Neuroscience 222 (2012) 124–135

CHRONIC HYPERDOPAMINERGIC ACTIVITY OF SCHIZOPHRENIA IS ASSOCIATED WITH INCREASED ΔFOSB LEVELS AND CDK-5 SIGNALING IN THE NUCLEUS ACCUMBENS

R. CANTRUP,[a] K. SATHANANTHAM,[a] W. J. RUSHLOW[a,b] AND N. RAJAKUMAR[a,b]*

[a] *Department of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada*

[b] *Department of Psychiatry, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada*

Abstract—Chronic subcortical hyperdopaminergic activity is associated with the positive symptoms of schizophrenia and is a hallmark feature of a number of animal models of the disorder. However, the molecular changes induced by increased dopaminergic activity associated with schizophrenia are not clear. Increased levels of ΔFosB have been found in association with chronic subcortical hyperdopaminergic activity following repeated cocaine or amphetamine administration. Therefore, we investigated ΔFosB signaling in a putative neurodevelopmental animal model of schizophrenia showing chronic subcortical hyperdopaminergic activity. Increased protein levels of the transcription factor ΔFosB as well as cyclin-dependent kinase-5 (cdk-5), p35, p25 and the GluR2 subunit of the AMPA glutamate receptor were observed in the nucleus accumbens (NA). Cdk-5, p35 and GlurR2 are all proteins regulated by ΔFosB, while p25 is a degradation product of p35. Increased total protein levels of cdk-5, p35 and p25 resulted in increased cdk-5 kinase activity as determined by increased phosphorylation of dopamine and adenosine regulated phosphoprotein-32 (DARPP32) at Thr^{75} in the NA. DARPP32 Thr^{75} is selectively phosphorylated by cdk-5 and phosphorylation of DARPP32 at Thr^{75} suppresses DARPP32 activity, a critical step in the regulation of both glutamatergic and dopaminergic activity in neurons. We also found that apomorphine-induced locomotor activity was further increased following intra-accumbens infusions of roscovitine, a cdk-5 blocker, in a dose-dependent manner. Our results indicate that chronic hyperdopaminergic activity, as seen in schizophrenia, may affect glutamate and dopamine function in the NA via ΔFosB-mediated transcriptional modulation.

Key words: dopamine, cdk-5, ΔFosB, DARPP32, nucleus accumbens, schizophrenia.

INTRODUCTION

Several lines of evidence have established a link between schizophrenia and chronic hyperdopaminergic activity. First, antipsychotics all possess D_2 dopamine receptor antagonism and D_2 dopamine receptor antagonism is essential for their clinical efficacy (Seeman and Lee, 1975; Creese et al., 1976; Sigmundson, 1994). Second, amphetamine, a drug that increases extracellular dopamine levels, results in paranoid schizophrenia-like symptoms in healthy human volunteers (Angrist et al., 1974, 1980), and provokes psychosis in schizophrenic patients who are in remission (Angrist and van Kammen, 1984; Lieberman et al., 1987). Finally, *in vivo* imaging studies have shown increased basal levels of dopamine in the striatum during illness exacerbation in schizophrenic patients (Abi-Dargham et al., 1998, 2000), and an increased amphetamine-evoked dopamine release in the striatum in patients compared to controls (Laruelle et al., 1996, 1999; Breier et al., 1997; Abi-Dargham and Laruelle, 2005). Therefore, increased dopaminergic activity is a key feature of schizophrenia (Abi-Dargham and Laruelle, 2005). In agreement with the dopamine theory of schizophrenia, several putative animal models of schizophrenia show evidence of chronically elevated dopaminergic activity in the striatum and the nucleus accumbens (NA) (Robinson and Becker, 1986; Lipska et al., 1993; Uehara et al., 2000; Cyr et al., 2003; Fiore et al., 2004; Rajakumar et al., 2004). However, despite a strong correlation between chronic hyperdopaminergic activity and schizophrenia, the molecular consequences associated with increased dopaminergic activity in schizophrenia are not known.

Animal studies have shown different patterns of gene expression following acute and chronic dopaminergic activation. For example, acute dopaminergic stimulation induces immediate early gene activation leading to increased proteins levels of c-Fos, FosB, Fra1 and Fra2, while repeated or chronic dopaminergic stimulation, results in increased levels of ΔFosB, a truncated isoform of FosB (Graybiel et al., 1990; Hope et al., 1992, 1994; Chao and Nestler, 2004; Nestler, 2004, 2008. Similarly, knockout of the dopamine transporter in mice results in striatal hyperdopaminergic activity and elevated ΔFosB levels in striatal neurons (Cyr et al., 2003). Finally, neonatal ventral hippocampal (nVH)

*Correspondence to: N. Rajakumar, Department of Anatomy & Cell Biology, Western University, London, ON, Canada N6A 5C1. Tel: +1-519-661-2111x80521; fax: +1-519-661-3936.
E-mail address: nrajakum@uwo.ca (N. Rajakumar).

