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Normalizing glucocorticoid levels attenuates metabolic and neuropathological symptoms in the R6/2 mouse model of huntington's disease

Brett D. Dufour[a,b], Jodi L. McBride[a,b,*]

[a] Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, United States
[b] Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, United States

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ABSTRACT

Huntington's disease (HD) is a fatal genetic neurological disorder caused by a mutation in the human Huntingtin (*HTT*) gene. This mutation confers a toxic gain of function of the encoded mutant huntingtin (mHTT) protein, leading to widespread neuropathology including the formation of mHTT-positive inclusion bodies, gene dysregulation, reduced levels of adult dentate gyrus neurogenesis and neuron loss throughout many regions of the brain. Additionally, because HTT is ubiquitously expressed, several peripheral tissues are also affected. HD patients suffer from progressive motor, cognitive, psychiatric, and metabolic symptoms, including weight loss and skeletal muscle wasting. HD patients also show neuroendocrine changes including a robust, significant elevation in circulating levels of the glucocorticoid, cortisol. Previously, we confirmed that the R6/2 mouse model of HD exhibits elevated corticosterone (the rodent homolog of cortisol) levels and demonstrated that experimentally elevated corticosterone exacerbates R6/2 HD symptomology, resulting in severe and rapid weight loss and a shorter latency to death. Given that efficacious therapeutics are lacking for HD, here we investigated whether normalizing glucocorticoid levels could serve as a viable therapeutic approach for this disease. We tested the hypothesis that normalizing glucocorticoids to wild-type levels would ameliorate HD symptomology. Wild-type (WT) and transgenic R6/2 mice were allocated to three treatment groups: 1) adrenalectomy with normalized, WT-level corticosterone replacement (10 μg/ml), 2) adrenalectomy with high HD-level corticosterone replacement (35 μg/ml), or 3) sham surgery with no corticosterone replacement. Normalizing corticosterone to WT levels led to an improvement in metabolic rate in male R6/2 mice, as indicated by indirect calorimetry, including a reduction in oxygen consumption and normalization of respiratory exchange ratio values ($p < .05$ for both). Normalizing corticosterone also ameliorated brain atrophy in female R6/2 mice and skeletal muscle wasting in both male and female R6/2 mice ($p < .05$ for all). Female R6/2 mice given WT-level corticosterone replacement also showed a reduction in HD neuropathological markers, including a reduction in mHTT inclusion burden in the striatum, cortex, and hippocampus ($p < .05$ for all). This data illustrates that ameliorating glucocorticoid dysregulation leads to a significant improvement in HD symptomology in the R6/2 mouse model and suggests that cortisol-reducing therapeutics may be of value in improving HD patient quality of life.

1. Introduction

Huntington's disease (HD) is a fatal genetic neurodegenerative disorder caused by a CAG triplet repeat expansion in the human huntingtin (*HTT*) gene (Huntington Disease Collaborative Research Group, 1993). If sufficiently long (40+ repeats), this mutation confers a toxic gain of function on the encoded mutant huntingtin protein (mHTT), leading to widespread neuropathology, including robust transcriptional abnormalities, the formation of mHTT+ inclusion bodies, regional reductions in brain volume, altered neurogenesis and neuroinflammation (astrogliosis and microgliosis) (Hodges et al., 2006; Huntington Disease Collaborative Research Group, 1993; Ross et al., 2014; Rub et al., 2015; Sapp et al., 2001; Vonsattel et al., 1985). The hallmark symptom of the disease is chorea, whereby patients show uncontrollable hyperkinetic

* Corresponding author at: Oregon Health & Science University, Oregon National Primate Research Center, 505 NW 185th Avenue, Beaverton 97006, OR, United States.
E-mail address: mcbridej@ohsu.edu (J.L. McBride).

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movements that become progressively more severe (Huntington Study Group, 1996; Ross et al., 2014). However, although HD is typically conceptualized as a movement disorder, patients also suffer from severe cognitive, psychiatric, and metabolic symptoms (Anderson and Marder, 2001; Bamford et al., 1995; Kirkwood et al., 2001; Ross et al., 2014; Ross and Tabrizi, 2011; van der Burg et al., 2009). Metabolic symptoms in HD include weight loss, muscle wasting and insulin resistance (Aziz et al., 2008; van der Burg et al., 2009; van der Burg et al., 2011). Calorimetry data demonstrates that patients are in a chronic catabolic state, and thus in negative energy balance (Goodman et al., 2008). Accordingly, at end stage of disease, patients are often severely cachexic (Djousse et al., 2002; Kosinski et al., 2007). The disease typically onsets in the 3rd to 4th decade of life, progresses over 15–20 years, and always leads to death (Ross et al., 2014).

Glucocorticoids, which are chronically elevated in Huntington's disease (HD), have long been suspected as a contributor to the metabolic phenotype shown by patients and in HD rodent models (Aziz et al., 2009; Bjorkqvist et al., 2006a; Goodman et al., 2008). This suspicion is logical, given that chronically elevated glucocorticoids in otherwise healthy individuals leads to widespread metabolic dysfunction, including muscle wasting (Braun et al., 2013; Grossberg et al., 2010; Lee et al., 2005), altered fat and protein metabolism (Lofberg et al., 2002), insulin resistance (Black et al., 1982), and osteoporosis (Moutsatsou et al., 2012; Toth and Grossman, 2013), all of which are symptoms of HD. There is also evidence that elevated glucocorticoids may potentially be exacerbating HD neuropathology, as chronically elevated cortisol in otherwise healthy individuals (i.e. Cushing's syndrome) is associated with reductions in regional and whole brain volume (Andela et al., 2013; Bourdeau et al., 2002) and reduced adult neurogenesis in the dentate gyrus (Brummelte and Galea, 2010; Cameron and Gould, 1994). Cell culture studies have demonstrated that glucocorticoids are also a potent modulator of mHTT inclusion formation (Diamond et al., 2000). Furthermore, elevated glucocorticoids are also known to exacerbate neuropathology in other neurodegenerative disease models. For example, in Alzheimer's disease rodent models, elevated glucocorticoids cause an increase in the accumulation of both amyloid beta plaques and hyperphosphorylated tau (Green et al., 2006), and blocking glucocorticoid receptors results in a reduction in both (Baglietto-Vargas et al., 2013).

