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Altered arginine metabolism in the hippocampus and prefrontal cortex of maternal immune activation rat offspring

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ABSTRACT

Altered arginine metabolism has been implicated in the pathogenesis of schizophrenia. The present study measured the levels of L-arginine and its downstream metabolites in the sub-regions of the hippocampus, prefrontal cortex and cerebellum in adult rats that had been exposed to maternal immune activation (MIA; a risk factor for schizophrenia). MIA significantly increased L-arginine, L-ornithine and putrescine levels and decreased agmatine levels in the hippocampus and prefrontal cortex in a region-specific manner. Correlational analysis revealed a significant neurochemical–behavioural correlation. Cluster analyses showed that L-arginine and its main metabolites formed distinct groups, which changed as a function of MIA. These results demonstrate, for the first time, that MIA leads to altered arginine metabolism in the hippocampus and prefrontal cortex of the adult offspring.

1. Introduction

Schizophrenia is a debilitating psychiatric disorder associated with prominent prefrontal and hippocampal dysfunction (Goldman-Rakic and Selemon, 1997; Harrison, 2004). Maternal immune activation (MIA) is a neurodevelopmental animal model of schizophrenia (Smith et al., 2007; Meyer et al., 2009b), based on epidemiological evidence that prenatal exposure to infections increases the risk of schizophrenia in adulthood (Brown et al., 2004; Patterson, 2007). As the maternal response to infection is thought to be a critical mediating factor, this model uses a single systemic administration of the synthetic cytokine inducer polyinosinic:polycytidilic acid (poly I:C) during mid-gestation to induce MIA in pregnant animals (reviews refer to Meyer et al., 2009a; Patterson, 2009). A number of morphological abnormalities, neurochemical changes, and behavioural features of schizophrenia are evident in the adult offspring of poly I:C treated dams, including hippocampal and prefrontal dysfunction (Shi et al., 2003; Zuckerman et al., 2003; Zuckerman and Weiner, 2005; Li et al., 2009; Winter et al., 2009; Wolff and Bilkey, 2010).

L-arginine is a semi-essential amino acid that can be metabolized to form a number of bioactive molecules (Fig. 1). Nitric oxide (NO) generated by NO synthase (NOS), for example, plays an important role in maintaining physiological function of the nervous system (Feil and Kleppisch, 2008; Steinert et al., 2010). Due to its properties as a free radical, however, it can be neurotoxic when present in excessive amounts (Calabrese et al., 2007). L-ornithine, the product of arginase, is the main precursor of polyamines putrescine, spermidine and spermine, which are essential in maintaining normal cellular function (Williams, 1997; Wu and Morris, 1998; Wallace et al., 2003). L-ornithine can also be channelled to produce glutamate, which can be further metabolized to generate γ-aminobutyric acid (GABA). Agmatine, the product of arginine decarboxylase (ADC), is a novel putative neurotransmitter and plays an important role in regulating the production of NO and polyamines (Wu and Morris, 1998; Reis and Regunathan, 2000; Halaris and Plietz, 2007).

Accumulating evidence suggests that arginine metabolism is altered in schizophrenia (Perez-Neri et al., 2006; Fiori and Turecki, 2008). For example, there are elevated NO and NOS expression levels in the brains and plasma of individuals with schizophrenia (Baba et al., 2004; Yao et al., 2004; Djordjevic et al., 2010), and human genetic studies have identified schizophrenia risk genes encoding nNOS and several downstream effectors of nNOS (Shinkai et al., 2002; Reif et al., 2006; Cui et al., 2010). Other studies have reported decreased plasma arginase

Abbreviations: ADC, arginine decarboxylase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; DG, dentate gyrus; GABA, γ-aminobutyric acid; MIA, maternal immune activation; NO, nitric oxide; NOS, nitric oxide synthase; PFC, prefrontal cortex; poly I:C, polyinosinic:polycytidilic acid; PPI, pre-pulse inhibition.

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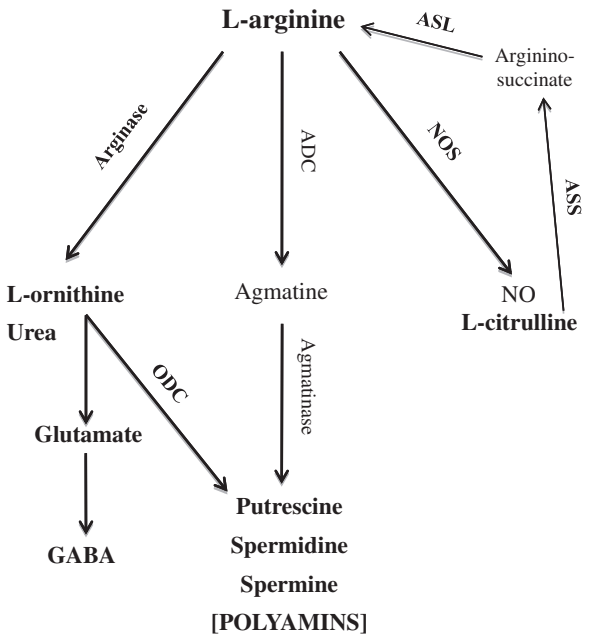

Fig. 1. Arginine metabolic pathways. L-arginine can be metabolized by nitric oxide synthase (NOS), arginase and arginine decarboxylase (ADC). ASL: argininosuccinate lyase; ASS: argininosuccinate synthetase; GABA: γ-aminobutyric acid; NO: nitric oxide; ODC: ornithine decarboxylase.

activity in schizophrenic patients (Yanik et al., 2003), and a positive correlation between the serum levels of L-ornithine and the duration of schizophrenia (Tomiya et al., 2007). Furthermore, it has been reported that polyamines and agmatine play a role in the etiology and pathology of several mental disorders, including schizophrenia (Fiori and Turecki, 2008).

