Psychoneuroendocrinology 74 (2016) 363–370

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

A pharmacological mouse model suggests a novel risk pathway for postpartum psychosis

Trevor Humby[a,b,c], Ellen S. Cross[b], Lauren Messer[a], Silvia Guerrero[d], William Davies[a,b,c,*]

[a] School of Psychology, Cardiff University, Tower Building, 70, Park Place, Cardiff, CF10 3AT, UK
[b] Neuroscience and Mental Health Research Institute, Cardiff University, Hadyn Ellis Building, Maindy Road, Cardiff, CF24 4HQ, UK
[c] Medical Research Council Centre for Neuropsychiatric Genetics and Genomics and Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Hadyn Ellis Building, Maindy Road, Cardiff, CF24 4HQ, UK
[d] University of Barcelona, Gran Via de les Corts Catalanes, 585 08007 Barcelona, Spain

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 8 September 2016
Accepted 21 September 2016

Keywords:
667-COUMATE
Elevated plus maze
Nephroblastoma-overexpressed
Startle response
Steroid sulfatase

ABSTRACT

Postpartum psychosis (PP) is a severe psychiatric disorder affecting a small proportion of new mothers shortly after childbirth. The molecular pathophysiology underlying the disorder is currently poorly understood, and there are no amenable animal models for the condition; maternal deficiency for the enzyme steroid sulfatase has been proposed as a potential risk mechanism. Here we show that inhibition of steroid sulfatase with 667-COUMATE (10 mg/kg p.o.) in new mouse mothers results in behavioural abnormalities that can be partially alleviated by the administration of the clinically-efficacious antipsychotic ziprasidone (0.3–1.0 mg/kg i.p.). The pattern of behavioural abnormalities in 667-COUMATE-treated mice implicated a genetic substrate at 21–23 cM on chromosome 15; of the 17 genes within this chromosomal interval, only one (*Nov*/*Ccn3*) was significantly differentially expressed in the brains of vehicle and 667-COUMATE-treated mice. Two additional members of the Ccn family (*Ccn2*/*Ctgf* and *Ccn4*/*Wisp1*) were also significantly differentially expressed between the two groups, as were three further genes co-expressed with *Nov*/*Ccn3* in brain (*Arhgdig*) or previously implicated in disorder risk by clinical studies (*Adcy8* and *Ccl2*). The expression of *Nov*/*Ccn3*, but not of the other differentially-expressed genes, could be normalised by ziprasidone administration (1.0 mg/kg). *NOV*/*CCN3* lies directly under a linkage peak for PP risk at 8q24, and the associated protein possesses numerous characteristics that make it an excellent candidate mediator of PP risk. Our data suggest the 667-COUMATE-treated mouse as a model for PP with some degree of face, construct, and predictive validity, and implicate a novel, and biologically-plausible, molecular risk pathway for PP.

1. Introduction

Postpartum psychosis (PP) is a severe psychiatric disorder occurring shortly after childbirth in 1–2 out of every 1000 mothers (Sit et al., 2006). The disorder is characterised by hallucinations, delusions, cognitive disorganisation and mood problems, and is associated with an increased risk of maternal suicide or infanticide (Sit et al., 2006). The pathophysiological basis of PP is poorly understood due, in part, to the lack of an amenable animal model. The identification of biomarkers associated with increased risk is a key goal for ensuring early clinical intervention.

Increased PP risk is associated with a personal or family history of psychotic disorder (notably bipolar disorder), with precipitous drops in circulating oestrogens following childbirth, with obstetric complications including pre-eclampsia, and with psychosocial stressors (Sit et al., 2006). Small genetic linkage and association studies have implicated regions of chromosome 16p13 and 8q24 (Jones et al., 2007) and serotonergic abnormalities (Kumar et al., 2007) respectively, but have not identified robust candidate genes. Recently, immune system (Bergink et al., 2013) and tryptophan-kynurenine pathway (Veen et al., 2016) disruptions have been demonstrated in PP, whilst regular smoking is associated with reduced risk (Di Florio et al., 2015).

Maternal deficiency for the enzyme steroid sulfatase (STS) may predispose to PP (Davies, 2012); STS converts sulfated steroids to their non-sulfated forms (e.g. dehydroepiandrosterone sulfate,

* Corresponding author at: Cardiff University School of Psychology, 70, Park Place, Cardiff CF10 3AT, UK.
E-mail addresses: humbyt@cardiff.ac.uk (T. Humby), CrossES@cardiff.ac.uk (E.S. Cross), laurenmesser94@gmail.com (L. Messer), silvia_2i2@hotmail.com (S. Guerrero), daviesw4@cardiff.ac.uk (W. Davies).

http://dx.doi.org/10.1016/j.psyneuen.2016.09.019

DHEAS, to DHEA) which may act as precursors for oestrogens and androgens (Davies, 2012). Maternal STS deficiency is associated with obstetric complications (Fernandes et al., 2010), whilst in healthy individuals, DHEAS serum levels positively correlate with postpartum psychoticism (Marrs et al., 2009); decreased postpartum DHEA levels are associated with activation of the maternal immune system (Tagawa et al., 2004).

In the first part of the study (Experiment 1), we tested whether acute inhibition of steroid sulfatase in new mouse mothers would elicit behavioural and gene expression changes of relevance to PP. STS was inhibited using 667-COUMATE (also known as STX64), a drug that systemically and irreversibly inhibits enzyme activity by >90% in rodents at the dose used here (Purohit et al., 2000). STS inhibition was indexed indirectly by measuring serum levels of DHEA(S), and levels of the stress hormone corticosterone were also measured. The behavioural tasks assayed aspects of emotional reactivity, activity and sensorimotor gating that are commonly perturbed in psychotic disorders; pup maltreatment or infanticide, which may represent a surrogate measure of PP in animals (Quilter et al., 2007) was also recorded. In Experiment 2, we tested whether 667-COUMATE-induced behavioural and gene expression changes could be reversed by administration of clinically-relevant doses of the atypical antipsychotic drug ziprasidone (Sit et al., 2006).

