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Regular Article

The influence of the HPG axis on stress response and depressive-like behaviour in a transgenic mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disease caused by a CAG tandem repeat mutation encoding a polyglutamine tract expansion in the huntingtin protein. Depression is among the most common affective symptoms in HD but the pathophysiology is unclear. We have previously discovered sexually dimorphic depressive-like behaviours in the R6/1 transgenic mouse model of HD at a pre-motor symptomatic age. Interestingly, only female R6/1 mice display this phenotype. Sexual dimorphism has not been explored in the human HD population despite the well-established knowledge that the clinical depression rate in females is almost twice that of males. Female susceptibility suggests a role of sex hormones, which have been shown to modulate stress response. There is evidence suggesting that the gonads are adversely affected in HD patients, which could alter sex hormone levels. The present study examined the role sex hormones play on stress response in the R6/1 mouse model of HD, in particular, its modulatory effect on the hypothalamic–pituitary–adrenal (HPA) axis and depression-like behaviour. We found that the gonads of female R6/1 mice show atrophy at an early age. Expression levels of gonadotropin-releasing hormone (GnRH) were decreased in the hypothalamus of female HD mice, relative to wild-type female littermates, as were serum testosterone levels. Female serum estradiol levels were not significantly changed. Gonadectomy surgery reduced HPA-axis activity in female mice but had no effect on behavioural phenotypes. Furthermore, expression of the oestrogen receptor (ER) α gene was found to be higher in the adrenal cells of female HD mice. Finally, administration of an ERβ agonist diarylpropionitrile (DPN) rescued depressive-like behaviour in the female HD mice. Our findings provide new insight into the pathogenesis of sexually dimorphic neuroendocrine, physiological and behavioural endophenotypes in HD, and suggest a new avenue for therapeutic intervention.

Introduction

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an autosomal dominant genetic mutation in the *huntingtin* gene (The Huntington's Disease Collaborative Research Group, 1993). Depression is an early-onset symptom (Duff et al., 2007; Kirkwood et al., 2001) and has estimated prevalence of approximately 50% (Gargiulo et al., 2009; Paulsen et al., 2001, 2005; Shiwach, 1994). Despite this, aetiology of depression in HD is not well understood.

The hypothalamic–pituitary–adrenal (HPA) axis is the endocrine system responsible for stress adaptation via the production of glucocorticoids. Its hyperactivity is the most consistently found biological abnormality in clinical depression (Lok et al., 2012; Pariante and Lightman, 2008; Stetler and Miller, 2011). In HD patients, hyperactivity of the HPA-axis has also been observed (Aziz et al., 2009; Bjorkqvist et al., 2006), although analysis of this phenotype is sparse. Abnormally increased HPA-axis function has also been observed in mouse models of HD (Bjorkqvist et al., 2006; Du et al., 2012). Prolonged exposure to elevated glucocorticoid has been found to be damaging to the brains of humans (Colla et al., 2007; Crochemore et al., 2005), rodents (Sapolsky et al., 1988) and monkeys (Sapolsky, 1990). This shared pathophysiology suggests that HPA-axis dysregulation may be a convergent mechanism contributing to depression in these two diseases.

In the general population, it has long been noted that females are twice as likely as males to develop clinical depression (Breslau et al., 1995; Kendler et al., 1993) as well as generalised anxiety disorder, which is often co-morbid with depression (Hoyer et al., 2001). The female sex hormone, especially the most potent oestrogen, 17β-estradiol (E2) has received much attention for its ability to influence stress adaptation (Young and Altemus, 2004). Abnormal decrease in oestrogen levels is linked to depression and altered regulation of the HPA-axis in females. Depressed women have been found to have reduced E2 (Schuschke et al., 2000). The hypothalamic–pituitary–gonadal (HPG) axis regulates the development and function of the gonads and in depressed patients this axis was found to be diminished (Baischer et al.,

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1995; Meller et al., 2001). Correspondingly, depression rates increase in women who are undergoing menopause (Freeman, 2010; Harsh et al., 2009). Supplementing E2 has had some success in treating post-partum (Dennis et al., 2008; Rasgon et al., 2007) and post-menopausal depression (Baksu et al., 2009; Studd and Panay, 2009). In rodent models, OVX surgery resulted in a heightened sensitivity to stress-induced depression-like behaviour as measured by the forced-swim test (FST) (Bekku and Yoshimura, 2005; Bekku et al., 2006; Nakagawasai et al., 2009). E2 supplementation was found to be anxiolytic in the FST and the tail-suspension test (Dhir and Kulkarni, 2008). These behavioural differences correlated with changes in HPA-axis regulation: aged rats with diminished E2 exhibited increased corticosterone response to ether stress and ACTH challenge whilst chronic treatment with E2 corrected the phenotype (Ferrini et al., 1999; Lo et al., 2006). Interestingly, the HPG-axis is also curtailed in HD patients (Markianos et al., 2005, 2007; Van Raamsdonk et al., 2007b) as well as in rodent models of HD (Hannan and Ransome, 2012; Papalexi et al., 2005; Van Raamsdonk et al., 2007b). Whilst there has only been a few studies examining the role sex hormones in HD, this evidence suggests that changes to E2 levels, subsequently leading to heightened HPA-axis activity, could contribute to depression in HD.