Winter et al. (2009) systematically investigated how MIA induced by poly I:C affected the basal levels of neurotransmitters in the adult mouse offspring, and found dramatic changes in dopamine and serotonin and their metabolites, as well as the inhibitory amino acid taurine, in a number of brain regions. There is, however, no previous research describing how the brain arginine metabolic profile changes following MIA. The present study, therefore, compared the tissue concentrations of L-arginine and its downstream metabolites in adult MIA rat offspring and their controls in the prefrontal cortex, hippocampus and cerebellum. Schizophrenia has previously been associated with prefrontal and hippocampal dysfunction (Goldman-Rakic and Selemon, 1997; Harrison, 2004), and there is also evidence to suggest an involvement of the cerebellum in the pathophysiology of the disease (Baldacara et al., 2008). Because there is a functional dissociation across the CA1, CA3, and dentate gyrus (DG) of the hippocampus (for a review see Kesner et al., 2004), the neurochemical changes in this region were examined at the sub-regional level.

2. Methods

2.1. Animals

Female Sprague–Dawley rats obtained from the University of Otago Animal Breeding Station were mated at 3 months of age. On gestational day 15, pregnant rats were anesthetized with halothane and given a single injection of either polyI:C (4.0 mg/kg, i.v.) dissolved in saline, or a saline injection equivalent. Pups were culled to give litters of 5–6 males. On postpartum day 21, pups were weaned and housed 3 to a cage based on the treatment group (MIA vs control) and handled similarly prior to tissue preparation at 3 months of age. All animals were maintained on a 12-h light–dark cycle (lights on 7 a.m.) with *ad lib* access to water and the same type of food. The MIA ($n = 6$, from 3 litters) and control ($n = 5$, from 3 litters) rats were a sub-set of animals used in the study of Wolff and Bilkey (2010), in which MIA offspring displayed impaired pre-pulse inhibition (PPI) in adulthood. PPI is an operational measure of sensorimotor gating and is known as a benchmark test for schizophrenia (Braff, 2010). Assessment of PPI and acoustic startle responses was conducted in a sound-attenuated SR-lab startle chamber using standard methods detailed in our previous publications (Wolff and Bilkey, 2008, 2010).

2.2. Tissue preparation

All rats were decapitated without anesthesia. The brains were rapidly removed and left in cold saline (4 °C) for at least 45 s. The CA1, CA2/3 and DG sub-regions of the hippocampus, prefrontal cortex (PFC) and cerebellum (CE) were dissected freshly on ice (Liu et al., 2008b, 2009b, 2010, 2011; Gupta et al., 2012). The tissues were weighed, homogenized in ice-cold 10% perchloric acid (~50 mg wet weight per millilitre) and centrifuged at 10,000 rpm for 10 min at 4 °C to precipitate protein. The supernatants (the perchloric acid extracts) were frozen immediately and stored at −80 °C until the assays.

2.3. Neurochemical procedures

The tissue concentrations of amino acids (L-arginine, L-citrulline, L-ornithine, glutamate and GABA) and polyamines spermidine and spermine were measured by the high performance liquid chromatographic methods, and the agmatine and putrescine levels were determined by a highly sensitive liquid chromatography/mass spectrometric method, as we have previously described (Liu et al., 2008a, 2008c, 2011; Gupta et al., 2012). All assays were performed in duplicate. For each brain region, samples from the control and MIA groups were assayed at the same time and the order was counterbalanced. High purity external and internal standards were used (Sigma, Sydney, Australia), and all other chemicals were of analytical grade. The concentrations of L-arginine and its eight downstream metabolites in tissue were calculated with reference to the peak area of external standards, and values (the mean of the duplicate) were expressed as μg/g wet tissue.

2.4. Statistical analysis

The neurochemical results were presented as mean ± SEM (the standard error of the mean). For each neurochemical variable in a given region, the comparison of interest was made between the control and MIA groups using unpaired student *t*-test. The significance level was set at $p \leq 0.025$ for all comparisons (Rothman, 1990; Zolman, 1993). All calculations were performed with the Prism program.

Cluster analysis is an exploratory data analysis tool to sort different variables into groups such that the degree of association between two variables is maximal if they belong to the same group. As we measured the levels of L-arginine and its three main metabolites (L-citrulline, L-ornithine and agmatine) in the sub-regions of the hippocampus, prefrontal cortex and cerebellum, cluster analysis was performed for each group and each brain region to determine which neurochemical variables co-varied, using Minitab 15 (Liu et al., 2010, 2011; Gupta et al., 2012). Prior to the analysis, the data were standardized to obtain z values. Agglomerative methods were then used on the correlation coefficient distance, which started with each observation as a cluster and then with each step combined observations to form clusters until there was only one large cluster. Comparisons between different cluster analysis algorithms indicated that Complete linkage, McQuitty linkage, Average linkage and Ward linkage all produced similar results (data not shown). Therefore, Ward linkage (based on the sum of squares between the two clusters, summed over all variables) was used for all

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