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Deficient Rab11 activity underlies glucose hypometabolism in primary neurons of Huntington's disease mice

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ABSTRACT

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a *CAG* repeat expansion in the *huntingtin* gene. Positron emission tomography studies have revealed a decline in glucose metabolism in the brain of patients with HD by a mechanism that has not been established. We examined glucose utilization in embryonic primary cortical neurons of wild-type (WT) and HD knock-in mice, which have 140 *CAG* repeats inserted in the endogenous mouse *huntingtin* gene ($HD^{140Q/140Q}$). Primary $HD^{140Q/140Q}$ cortical neurons took up significantly less glucose than did WT neurons. Expression of permanently inactive and permanently active forms of Rab11 correspondingly altered glucose uptake in WT neurons, suggesting that normal activity of Rab11 is needed for neuronal uptake of glucose. It is known that Rab11 activity is diminished in $HD^{140Q/140Q}$ neurons. Expression of dominant active Rab11 to enhance the activity of Rab11 normalized glucose uptake in $HD^{140Q/140Q}$ neurons. These results suggest that deficient activity of Rab11 is a novel mechanism for glucose hypometabolism in HD.

1. Introduction

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by progressive uncontrolled movements, cognitive impairments, and personality alterations [1]. There is a selective loss of neurons initially in the striatum and cortex, and severe whole brain atrophy as the disease progresses [2,3]. Most persons carrying the HD gene develop symptoms in midlife and die within 20 years of disease onset. To date there is no cure or effective treatment for HD to slow disease progression. The HD mutation causes an elongated tract of polyglutamines (polyQ) near the NH_2-terminus of the protein huntingtin (Htt) [4]. There are multiple views of how polyglutamine expansion in Htt leads to neurodegeneration, including deregulated gene transcription, impaired protein clearance, aberrant vesicular trafficking, oxidative stress and defective energy metabolism [5–11].

Glucose is the primary source of metabolic energy for the brain [12]. Defects associated with glucose utilization can affect virtually all brain activities. Positron emission tomography (PET) scans with ^{18}F-fluorodeoxyglucose (^{18}FDG) reveal a regional loss of glucose utilization in the striatum (caudate and putamen) and cortex (frontal and temporal lobes) of HD patients [13–18]. The decrease in glucose utilization in HD is seen in the absence of visible symptoms and precedes bulk loss of brain tissue. However, it remains undetermined whether and how deficient glucose utilization occurs in HD neurons.

In this study, we addressed if glucose utilization is impaired in HD neurons. We examined glucose uptake in neurons cultured from embryonic cortex of mice bearing 140 CAG repeats in the endogenous mouse Htt gene (HD^{140Q} knock-in mice). These mice express mutant Htt at the same levels as WT Htt, and therefore provide a model that best replicates the situation in human disease [19]. We found that primary $HD^{140Q/140Q}$ cortical neurons took up less glucose than did WT cortical neurons. Expression of constitutively active Rab11 restored the capacity of $HD^{140Q/140Q}$ neurons to intake glucose. Our study for the first time provides direct experimental evidence to show that HD neurons are defective in utilizing glucose.

2. Material and methods

2.1. Preparation and culture of primary cortical neurons

Primary mouse cortical neurons were isolated and cultured as described previously. All reagents used for preparation and culturing of primary neurons were obtained from Invitrogen, except where indicated. As previously described [20], 16–18 day embryos were collected from pregnant mice (WT and $HD^{140Q/140Q}$) and the brains were removed. Cortices were dissected, pooled, and incubated in PBS containing penicillin, streptomycin, neomycin and 0.25% trypsin for 10 min at 37 °C. Cells released from cortices upon

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digestion with trypsin were washed twice in PBS supplemented with calcium and magnesium, and resuspended in Neurobasal DMEM medium containing B27 supplement, N2 supplement, 25 mM mercaptoethanol and L-glutamine (NBM), seeded in dishes (BD Biosciences) with or without glass coverslips (Warner Instruments) and cultured in a 37 °C cell culture incubator. Culture dishes and glass coverslips were coated with poly-L-lysine. Twenty-four to 48 h after seeding, the cultures were treated with cytosine arabinoside (AraC) for 24 h to inhibit proliferation of glial cells. Then, fresh NBM media was added to the cultures. Upon AraC treatment, more than 99.5% of cells in the cultures were neurons based on our immunohistochemical analysis of the primary cultures using antibodies against GFAP, CD68, and β-III-tubulin which identify astrocytes, microglia, and neurons, respectively. Neuronal enriched cultures at DIV8 were used for studies.

