and early impairment in the maintenance of synaptic transmission in basal ganglia. Such a failure in ascorbic acid uptake in symptomatic HD subjects could promote neuronal death.

Results

Glial-neuron ascorbic acid flux is impaired in R6/2 mice. Ascorbic acid flux from astrocytes to neurons during synaptic activity is essential to protect neurons against oxidative damage and for modulation of neuronal metabolism, thus permitting optimal ATP production. According to our previous data, ascorbic acid inhibits glucose consumption and stimulates lactate uptake during glutamatergic synaptic activity (ascorbic acid metabolic switch[19,21]). We were interested in determining whether the ascorbic acid metabolic switch functions in R6/2 transgenic mice that carry HD gene, as HD mice have been shown to be unable to maintain adequate levels of ascorbic acid during behavioural activation[22,24] and HD has also been associated with a failure in brain energy metabolism. In our studies of mice striatal slices, we have shown that intracellular ascorbic acid inhibits the ability of glucose to sustain excitatory postsynaptic currents (EPSCs) when neuronal lactate uptake is inhibited (Fig. 1, Table 1). EPSCs were evoked by stimulating the corticostriatal pathway (Fig. 1a). After glucose deprivation, glucose application produced a fast recovery of synaptic responses (Fig. 1b), as much in WT mice as in both presymptomatic and symptomatic R6/2 mice (Fig. 1c, control). In the presence of external 100 µM alpha-cyano-4-hydroxycinnamic acid (4-CIN), an inhibitor of the neuronal monocarboxylate transporter MCT2, glucose was able to restore EPSCs only in presymptomatic (80.0 ± 14.1%) and symptomatic (72.3 ± 16.4%) R6/2 mice (Fig. 1c, 4-CIN). Thus, in WT mice, glucose is not able to sustain neural function when monocarboxylate uptake is inhibited (27.8 ± 12.0% and 44.8 ± 10.6% for EPSC amplitudes after a period of glucose deprivation in presymptomatic and symptomatic WT slices, respectively). In presymptomatic and symptomatic R6/2 mice, 4-CIN did not affect the ability of glucose to sustain EPSCs. Given these findings, our experiments strongly suggest that the ANLS (astrocyte-neuron lactate shuttle) is necessary to maintain adequate ATP levels in healthy cells, a normal mechanism that is impaired in HD neurons. When extracellular ascorbic acid was applied, we observed a strong decrease in the amplitude of the EPSCs after a period of glucose deprivation in presymptomatic R6/2 slices (Fig. 1c, 4-CIN/Asc). In symptomatic R6/2 slices, we saw no significant decrease in the amplitude of EPSCs after glucose deprivation (Fig. 1c, 4-CIN/Asc). So, ANLS would seem to function in presymptomatic R6/2 slices by over-supplementation of extracellular ascorbic acid. This effect was reversed when ascorbic acid uptake was inhibited (by including an anti-SVCT2 antibody in the recording pipette; Fig. 1c, 4-CIN/Asc/anti-SVCT2). This antibody inhibits ascorbic acid uptake by binding to the endofacial side of SVCT2[19]. In conclusion, the ANLS and the ascorbic acid metabolic switch do not function in the striatum of presymptomatic R6/2 mice due to the fact that ascorbic acid release from glial cells seems to be decreased in these mice. The ANLS and ascorbic acid metabolic switch is also impaired in symptomatic R6/2 mice, though in these mice, this dysfunction appears to arise from a failure in ascorbic acid uptake rather than from a decreased release of ascorbic acid from glial cells.

To investigate whether the failure in ascorbic acid uptake may be related to a deficiency in SVCT2 expression, we analysed mRNA and protein levels in samples obtained from mice striata. RT–qPCR analyses showed increased levels of mRNA coding for SVCT2 in symptomatic R6/2 mice, while mRNA levels were not affected in presymptomatic R6/2 mice (Fig. 1d,e). Relative levels of SVCT2 were also increased in symptomatic R6/2 mice as seen by immunofluorescence analysis (Fig. 1f). In presymptomatic R6/2 mice, SVCT2 expression did not seem to be altered (Fig. 1d–f). Thus, before the onset of HD-like symptoms, SVCT2 expression and function should be normal. After the onset of HD-like symptoms, ascorbic acid uptake seems to be impaired, although SVCT2 expression is increased.

Ascorbic acid transport in cellular model of HD is impaired. To visualize the possibility that neuronal ascorbic acid transport is impaired in a cellular model of HD, we studied SVCT2 expression and function in STHdhQ7 and STHdhQ111 cell lines.