Despite the extremely high prevalence of depression in the HD population, only very recently have studies examined whether sex differences exist. Investigating antidepressant usage in prodromal HD patients, Rowe and colleagues found that more female HD patients were prescribed antidepressants than males (Rowe et al., 2012), an indicator of higher rates of depression. This finding is further endorsed by another study which found that female HD patients had significantly higher rates of past and current depression than male patients (Zielonka et al., 2012). But whether sex hormones are involved is yet to be determined. The only clinical study looking at sex hormones in female HD patients measured serum testosterone levels but conspicuously not E2 levels (Markianos et al., 2007).

We have previously described a female-specific depression-related behavioural phenotype in the R6/1 transgenic mouse model of HD (Pang et al., 2009; Renoir et al., 2011, 2012), which also displayed HPA-axis hyperactivity (Du et al., 2012). Here, we investigated whether the sex-dimorphism of the phenotypes is due to modulatory effects of sex hormones. To our knowledge, this is the first study to directly examine the role of sex hormones in the context of depression in a model of Huntington's disease. We hypothesised that the HPG-axis would be dysfunctional in female R6/1 mice and that this would result in altered sex-hormone regulation of the HPA-axis, leading to its hyperactivity and subsequently depression-like behavioural phenotypes.

Methods

Animals

R6/1 transgenic mice and wild-type (WT) littermates (female only) were bred from a colony maintained at the Florey Institute of Neuroscience and Mental Health. Genotype was determined by polymerase chain reaction (PCR) with genomic DNA from tail biopsies. CAG repeat length was sequenced for all studs used for breeding purposes using the Roche FastStart PCR Master (Roche Applied Science, Indianapolis, USA) and then repeat numbers were determined by the University of Melbourne Pathology. Animals were randomly weaned into groups of 4–5 per cage (15 × 30 × 12 cm), other than ensuring that a mix of genotypes was present in each cage. All experiments were conducted at either 8 or 12 weeks of age. All mice were housed together in a room with 12 hour light/dark cycle and had access to food and water ad libitum. All experiments were approved by the Florey Institute Animal Ethics Committee in accordance to the guidelines of the National Health and Medical Research Council (NHMRC) Australia.

Gonadal weight measurements

Mice were killed via cervical dislocation. Ovaries were dissected out, surrounding fat removed and immediately weighed. Weight reported for each individual mouse is the total weight of the two ovaries in grammes. Ovary weights were measured at 8 and 12 weeks of age.

Serum estradiol measure

Serum E2 levels of 12-week-old mice were measured. The phase of the estrus cycle for each mouse was determined by using the Diff Quick staining reagent (Thermo Fisher Scientific, Scoresby, VIC, Australia) according to the manufacturer's instructions. Samples were collected in the proestrus phase, when E2 levels are at their peak. Mice were anaesthetised using isoflurane (4% initially then 2% to maintain anaesthesia). Blood was collected via cardiac puncture. Blood samples were put into 1.5 ml Eppendorf tubes and left at room temperature for 30 min to allow coagulation. They were then centrifuged at room temperature (1090 rcf for 15 min) and serum was collected and frozen immediately at −20 °C. Samples were outsourced to Monash Institute of Medical Research (Clayton, Vic, Australia), Endocrinology and Immunophysiology Laboratory and analysed via radioimmunoassay.

Serum testosterone measure

Serum testosterone concentrations were quantified in 12-week-old mice using enzyme-linked immune sorbent assay (EIA) (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions.

DEX–ACTH challenges

For the DEX–ACTH challenge, 12-week-old mice were given i.p. administration of dexamethasone (DEX) (0.1 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA) between 0800 and 1000 H. Six hours after DEX administration, mice received ACTH (i.p., 500 μg/kg body weight; ProSpec, Rehovot, Israel). Thirty minutes post-ACTH injection, mice were killed and trunk blood was collected for corticosterone analysis.

Quantification of corticosterone

Serum corticosterone concentrations were quantified using EIA (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

OVX

Mice were subjected to OVX surgeries at 8 weeks of age to allow time for healing and for the ovary-derived endogenous E2 to be cleared when experiments were carried out at 12 weeks of age. Mice were anaesthetised with isoflurane (4% initially, then 2% to maintain anaesthesia). Two incisions were made on the dorsal side above the location of the ovaries. Ovaries were removed and incisions were sutured. Betadine ointment was used on the incisions and a single injection of meloxicam (1–3 mg/kg) was given. Mice were placed under a heat lamp individually until fully awake and moving freely. Mice were then re-housed together with original cage-mates and were given soy-free diet (SF06-053, Specialty Feeds, Glen Forrest, WA, Australia) for the duration of their housing. Sham surgeries were identical to OVX surgeries except that the ovaries were left intact.

Locomotor activity

Locomotor activity was tested during the light phase, mostly in the morning. Mice were acclimatised to the experimental room for 1 h