2.2. Construction of Rab11 mutant lentiviral plasmids and packaging of lentivirus

The DNA fragment encoding HA-tagged Rab11Q70L or Rab11S25N was obtained by digestion of pcNDA3-HA/Rab11Q70L or pcDNA3-HA/Rab11S25N with Nhe I and Xho I and ligated into the Nhe I/Xho I sites of CSCW2-pgk-IRES-eGFP lentiviral vector. The constructs were confirmed by DNA sequencing. Viral packaging was carried out as previously described [21]. Titers of virus were determined by infecting 5×10^5 of 293T cells with 0.1, 1 and 10 μl of virus. All cells were positive for eGFP when infected with 10 μl of virus, whereas when infected with 0.1 μl of virus very few cells had eGFP signal. Therefore cells infected with 1 μl of virus were used for determining titers of virus. The numbers of eGFP positive and negative cells in three randomly selected imaging fields were counted with immunofluorescence microcopy. The mean percentage of eGFP positive cells was determined to be 5.3×10^7 pfu/ml for lenti-Rab11S25N/eGFP and 6.2×10^7 pfu/ml for lenti-Rab11Q70L/eGFP, respectively. This titer yielded over 90% infection of neurons in primary cultures.

2.3. Glucose uptake

Cells from both WT and $HD^{140Q/140Q}$ mouse embryonic cortex were cultured in six-well plates for 24–48 h and then treated with AraC for 24 h. After a change into fresh media, cells were either routinely cultured or infected with virus expressing Rab11 mutants. The efficiency of viral infection was examined by visualizing the signals of eGFP in neurons with immunofluorescence microscopy. Cells with more than 90% efficiency of infection were used for experiments. Primary neurons at DIV8 were washed twice in pre-warmed PBS, cultured in PBS for 1 h at 37 °C and incubated with 2 μl of [^{3}H]-deoxyglucose (1.66–2.22Gbq/mmol, PerkinElmer) at room temperature for 20 min in PBS containing 1% BSA. After glucose uptake was stopped, cells were lysed in 0.5 ml lysis buffer containing 1% Triton X-100 and protease inhibitors. Radioactivity in 50 μl of cell lysates was measured with liquid scintillation counting. Concentration of proteins in cell lysates was determined using the Bio-Rad protein assay kit.

3. Results

3.1. Reduced glucose uptake in HD neurons

We investigated if neurons from HD mice are impaired in taking up glucose, a necessary step for neurons to utilize glucose to generate bioenergy. Neurons were isolated and cultured from embryonic cortex of 140 *CAG* repeats knock-in mice (HD^{140Q} mice). These mice have human exon 1 with 140 *CAG*s inserted into the endogenous mouse Htt gene. Mutant Htt is expressed at the same level as the endogenous mouse Htt. We used embryonic $HD^{140Q/140Q}$ neurons cultured for 8 days in vitro (DIV8) since previously we found HD neurons at this stage were deficient compared to WT neurons in uptake of cysteine by the EAAC1 transporter. At DIV8 $HD^{140Q/140Q}$ neurons took up significantly less glucose than did WT neurons ($n = 3$, Mean ± SD of cpm per mg of proteins, HD *vs.* WT: 324.53 ± 19.75 *vs.* 519.81 ± 18.92; two-tailed Student *t*-test: $p < 0001$; Fig. 1), indicating that glucose utilization is reduced in HD neurons.

