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Developmental vitamin D deficiency alters multiple neurotransmitter systems in the neonatal rat brain

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ABSTRACT

Background: Epidemiological evidence suggests that developmental vitamin D (DVD) deficiency is a risk factor for neuropsychiatric disorders, such as schizophrenia. DVD deficiency in rats is associated with altered brain structure and adult behaviours indicating alterations in dopamine and glutamate signalling. Developmental alterations in dopamine neurotransmission have also been observed in DVD-deficient rats but a comprehensive assessment of brain neurochemistry has not been undertaken. Thus, the current study determined the regional concentrations of dopamine, noradrenaline, serotonin, glutamine, glutamate and γ-aminobutyric acid (GABA), and associated metabolites, in DVD-deficient neonates.

Methods: Sprague-Dawley rats were fed a vitamin D deficient diet or control diet six weeks prior to mating until birth and housed under UVB-free lighting conditions. Neurotransmitter concentration was assessed by high-performance liquid chromatography on post-mortem neonatal brain tissue.

Results: Ubiquitous reductions in the levels of glutamine (12–24%) were observed in DVD-deficient neonates compared with control neonates. Similarly, in multiple brain regions DVD-deficient neonates had increased levels of noradrenaline and serine compared with control neonates. In contrast, increased levels of dopamine and decreased levels of serotonin in DVD-deficient neonates were limited to striatal subregions compared with controls.

Conclusions: Our results confirm that DVD deficiency leads to changes in multiple neurotransmitter systems in the neonate brain. Importantly, this regionally-based assessment in DVD-deficient neonates identified both widespread neurotransmitter changes (glutamine/noradrenaline) and regionally selective neurotransmitter changes (dopamine/serotonin). Thus, vitamin D may have both general and local actions depending on the neurotransmitter system being investigated. Taken together, these data suggest that DVD deficiency alters neurotransmitter systems relevant to schizophrenia in the developing rat brain.

1. Introduction

Vitamin D is a fat soluble steroid synthesised from cholesterol either through dietary sources, or through the conversion of 7-dehydrocholesterol in the skin following exposure to ultraviolet B radiation (Holick, 2004). In addition to peripheral calcium homeostasis, vitamin D has a diverse range of brain functions including the regulation of neurotrophic signalling, immune modulation and neuroprotection (Kesby et al., 2011). There is widespread expression of the vitamin D receptor (VDR) throughout the human (Sutherland et al., 1992; Zehnder et al., 2001; Eyles et al., 2005) and rat brain (Clemens et al., 1988; Fu et al., 1997; Prufer et al., 1999) suggesting a direct role in brain function. The expression of the VDR in the developing rodent brain (Veenstra et al., 1998; Erben et al., 2002) also indicates a role in brain development. Furthermore, neonatal vitamin D deficiency has been associated with an increased risk of schizophrenia (McGrath et al., 2010) that may be related to the early disruption of neurotransmitter function (Kesby et al., 2013). Thus, understanding the role of vitamin D in early brain development and neurotransmission may shed light upon the biological plausibility of vitamin D's role in neurodevelopmental psychiatric disorders.

Developmental vitamin D (DVD) deficiency results in long lasting changes in the behaviour of adult rats. Alterations in dopamine signaling have been consistently observed in DVD-deficient rats. Female adult DVD-deficient rats show significantly increased dopamine transporter density and binding affinity in basal ganglia (BG) sub regions,

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which may be associated with an increased locomotor sensitivity to the psychomimetic drug amphetamine (Kesby et al., 2010). Moreover, adult DVD-deficient rats display novelty-induced hyperlocomotion (Burne et al., 2004, 2006; Kesby et al., 2006), a behaviour reliant on mesolimbic dopaminergic circuitry (Hooks and Kalivas, 1995). In neonatal rats, DVD deficiency decreases forebrain dopaminergic turnover by a reduction in catechol-O-methyl transferase (COMT) enzyme expression (Kesby et al., 2009), which is an enzyme responsible for the metabolism of dihydroxyphenylacetic acid (DOPAC) and dopamine. DVD deficiency has also been shown to transiently reduce factors crucial for specifying dopaminergic phenotype, such as Nurr1 and p57Kip2 during embryonic development (Cui et al., 2010). The VDR is present in the nucleus of tyrosine hydroxylase-positive neurons in both human and rat substantia nigra, emerging early in rat brain development (Cui et al., 2013). Thus, vitamin D may act directly on dopaminergic systems in the brain leading to the consistently observed abnormalities in dopaminergic function after DVD deficiency.

Other relevant neurotransmitter systems have also been associated with vitamin D deficiency. DVD-deficient rats are more sensitive to the locomotor-enhancing (Kesby et al., 2006; O'Loan et al., 2007; Kesby et al., 2012) and startle eliciting (Kesby et al., 2012) effects of the *N*-Methyl-D-aspartate (NMDA) receptor antagonist, MK-801. NMDA receptor antagonists produce a symptom profile very similar to that observed in schizophrenia (Lahti et al., 1995, 2001), suggesting alterations in glutamatergic systems. On the other hand, recent work has implicated vitamin D in serotonergic synthesis and metabolism, and developmental periods may be more susceptible to vitamin D-related effects (Patrick and Ames, 2015). Thus, these data suggest the absence of vitamin D during brain development may induce persistent alterations in multiple neurotransmitter systems but highlights the need for a more detailed investigation into the effects of DVD deficiency on neurotransmission.