2. Materials and methods

2.1. Subjects and husbandry

Virgin C57BL/6JOlaHsd female mice (aged 12–26 weeks, originally obtained from Envigo UK and bred within Cardiff University School of Psychology), were housed in trios with males of the same strain. 3–5 days before giving birth, females were housed individually and were monitored closely until birth. Mice were maintained on *ad libitum* food and water, in a temperature, humidity and light-controlled room (21 ± 2 °C, 50 ± 10% humidity, lights on at 0700hr for 12 h) and were regularly inspected for signs of ill health. Experiments were performed according to the UK Animal Scientific Procedures Act (1986).

2.2. Drug administration

2.2.1. Experiment 1

<12 h after giving birth, mothers were injected *per os* (p.o.) with either vehicle solution (tetrahydrofuran:polyethylene glycol 400: distilled water in a 1:6:3 ratio (Ireson et al., 2004)), n = 14) or 667-COUMATE (10 mg/kg, Sigma-Aldrich, UK) in the same vehicle (n = 17) in a pseudo-randomised manner. Mothers were administered the same treatment 48 h after this first injection. The 667-COUMATE administration regime was based upon previously-published pharmacokinetic data in rodents (Ireson et al., 2004; Purohit et al., 2000) and was intended to provide maximal enzyme inhibition across the postpartum period whilst minimising off-target effects. Injections were performed between 09:00–10:00 h. Behavioural testing was carried out 24 h after the second injection between 09:00–13:00 h.

2.2.2. Experiment 2

<12 h after giving birth, mothers were injected *per os* (p.o.) with 667-COUMATE (10 mg/kg) as above. 24 h after the first injection, mice were injected intraperitoneally (i.p.) with either vehicle solution (1% methylcellulose in 0.9% saline) or one of two doses of ziprasidone hydrochloride (0.3 mg/kg or 1.0 mg/kg (free-base concentrations) Sigma-Aldrich, UK) in the same vehicle in a pseudo-randomised manner. 24 h later, mice received a second injection of 667-COUMATE (10 mg/kg), and 23 h after this, mice received a final injection of vehicle, 0.3 mg/kg, or 1.0 mg/kg ziprasidone. Injections were performed between 08:30–10:00h. The three experimental groups were: mice which received 667-COUMATE (10 mg/kg) with ziprasidone (0 mg/kg)(CVCV group, n = 16), mice which received 667-COUMATE (10 mg/kg) with ziprasidone (0.3 mg/kg)(CZCZ0.3 group, n = 16), and mice which received 667-COUMATE (10 mg/kg) with ziprasidone (1.0 mg/kg)(CZCZ1.0 group, n = 8). Ziprasidone doses were selected to have minimal effects on activity (Kalinichev and Dawson, 2011). Behavioural testing was carried out 1hr after the final injection between the hours of 09:30–13:00 h.

2.3. Homecage monitoring and behavioural analysis

Prior to injections, mother/pup health, litter sizes and weights, and maternal weights were recorded; pup deaths or signs of maternal aggression towards the pups were noted. These measures allowed us to assess whether the drug regimes were adversely affecting gross maternal and/or pup health, and/or pup maltreatment or infanticide; we were particularly concerned that inhibition of STS in the mother's mammary gland may affect provisioning of her pups. Only mothers who gave birth to at least one live pup were included in the study.

Mothers were initially behaviourally tested on an elevated plus-maze (Isles et al., 2004) to assay anxiety-related and exploratory phenotypes. Mice were placed in a closed arm, and allowed to explore the apparatus freely for 5mins. Their lateral and vertical ('rearing') activity was objectively tracked using Ethovision Observer software Version 3.0.15 (Noldus Information Technology, The Netherlands); additional measures of vertical and lateral exploration (head dips from the open arms and stretch attend postures) and emotional reactivity (defecation) were recorded manually. Key measures of interest were the ratio of open arm:closed arm time and latency to first entry of the open arms (indexing anxiety), entries into the closed arms (an index of within-maze activity minimally confounded by anxiety), numbers of rears/head dips and stretch-attend postures, and numbers of fecal boli. Two seconds per animal were added to the open arm latency measure obtained from Ethovision to account for the time lag between putting the animal on the maze and initiating tracking. Following testing on the elevated plus-maze, basal activity level was tested in the dark using locomotor cages (Isles et al., 2004). The number of infrared beam breaks made over a 1hr session (4 × 15 min bins) was recorded. Finally, sensorimotor gating was assayed using a startle and prepulse inhibition (PPI) paradigm (Dent et al., 2014); the main measures of interest were startle response over first three pulse alone trials (an index of emotional reactivity), the startle response for four levels of prepulse at 0, 4, 8 and 16 dB above background (P120, PP4P120, PP8P120 and PP16P120 respectively, an index of habituated startle and PPI), and startle response over pulse-alone trials with varying stimulus intensity (an index of auditory acuity). The behavioural tests were administered in order of increasing severity such that performance on the latter tests would not be substantially influenced by prior exposure to potential stressors. Mice were returned to their homecage for 5–10min between behavioural tests.

2.4. Culling and tissue collection

3 h after behavioural testing, subjects were culled by cervical dislocation. Trunk blood was collected in BD SST Microtainer Gold tubes (BD Biosciences) and serum extracted according to the manufacturer's instructions prior to storage at −80 °C. Whole brains were removed, bisected sagitally, and frozen on dry ice.