3.2. Rab11 modulates glucose uptake in neurons

Rab11 is known to regulate uptake of nutrients, for example iron (in complex with transferrin) and cysteine [20,22]. To examine if Rab11 activity modulates glucose uptake in neurons, we expressed dominant negative and dominant active mutants of Rab11 in primary neurons as these mutant forms of Rab11 are widely used tools for modulating the function of Rab11. WT neurons at DIV8 were infected with virus expressing Rab11S25N (dominant negative) or virus expressing Rab11Q70L (dominant active) or left uninfected. We measured glucose uptake two days after viral infection. Compared with no infection of virus, infection of virus delivering Rab11S25N markedly reduced glucose uptake in neurons, whereas expression of virus delivering Rab11Q70L enhanced neuronal uptake of glucose ($n = 3$, Mean ± SD percentage of No-virus, two-tailed Student *t*-test: **No-virus** *vs.* **Rab11S25N**, 100 ± 2.43 *vs.* 48.79 ± 2.64, $p < 0.0001$; **No-virus** *vs.* **Rab11Q70L**, 100 ± 2.43 *vs.* 114.1 ± 3.84, $p < 0.01$; Fig. 2). These data indicate that normal function of Rab11 is needed for glucose uptake in neurons.

3.3. Glucose uptake in HD neurons is improved by reinforcing Rab11 function

Having shown above the requirement of Rab11 activity for glucose uptake in neurons and in previous studies insufficient activity of Rab11 in HD [20,21,23], we speculated that expression of Rab11Q70L in $HD^{140Q/140Q}$ neurons might improve glucose uptake. Primary WT and $HD^{140Q/140Q}$ cortical neurons at DIV3 were infected with lentivirus expressing enhanced green fluorescent protein (eGFP) alone or a combination of Rab11Q70L and eGFP driven by a bicistronic promoter. The efficiency of viral infection in the neurons was more than 90% according to the presence of eGFP signal detected with immunofluorescence microscopy. Five

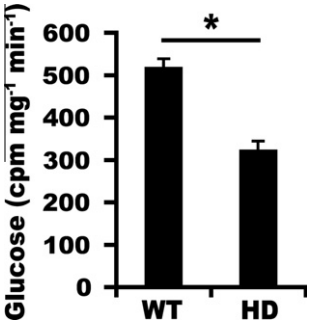

Fig. 1. Glucose uptake in primary cortical neurons. Neuronal enriched cultures were prepared from embryonic cortex of WT and $HD^{140Q/140Q}$ pregnant mice as described in Section 2. At DIV8, primary embryonic cortical neurons in six-well plates were used for conducting uptake of [^{3}H]-deoxyglucose and harvested for preparing cell lysates. The amount of [^{3}H]-deoxyglucose in cell lysates from each of three wells was determined by liquid scintillation count. Mean cpm per mg of proteins per minute with standard deviation were calculated and graphed (two-tailed Student *t*-test: $^*p < 0.001$).

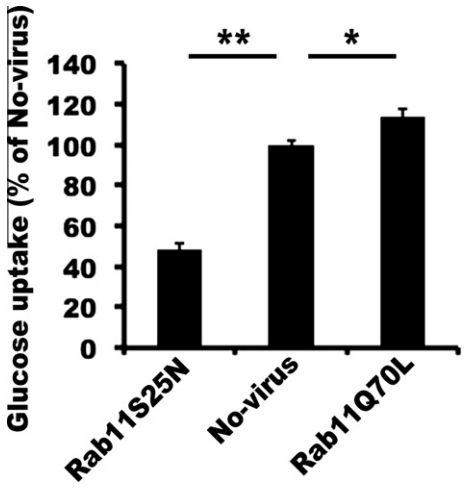

Fig. 2. Requirement of Rab11 function in neuronal glucose uptake. At DIV8, WT neurons in 6-well plates were infected with lentivirus expressing Rab11S25N, or virus expressing Rab11Q70L, or uninfected. Each treatment was conducted using 3 wells of neurons. Uptake of [^{3}H]-deoxyglucose was performed 2 days after viral infection. Equal volume of lysates from each well was used for scintillation counting. The count in lysates from each well of neurons infected with virus was used for calculating the percentage of [^{3}H]-deoxyglucose uptake relative to the mean count in lysates from three wells of neurons with no virus infection. [^{3}H]-Deoxyglucose uptake in neurons with no virus infection was set as 100%. The mean percentage with standard deviation was graphed (two-tailed Student *t*-test: $^*p < 0.01$; $^{**}p < 0.00001$).