Comprehensive assessments of whole brain neurotransmitter levels in adult vitamin D-deficient mice have demonstrated a broad range of effects on neurotransmitter levels (Groves et al., 2013). For example, vitamin D deficient C57BL/6J mice had changes in dopamine and serotonin turnover. In contrast, vitamin D deficient BALB/c mice had decreased levels of glutamate and glutamine, and increased levels of γ-aminobutyric acid (GABA). Thus, evidence suggests that adult vitamin D deficiency in the mouse can impact a range of neurotransmitter systems. However, the effects of DVD deficiency on dopamine, serotonin, glutamate and GABA systems have not been well categorised in the developing rat brain. Moreover, whether vitamin D deficiency alters neurotransmitter systems locally, in a regionally-specific manner, or more globally has not been determined given the prior use of only whole brain and forebrain samples.

Therefore, the aim of the present study was to comprehensively examine brain tissue levels of neurotransmitters and amino acids by high-performance liquid chromatography (HPLC) in DVD-deficient newborn Sprague-Dawley rats. To assess dopaminergic and serotonergic function, levels of dopamine and its metabolites DOPAC and homovanillic acid (HVA), in addition to serotonin and its major metabolite 5-hydroxy-indoleacetic acid (5-HIAA) were determined. Levels of amino acids directly involved in neurotransmission including GABA and glutamate, in addition to associated substrates and co-agonists including glutamine, glycine, serine, aspartate and taurine were also determined. Numerous brain areas, including the medial prefrontal cortex (mPFC), hippocampus, caudate putamen (CPu), hypothalamus, thalamus, BG, midbrain and cerebellum, were included to determine both regionally-specific and global outcomes.

2. Materials and methods

2.1. Animals

All procedures were performed with approval from the University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia. To induce vitamin D depletion, four-week old female Sprague-Dawley rats (Herston Animal Facility, Queensland, Australia) were kept on a vitamin D deficient diet (AIN93G with 0 I.U. vitamin D_3; Specialty Feeds, WA, Australia). Animals were housed on a 12-h light/dark cycle (lights on at 06:00 h) using incandescent lighting, to avoid ultraviolet radiation within the vitamin D action spectrum. These conditions were maintained for 6 weeks prior to mating and throughout gestation. This breeding protocol is sufficient to deplete serum vitamin D in dams and offspring without altering neonatal blood calcium levels (Eyles et al., 2003; O'Loan et al., 2007; Eyles et al., 2011). Control animals were kept under similar conditions except they received a standard vitamin D containing rat chow (AIN93G with 1000 I.U. vitamin D_3).

2.1.1. Tissue collection

At postnatal day 0, pups were sacrificed by decapitation between 09:00 and 12:00 h and brains were removed rapidly and dissected freehand into multiple brain regions guided by standard landmarks using an atlas of prenatal rat brain development (Altman, 1995). Two cohorts were used; one for the collection of mPFC, hippocampus, and CPu (n = 14–16/group), and another for the collection of hypothalamus, thalamus, BG (including, striatum, nucleus accumbens and globus pallidus), midbrain and cerebellum (n = 21–22/group). Two cohorts were used because the protocol and time taken for dissection of the mPFC and hippocampus was prohibitive to the confident collection of the hypothalamus and thalamus. Pups were taken from 3 separate litters/diet/cohort. Tissue samples were rapidly frozen on dry ice and stored at −80 °C prior to use.

2.1.2. High performance liquid chromatography

Catecholamines and amino acids from brain tissue were measured by HPLC with electrochemical detection for catecholamines and fluorescent detection for amino acids (Kesby et al., 2009; Groves et al., 2013). Brain regions were weighed (wet weight) and diluted based on weight with a minimum volume of 0.1 ml of 0.1 M perchloric acid with 50 ng/ml deoxyepinephrine (catecholamine internal standard). They were then homogenised on ice using probe sonication (2 × 5 s at 60% amplitude; Vibra-Cell, Sonics & Materials, CT, USA) and centrifuged at 13,000 rpm for 5 min at 4 °C with the supernatant filtered by a 4 mm 0.22 μM nylon syringe filter (PM Separations, QLD, Australia). This method produces more sample supernatant than is required for the assessment of both catecholamine and amino acids. One 10 μl aliquot of sample supernatant was injected into the HPLC system, which consisted of a degasser, autosampler and an isocratic HPLC pump (Model 1100, Agilent Technologies, CA, USA), a Sunfire C18 column, 4.6 mm × 150 mm, 5 um; (Waters Corporation, MA, USA) and a Coulochem III (ESA Laboratories, MA, USA) electrochemical detector. The mobile phase consisted of a 12% acetonitrile/75 mM potassium dihydrogen phosphate buffer containing 25 μM EDTA and 1.7 mM octane sulfonic acid adjusted to pH 4.13 with phosphoric acid. Flow rate was 1.2 ml/min. Detector settings were as follows: conditioning cell (Model 5020, ESA Laboratories) at +350 mV; analytical cell (Model 5014B, ESA Laboratories) with the first and second electrodes maintained at −150 and +250 mV, respectively. Amino acids were analysed by HPLC using pre-column derivatisation and fluorescence detection. Samples were maintained at 4 °C and the derivatisation protocol was conducted by the autosampler as follows: −10 μl of 1 nM/μl homoserine (amino acid internal standard) was mixed with a 10 μl aliquot of sample supernatant; then 100 μl of diluent (methanol/water, 50:50, v/v) was added and mixed; 2 μl of borate buffer (Agilent Technologies) was drawn into the loop; then 1 μl was drawn from the sample and mixed in the loop; finally 0.5 μl of OPA reagent (Agilent Technologies) was drawn and mixed in the loop; after a 1 min wait this was then injected into the system. The system consisted of an Agilent 1200 degasser, binary pump, autosampler with thermostat and fluorescence detector