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Behavioral disturbances in adult mice following neonatal virus infection or kynurenine treatment – Role of brain kynurenic acid

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ABSTRACT

Exposure to infections in early life is considered a risk-factor for developing schizophrenia. Recently we reported that a neonatal CNS infection with influenza A virus in mice resulted in a transient induction of the brain kynurenine pathway, and subsequent behavioral disturbances in immune-deficient adult mice. The aim of the present study was to investigate a potential role in this regard of kynurenic acid (KYNA), an endogenous antagonist at the glycine site of the N-methyl-D-aspartic acid (NMDA) receptor and at the cholinergic α7 nicotinic receptor. C57BL/6 mice were injected i.p. with neurotropic influenza A/WSN/33 virus (2400 plaque-forming units) at postnatal day (P) 3 or with L-kynurenine (2 × 200 mg/kg/day) at P7–16. In mice neonatally treated with L-kynurenine prepulse inhibition of the acoustic startle, anxiety, and learning and memory were also assessed. Neonatally infected mice showed enhanced sensitivity to d-amphetamine-induced (5 mg/kg i.p.) increase in locomotor activity as adults. Neonatally L-kynurenine treated mice showed enhanced sensitivity to d-amphetamine-induced (5 mg/kg i.p.) increase in locomotor activity as well as mild impairments in prepulse inhibition and memory. Also, d-amphetamine tended to potentiate dopamine release in the striatum in kynurenine-treated mice. These long-lasting behavioral and neurochemical alterations suggest that the kynurenine pathway can link early-life infection with the development of neuropsychiatric disturbances in adulthood.

1. Introduction

Schizophrenia is a mental disorder, usually emerging in adolescence or early adulthood (Andreasen, 1995) and is characterized by a large diversity of positive and negative symptoms as well as cognitive deficits. Aberrations in brain dopamine (DA) neurotransmission as part of the pathophysiology has for long been the predominant biological hypothesis of schizophrenia (Carlsson and Lindqvist, 1963; Carlsson and Carlsson, 2006). The DA hypothesis arises from the finding of amelioration in positive symptoms following blockade of DA D_2-receptors, as well as from observations of psychosis following frequent abuse of the indirect DA agonist d-amphetamine (Angrist and Gershon, 1970; Cruickshank and Dyer, 2009; Griffith et al., 1972). Further supporting the DA hypothesis, brain imaging studies reveal an enhanced DA release following administration of d-amphetamine in patients with schizophrenia (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). Research during the last decade however, proposes that DA only plays an intermediary role in the pathophysiology and that deficits in brain glutamatergic systems are of major importance for the disease (Carlsson et al., 2001; Javitt, 2004; Jentsch and Roth, 1999). For example, administration of N-methyl-D-aspartic acid (NMDA) receptor antagonists (e.g. phencyclidine and ketamine) evokes behavior similar to schizophrenia symptoms in healthy individuals, and exacerbates symptoms in patients with schizophrenia (Adler et al., 1999; Javitt and Zukin, 1991; Luby et al., 1959). Providing strong support for a dysfunction of glutamatergic transmission in schizophrenia, the concentration of kynurenic acid (KYNA) is elevated in the cerebrospinal fluid (CSF) and in post mortem brain of patients with schizophrenia (Erhardt et al., 2001a; Linderholm et al., 2012; Nilsson et al., 2005; Sathyasaikumar et al., 2011; Schwarcz et al., 2001). KYNA is a tryptophan metabolite, synthesized in astrocytes via the kynurenine pathway. At nanomolar concentrations, KYNA antagonizes the glycine-site of the NMDA receptor as well as the cholinergic α7 nicotinic receptor (α7nAChR; Schwarzc et al., 2012). At higher,

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micromolar concentrations, KYNA blocks the glutamate recognition-site of the NMDA receptor (Kessler et al., 1989). Notably, endogenous concentrations of KYNA tonically control DA transmission in the rat brain (Erhardt et al., 2009; Linderholm et al., 2007; Amori et al., 2009b; Schwieler et al., 2008), findings functionally linking the DA hypothesis to the glutamate deficiency theory of schizophrenia.