days after viral infection (DIV8), primary neuronal cultures were evaluated for glucose uptake. Compared with expression of eGFP alone, bicistronic expression of Rab11Q70L and eGFP significantly increased glucose uptake in both HD$^{140Q/140Q}$ and WT neurons ($n = 3$, Mean ± SD of cpm per mg of protein, two-tailed Student *t*-test, **WT**: eGFP *vs.* Rab11Q70L, 492.52 ± 19.87 *vs.* 574.05 ± 21.21, $p < 0.05$; **HD**: eGFP *vs.* Rab11Q70L, 293.79 ± 18.98 *vs.* 499.96 ± 19.32, $p < 0.001$; Fig. 3). Levels of glucose uptake in HD$^{140Q/140Q}$ neurons expressing Rab11Q70L were not significantly different from levels of glucose uptake in WT neurons expressing eGFP alone (HD-Rab11Q70L *vs.* WT-eGFP, 492.52 ± 19.87 *vs.* 499.96 ± 19.32, two-tailed Student *t*-test: $p = 0.666$; Fig. 3). These data show that

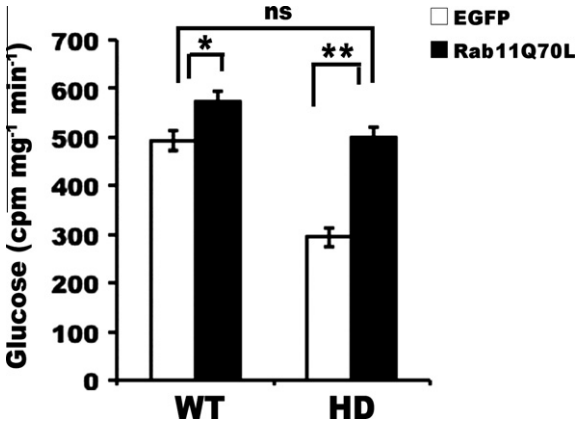

Fig. 3. Expression of Rab11Q70L improves glucose uptake in HD neurons. At DIV3, primary WT and HD$^{140Q/140Q}$ neurons in 6-well plates were infected with lentivirus expressing Rab11Q70L or lentivirus expressing eGFP alone. Viral infection efficiency was determined by observation of eGFP signal in neurons with immunofluorescence microscopy. Five days after viral infection, neurons were used for performing [^{3}H]-deoxyglucose uptake. The amount of [^{3}H]-deoxyglucose in lysates from each of three wells of neurons was measured by liquid scintillation counting. Mean cpm per mg of protein per minute with standard deviation was graphed (two-tailed Student *t*-test: ns, not significant; $^*p < 0.05$; $^{**}p < 0.001$).

enhancement of Rab11 activity is able to normalize uptake of glucose in HD neurons.

4. Discussion

Energy metabolism is reduced in HD and may arise from multiple factors including deficient utilization of glucose in the brain. A mechanism for impaired glucose utilization in HD is unknown. Studies in immortalized striatal cell lines from HD knock-in mice (Q111) have shown reduced ATP/ADP ratio suggestive of an energy deficiency [24]. Interestingly, the source of the energy deficiency was thought to involve an extra-mitochondrial pathway [25]. In this study we show that neurons cultured from embryonic cortex of HD$^{140Q/140Q}$ mice were defective in taking up glucose. The defect in glucose uptake was attenuated upon enhancement of Rab11 activity.

We found that glucose uptake in neurons was modulated by Rab11, a GTPase that governs numerous endocytosed proteins recycling back to the cell surface for reuse [22,26]. Glucose is a polar molecule and its entry into cells requires a transporter. The family of facilitative glucose transporter (Glut) proteins is considered to mediate most of glucose uptake in cells [27]. Several Glut isoforms, specifically Glut-2, Glut-3, Glut-4 and Glut-8, are detected in neurons [27]. Characterization of the Glut isoform that is regulated by Rab11 in neurons is worthy of investigation in future studies.

In summary, we elucidate a novel mechanism for glucose hypometabolism in HD involving diminished activity of Rab11. This study strengthens the idea that strategies to elevate Rab11 activity may be beneficial in treating HD.

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