Pharmacology, Biochemistry and Behavior 127 (2014) 15–20

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Sexually dimorphic dopaminergic dysfunction in a transgenic mouse model of Huntington's disease

Thibault Renoir [a,*], Andrew Argyropoulos [a,b], Caroline Chevarin [c,d], Laurence Lanfumey [c,d], Anthony J. Hannan [a,b]

[a] Florey Institute of Neuroscience and Mental Health, Melbourne Brain Centre, University of Melbourne, Parkville, Australia
[b] Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Australia
[c] Inserm UMR S894, F-75013 Paris, France
[d] UPMC, University of Paris 06, UMR S894, F-75013 Paris, France

ARTICLE INFO

Article history:
Received 16 June 2014
Received in revised form 2 October 2014
Accepted 6 October 2014
Available online 12 October 2014

Keywords:
Huntington's disease
Locomotor activity
Dopamine
Sex differences
Bupropion
Tandem repeat disorder

ABSTRACT

Background: Using the R6/1 transgenic mouse model of Huntington's disease (HD), we have recently shown that acute administration with the dopamine–norepinephrine reuptake inhibitor bupropion was able to rescue depressive-like behaviours in female HD mice at 12 weeks of age.
Objective: In this present study, we aimed to further investigate the dopamine system as well as specifically measure dopamine transporter (DAT) and D1 receptor function in female versus male R6/1 HD mice at a very early stage of the disease.
Methods: We assessed the effects of acute administration of bupropion and the dopamine D1 receptor agonist SKF-8129 on spontaneous locomotor activity in 8-week-old HD and wild-type (WT) mice. We also measured dopamine levels in striatum via high performance liquid chromatography (HPLC).
Results: We found that female (but not male) HD mice were hyposensitive to bupropion when compared to WT littermates. However, both female and male HD mice were less sensitive to SKF-81297 locomotor effects. We also found that striatal dopamine levels and dopamine turnover were reduced in HD animals, regardless of sex.
Conclusion: Our present findings suggest that whereas only female HD mice exhibit an impaired response to bupropion, dopamine D1 receptor function is altered in both female and male HD animals. These data are the first in vivo evidence of impaired dopamine D1 receptor-dependent function in pre-motor symptomatic HD mice suggesting that this is a candidate target for early therapeutic interventions.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an abnormal expansion of CAG repeats in exon 1 of the *huntingtin* gene (The Huntington's Disease Collaborative Research Group, 1993). HD encompasses a triad of psychiatric, cognitive and motor dysfunctions. The majority of HD mutation carriers experience some psychopathology during their lifetime (van Duijn et al., 2014), including mood disturbances such as depression, anxiety and irritability, which often start before the onset of motor symptoms (Pla et al., 2014). We have previously discovered that expression of the mutant *huntingtin* gene was sufficient to cause depression-like behaviours in an animal model of HD, R6/1 transgenic mice (Grote et al., 2005; Pang et al., 2009; Renoir et al., 2012). Another interesting finding was that female – and not male – R6/1 HD mice exhibited a depressive-like phenotype (Pang et al., 2009; Renoir et al., 2011). We have previously identified dysregulation in serotonin signalling as possibly underlying the HD mutation-induced depression-like behaviours (Pang et al., 2009; Renoir et al., 2011). However, early neurochemical changes related to the dopamine (DA) system could also be the cause of the neuropsychiatric symptoms preceding the locomotor impairment. Indeed, clinical data indicate that dopaminergic dysfunction is common in both symptomatic and pre-manifest HD gene carriers (Andrews et al., 1999; Ginovart et al., 1997; Pavese et al., 2010) suggesting that this early event in HD pathophysiology could contribute to neuropsychiatric disorders. Animal studies have also reported reduction in specific dopamine receptors in HD mice associated with the onset of locomotor symptoms (Bibb et al., 2000; Cha et al., 1998). Alterations in dopaminergic signalling may underlie early cognitive and affective dysfunction in HD (Cummings et al., 2006; Dallerac et al., 2011).