However, while these findings provide abundant circumstantial evidence for a role for glucocorticoids in HD metabolic symptoms and neuropathology, there has been little direct evidence to support this speculation. Of the existing HD mouse models, a spontaneous elevation in corticosterone, the rodent homolog of cortisol, has only been described in R6/2 mice (Bjorkqvist et al., 2006a; Dufour and McBride, 2016). R6/2 transgenic mice show robust metabolic symptomology including severe and progressive weight-loss, muscle wasting, and insulin resistance (Bjorkqvist et al., 2005; Luthi-Carter et al., 2002; van der Burg et al., 2009). R6/2 mice are also in negative energy balance (Goodman et al., 2008), with indirect calorimetry measures showing that this may be driven by a chronic increase in metabolic rate, indicated by an increase in oxygen consumption (Bjorkqvist et al., 2006b; Goodman et al., 2008). While elevated corticosterone levels and metabolic symptomology co-occur in R6/2 mice, it remains unclear whether these metabolic symptoms are directly affected or even mediated by this spontaneous elevation in glucocorticoid levels, or whether it is driven by the widespread deleterious consequences of mHTT toxicity alone. Thus, this speculative relationship between corticosterone and metabolic symptoms in R6/2 mice has remained largely correlational. The role of glucocorticoid dysregulation has been more directly investigated in R6/1 mice, another transgenic HD line. Although R6/1 mice have not been shown to have a spontaneous elevation in corticosterone, experimental elevations in corticosterone, as well as experimenter induced stress, worsen HD cognitive symptoms and reduce hippocampal neurogenesis (Du et al., 2012; Grote et al., 2005; Lazic et al., 2004; Mo et al., 2014a; Mo et al., 2014b; Mo et al., 2014c). Surprisingly, experimentally elevated corticosterone only caused a mild and transient weight-loss in male R6/1 mice (Mo et al., 2014a).

We recently demonstrated that experimental elevation of corticosterone in adrenalectomized R6/2 mice dramatically exacerbates weight loss and shortens the latency to death (Dufour and McBride, 2016). While this provides direct evidence that an elevation in glucocorticoids can exacerbate HD symptomology, it remains unclear whether normalizing glucocorticoids to wild-type (WT) levels in HD mice would conversely attenuate HD symptomology. Given that there are no current FDA-approved medications that can slow the progression of HD symptoms, we investigated whether glucocorticoid dysregulation in HD could serve as a novel point of therapeutic intervention to alleviate HD symptomology. Thus, here, we tested the hypothesis that normalizing corticosterone to wild-type levels in R6/2 mice would attenuate the metabolic and neuropathological symptomology germane to this model as well as seen in patients suffering from HD.

2. Materials and methods

2.1. Animals

All animals were group housed with littermates (3–5 mice per cage) under controlled conditions of temperature and light (12 h light/dark cycle). Food (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO) and water were provided ad libitum. R6/2 mice (stock # 002810, carrying 160 ± 5 cag repeats) were obtained from Jackson Laboratories (Bar Harbor, ME) and bred in the vivarium at the Oregon National Primate Research Center (ONPRC). Wild-type males were mated with ovary transplanted wild-type females. Transgenic mice were genotyped using primers specific for the mutant human HTT transgene (forward primer 5' TCATCAGCTTTTCCAGGGTCGCCAT and reverse primer 5' CGCAGG CTAGGGCTGTCAATCATGCT), and age-matched wild-type littermates were used for the indicated experiments. Average CAG repeat length for the colony was assessed from a subset of animals by sequencing, with mice from the colony showing an average of 165 ± 5 (SD) CAG repeats (Laragen Sequencing and Genotyping). Body weights of all animals were recorded weekly. All experimental procedures were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals, and were approved by the Oregon Health and Science University (OHSU) and ONPRC Institutional Animal Care and Use Committee.

2.2. Adrenalectomy surgery

Mice were anesthetized with 3% isoflurane. A 2 cm × 2 cm square was shaved on their lower dorsal surface and an incision was made in the skin at the animal's dorsal midline (this opening was used for the removal of both adrenal glands). Next, a small (3-5 mm) incision in the muscular wall was made, directly above the kidney. Small tweezers were used to grasp and gently remove the adrenal gland. This procedure was repeated for the animal's contralateral side (bilateral adrenalectomy). The muscle incisions were closed using absorbable suture and the dorsal skin was closed with wound clips.

2.3. Corticosterone replacement

Corticosterone replacement was provided in the animals' drinking water, which was available ad-libitum. Corticosterone (Sigma, cat. #27840) was dissolved in a small volume of ethanol (0.6%), and then added to dH_20. Since the aldosterone producing cells of the adrenal gland are lost to adrenalectomy, NaCl (0.9%) was also added to replace salt loss. Sucrose (2.0%) was also added to increase palatability of the solution. A dose of 10 μg/ml corticosterone was used for the physiological/WT level replacement group (therapeutic group) and 35 μg/ml of corticosterone was used for the high/HD level replacement group. Vehicle alone (2% sucrose, 0.9% NaCl, and 0.6% ethanol in dH_20) was