Synthesis of KYNA is induced following immune activation (Dantzer et al., 2008). Indeed, CSF KYNA is elevated in various infectious diseases (Heyes et al., 1992), such as those caused by human immunodeficiency virus-1 or tick-borne encephalitis (Atlas et al., 2007; Holtze et al., 2012) or during influenza A virus infections of neuron or glial cultures in vitro (Holtze et al., 2008). Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO), the initial and rate-limiting enzymes in the production of KYNA, are induced by interferon-γ or other pro-inflammatory cytokines (Schwarcz et al., 2012) and are important in controlling microbial growth as well as the host immune response (King and Thomas, 2007). Moreover, activation of the pro-inflammatory cytokine interleukin (IL)-1β in CSF from first-episode patients with schizophrenia was recently reported, strongly indicating immune dysregulation in the disease (Soderlund et al., 2009). Interestingly, exposure to infections, including influenza A virus, during early-life appear to increase the risk for the future development of schizophrenia and related psychotic disorders (Blomström et al., 2012; Brown and Derkits, 2010; Dalman et al., 2008; Ellman et al., 2009; Yolken and Torrey, 2008). While a causal link between early-life exposure to infections and the later development of psychoses is still missing, several animal studies have demonstrated deficits in prepulse inhibition (PPI), a cross-species measure of sensorimotor gating which is impaired in patients with schizophrenia (Braff et al., 2001), in adult animals following either prenatal or early-life exposure to immunostimulatory agents (Crnic and Pizer, 1988; Engel et al., 2000; Gold et al., 1994; Meyer and Feldon, 2010; Tohmi et al., 2004). We recently reported that a neonatal infection with neurotropic influenza A/WSN/33 virus transiently increases brain KYNA concentrations in wild type mice as well as in immunodeficient mice with a targeted disruption of the gene-encoding transporter associated with antigen processing 1 (*Tap1*$^{-/-}$), leading to a lack of MHC class I expression and functional CD8+ T cells (Asp et al., 2010; Holtze et al., 2008). The neonatal infection was associated with deficits in PPI and working memory as well as with increased rearing and anxiety in adult immunodeficient mice, but not in adult wild type mice (Asp et al., 2009, 2010). A recent study from our laboratory also shows that mice with subchronically elevated levels of KYNA in adulthood have normal spontaneous locomotor activity but a potentiated locomotor response to d-amphetamine (Olsson et al., 2012a). Since our previous studies showed that wild type mice neonatally infected with influenza A virus do not show aberrant behavior under baseline conditions, in the present study we investigated the locomotor response to challenge with d-amphetamine. A second aim of the present study was to investigate whether neonatally elevated brain KYNA is associated with the disturbed behavior in adulthood seen after infection in early life.

2. Materials and methods

2.1. Animals

C57BL/6 mice were used for all experiments. Pregnant mice were obtained from Scanbur AB, Sweden and kept in a single cage until delivery. All pups (males and females) stayed with their mothers until weaning (5–8 mice per cage). After weaning at the age of four weeks, all mice were group-housed in standard transparent cages (2–5 mice per cage, as determined by the number of male mice in each litter) because isolation rearing (i.e. housing mice one per cage) can affect amount of PPI and locomotor response to amphetamine. All mice were handled according to institutional guidelines and environmental conditions and checked daily. The animals were maintained under standard laboratory conditions with free access to food and tap water in a light-controlled room (12 h light/dark cycle, light on at 6.00 a.m.), under constant temperature (22 °C) and humidity (40–60%). To check for confounding factors regarding differences in litter size we plotted individual test responses with regard to PPI. The responses were found to be almost similar in all mice with no deviation with regard to litter sizes. Two separate sets of pups were neonatally treated with L-kynurenine or saline. In the first set, only male mice were used and locomotor activity and PPI assessed. In the second set, both male and female mice were neonatally treated with L-kynurenine or saline and assessed in the light dark box and the elevated plus maze. Thereafter female mice were assessed for trace fear conditioning and male mice were assessed biochemically utilizing in vivo microdialysis. In total, 79 male and 35 female mice were used in these experiments, including a smaller batch of mice used for verification of brain KYNA levels at P16. All experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. All efforts were made to minimize the number of animals used and their suffering.

2.2. Neonatal influenza A/WSN/33 infection

The neonatal infection model has previously been described in detail in Asp et al. (2007, 2009). Male C57BL/6 mice (n = 9 from a total of three litters) were infected intraperitoneally (i.p. thus mimicking a hematogenous route of infection) with 2400 plaque-forming units of mouse adapted neurotropic influenza A/WSN/33 virus (obtained from Dr. S Nakajima, Institute of Public Health, Tokyo, Japan) suspended in 30 µl of phosphate buffered saline (PBS; Gibco), at 3.5 cm head-to-rump length, i.e. at postnatal day (P)3 or P4. Control mice were injected similarly with PBS (n = 10 from a total of four litters).

2.3. Neonatal kynurenine treatment

Two cohorts of male and female C57BL/6 mice were i.p. injected with L-kynurenine sulfate salt (2 × 200 mg/kg/day; Sigma Aldrich), adjusted to pH ~8.2, every 12th hour for ten days to mimic the increase in brain KYNA concentration following influenza infection (Holtze et al., 2008). Administration of L-kynurenine started at P7 (first cohort; n = 8 (only males) from a total of three litters, second cohort; n = 19 males and 18 females from a total of 15 litters). Similarly, control mice were injected with saline (0.9% NaCl; first cohort; n = 10 (only males) from a total of two litters, second cohort: n = 17 males and 17 females from a total of 15 litters).

2.4. Behavioral and neurochemical assessments in adult life

Locomotor activity of influenza A virus-infected male mice and uninfected male controls was assessed at the age of 5–6 months (Fig. 1A). The L-kynurenine treated mice and saline controls were tested at the age of 3–4 months. In the first cohort of mice neonatally treated with L-kynurenine or saline and in the influenza infected mice, startle response and PPI tests were performed approximately one week prior to the locomotor activity recording. No difference in body weight was observed between influenza A virus-infected mice and uninfected controls nor between L-kynurenine treated mice and saline controls at the time of these behavior experiments. In the second cohort of mice (both males and