However, there is yet to be a study assessing dopamine signalling function in female versus male HD mice at an early stage of the disease. In that context, the present study aimed to functionally investigate the dopaminergic system of asymptomatic R6/1 HD mice (8-week-old female and male animals) measuring the effect of bupropion (a dopamine reuptake inhibitor) as well as the dopamine D1 receptor agonist, SKF-81297, on locomotor activity.

* Corresponding author at: Florey Institute of Neuroscience and Mental Health, Melbourne Brain Centre, University of Melbourne, VIC 3010, Melbourne, Australia. Tel.: +61 3 9035 6614; fax: +61 3 9035 3107.
E-mail addresses: thibault.renoir@unimelb.edu.au, tibo.renoir@gmail.com (T. Renoir).

http://dx.doi.org/10.1016/j.pbb.2014.10.004
0091-3057/

2. Materials and methods

2.1. Ethics statement

All experiments were performed in accordance with the guidelines of the Howard Florey Institute Animal Ethics Committee (approval ID, AEC #10-050) and the National Health and Medical Research Council (NHMRC).

2.2. Animals

R6/1 transgenic hemizygote males were originally obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and bred with CBB6 (CBA6C57/B6) F1 females to establish the R6/1 colony at the Florey Institute. The CAG repeat length of transgenic mice in the colony at the time of cohort generation was within the range 127–135 (Pathology Department, University of Melbourne, Australia). After weaning, animals were group-housed (4 mice per cage with 2 of each genotype) and maintained on a 12 h light/dark cycle with access to food and water ad libitum. All experiments were performed on male and female wild-type (WT) and R6/1 (HD) mice at 8 weeks of age. Each animal was only exposed to a unique behavioural test.

2.3. Drugs

The dopamine–norepinephrine reuptake inhibitor, bupropion, and the dopamine D1 receptor agonist, SKF-8129, were supplied by Sigma-Aldrich Australia.

2.4. Whole tissue measurements of dopamine and its metabolites

Tissue levels of endogenous dopamine (DA) and its metabolites homovanillic acid (HVA) 3,4-dihydroxyphenylacetic acid (DOPAC) were determined as previously published (Renoir et al., 2011; PMID: 22266953). Dissected striatum were homogenized in 5–10 volumes (v/w) of ice-cold 0.1 M $HClO_4$ containing 1.34 mM disodium EDTA and 0.05% $Na_2S_2O_5$. Homogenates were centrifuged at 30,000 *g* for 20 min at 4 °C. After neutralization with 2 M KH_2PO_4/K_2HPO_4, pH 7.4, containing 0.01 mg/mL ascorbate oxidase (Boehringer Mannheim, Meylan, France), supernatants were further centrifuged at 30,000 *g* for 20 min. Aliquots (10 μL) of clear supernatants were injected into a high performance liquid chromatography (HPLC) column (Ultrasphere IP, Beckman, Gagny, France; 25 × 0.46 cm, C18 reversed phase, particle size 5 μm) protected with a Brownlee pre-column (3 cm, 5 μm). The mobile phase for the elution (at a flow rate of 1 mL/min) consisted of (in mM): KH_2PO_4, 70; triethylamine, 3.1; disodium EDTA, 0.1; octane sulphonate, 1.05; and methanol, 16%, adjusted to pH 3.02 with solid citric acid. The electrochemical detection system (ESA 5011, Bedford, MA,, USA) comprises an analytical cell with dual coulometric monitoring electrodes (+50 and +350 mV). The generated signals were integrated by a computing integrator (Millenium 32, Waters, Saint Quentin Fallavier, France). Overall, 27 mice (13 WT and 14 HD) were used for the HPLC measurements.

2.5. Exploratory activity assessment

Mice were acclimatised to the room for 1 h prior to testing. Animals were then individually placed in a square clear acrylic box (26 × 26 × 38 cm) for another 30 min habituation period and then intra-peritoneally (i.p.) injected with either saline solution (0.9% NaCl, 1 ml/100 g body weight), bupropion (10–20 mg/kg) or the dopamine D1 receptor agonist SKF-8129 (5 mg/kg). Total distance travelled (in the horizontal plane) was assessed using locomotor cells (TruScan Photobeam Arenas E63-12, Coulbourn Instruments, Allentown, PA, USA) for another 35 min post-injection. Overall, 125 mice (63 WT and 62 HD) were used to study the effect of bupropion. Another 67 animals (34 WT and 33 HD) were used to study the effect of SKF-8129.

2.6. Statistical analysis

Analyses of variance (ANOVAs) were used to examine main effects and/or interactions. Genotype and sex were analysed in the HLPC experiment measuring levels of dopamine and its metabolites. Repeated measures ANOVA with time and/or doses as the within-subjects factor and treatment as the between-subjects factor was used to analyse the over-time effects of bupropion and SKF-8129 on spontaneous locomotor activity. Genotype and treatment were subsequently re-analysed by a 2-way ANOVA when analysing the area under curve (AUC) representing the overall locomotor effect following drug injections. To determine specific group differences in case of significant main effects (or interaction), the ANOVAs were followed by Fisher's LSD or Bonferroni post-hoc tests. In all cases, the significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS statistics 17.0 and Prism 5.

3. Results

3.1. Reduced striatal dopamine levels in HD mice

As shown in Fig. 1, the HD mutation had a significant effect on striatal levels of dopamine (DA; $F_{1,23} = 8.04$; $p < 0.01$) and homovanillic acid (HVA; $F_{1,23} = 22.17$; $p < 0.001$), whereas tissue levels of 3,4-dihydroxyphenylacetic acid (DOPAC) were only affected by sex ($F_{1,23} = 4.63$; $p < 0.05$) but not the HD mutation ($F_{1,23} = 3.10$; $p = 0.09$).

3.2. Altered dopamine turnover in HD mice

As shown in Fig. 2, striatal DOPAC/DA ratios were not altered by the genotype ($F_{1,23} = 2.86$; $p = 0.10$) or the sex ($F_{1,23} = 2.86$; $p = 0.47$). In contrast, HVA/DA ratios were reduced in HD mice ($F_{1,23} = 6.43$; $p < 0.05$). There was also an overall effect of sex ($F_{1,23} = 6.99$; $p < 0.05$) on the striatal HVA/DA ratios, but no significant interaction.

3.3. Reduced effect of acute bupropion on locomotor activity in female HD only

Acute administration of the dopamine transporter inhibitor, bupropion (10–20 mg/kg), resulted in a dose-dependent increase of locomotor activity (Fig. 3, A–D), with significant effects of time and treatment as well as significant time * treatment interactions in both female (WT: $F_{(24,204)} = 10.4$, $P < 0.001$; HD: $F_{(24,252)} = 4.15$, $P < 0.001$) and male (WT: $F_{(24,480)} = 14.3$, $P < 0.001$; HD: $F_{(24,420)} = 8.69$, $P < 0.001$) animals.

Post-hoc analysis revealed that there were no significant increases in activity of both WT and HD females treated with the lowest dose tested (10 mg/kg) following treatment. Conversely, a dose of 20 mg/kg of bupropion was sufficient to significantly enhance the locomotor activity of both WT and HD females during most of the time periods recorded. Interestingly, the increased locomotor activity observed 5 and 10 min following administration with the high dose of bupropion was less pronounced in female HD mice when compared to female WT animals ($p < 0.05$). A significant effect of the HD mutation was also found in female mice ($F_{1,38} = 5.61$; $p < 0.05$), when analysing the area under curve (AUC) representing the overall locomotor effect following bupropion injection (Fig. 3E). In contrast, there was no effect of genotype in male mice ($F_{1,75} = 0.19$; $p > 0.05$).