Abbreviations: ANOVA, analysis of variance; cdk-5, cyclin-dependent kinase-5; DARPP32, dopamine and adenosine regulated phosphoprotein-32; DISC-1, disrupted in schizophrenia-1; DMSO, dimethy sulfoxide; NA, nucleus accumbens; NUDEL, nuclear distribution protein nudE-like 1; nVH, neonatal ventral hippocampal; PKA, protein kinase A; PB, phosphate buffer; PP-1, protein phosphatase-1.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2012.07.027

lesioning creates a putative rat model of schizophrenia associated with chronic subcortical hyperdopaminergic activity and also shows increased numbers of ΔFosB-labeled neurons in the striatum (Powell et al., 2003).

ΔFosB is a transcription factor resistant to proteolytic degradation and accumulates following chronic dopaminergic activation (Chen et al., 1997; Nestler, 2008). ΔFosB dimerizes with the Jun family of transcription factors and the resulting dimmer binds to AP-1 transcriptional regulator sites in target genes (Nakabeppu and Nathans, 1991). Studies using ΔFosB over-expressing transgenic mice have identified several downstream genes that are activated by ΔFosB in NA neurons including cyclin-dependent kinase-5 (cdk-5), p35, an activator of cdk-5, and GluR2 subunit of the AMPA receptor (Kelz et al., 1999; Chen et al., 2000; McClung and Nestler, 2003).

We have previously shown that infusions of p75 neurotrophin receptor antibody conjugated to saporin into the developing prefrontal cortex in neonatal rats produce a putative neurodevelopmental model of schizophrenia (p75-PFC model; Rajakumar et al., 2004). This model is characterized by adult emergence of behavioral features reminiscent of subcortical hyperdopaminergic activity that lasts their lifetime (Rajakumar et al., 2004). The present study investigated whether ΔFosB levels are increased in the NA of p75-PFC model rats with chronic subcortical hyperdopaminergic activity compared to control animals. In addition, the downstream targets of ΔFosB were also examined to determine if there are function consequences associated with increased levels of ΔFosB.

EXPERIMENTAL PROCEDURES

Animals

Sprague–Dawley rats (Charles River, PQ, Canada) were used throughout the study. All procedures were approved by the Institutional Animal Care Committee and are in compliance with the Canadian and National Institute of Health Guides for Care and Use of Laboratory Animals (NIH Publication #80-23). Efforts were taken to reduce pain and suffering of the animals. The rats were maintained in a room with a 12-h on and 12-h off light/dark cycle and constant temperature and humidity with free access to food and water.

Neonatal lesioning

For each lesioning session, three-time pregnant mothers were purchased. On the day of birth (P0), male pups from multiple litters were randomly assigned to two of the mothers (maximum of 10 pups per mother). From each of the surrogate litters, five pups were randomly selected and lesioned and the remaining five received control infusions. Neonatal lesioning was done according to the methods described previously (Rajakumar et al., 2004). Briefly, at post-natal day 1 (P1), each pup was separated from the mother for 10 min and kept under warm light. The scalp was anesthetized with lidocaine and a 30-gauge needle connected to a Hamilton syringe by Teflon tubing was lowered through the scalp into the brain. Bilateral injections were made into the developing PFC (0.5 mm anterior to Bregma, 0.5 mm lateral to the midline and 1.5 mm deep to the skin surface), using the stereotaxic coordinates of Paxinos et al. (1994). Each pup received bilateral injections of a depot preparation of a saporin-conjugated mouse monoclonal antibody directed against the p75 neurotrophin receptor (0.75 μl/site; 192 IgG-saporin, Chemicon, Temecula, CA, USA) or sterile saline (0.75 μl/site). Following treatment, the rats were returned to their surrogate mother and raised under standard conditions until weaning. Rat pups were weaned at 21 days of age and housed two per cage.

Experimental design

Stress- or amphetamine-induced locomotor hyperactivity, a feature reminiscent of subcortical hyperdopaminergic activity, emerges in lesioned p75-PFC model rats by 8 weeks of age and persists throughout life (Rajakumar et al., 2004). Lesioned and sham control rats at 9 weeks of age were subjected to stress-induced locomotor activity. A minimum of twofold increase in locomotion compared to the mean of sham control rats was set as the threshold for a rat to be considered to be hyperdopaminergic, and included in the study. Our objective was to determine if chronic subcortical hyperdopaminergic activity is associated with changes in ΔFosB signaling, and therefore, we compared animals showing behavioral features of hyperdopaminergic activity for <2 weeks (subacute) to those showing hyperdopaminergic activity for >10 weeks (chronic). Because it is not possible to ascertain the completeness of lesioning, we decided not to include a lesion group prior to manifesting features of hyperdopaminergic activity (i.e., <8 weeks of age).

Randomly selected groups of lesioned and sham control rats at 10 weeks of age (presumably have <2 weeks of hyperdopaminergic activity) were decapitated ($n = 15$, each) and protein extracted from the NA for Western blotting or perfusion-fixed ($n = 10$, each) for histological examination. The remaining lesioned and sham control rats were allowed to survive until week 19. On week 19, they were subjected to the same stress-induced locomotion testing to verify the continued presence of hyperdopaminergic activity. Randomly selected rats at 20 weeks (presumably have >10 weeks of hyperdopaminergic activity) were decapitated ($n = 15$, each) and protein extracted form the NA for Western blotting or perfusion-fixed ($n = 10$, each) for histological examination, and results were compared to samples obtained from 10-week-old animals. The remaining rats ($n = 40$, each) were used to investigate the effect of blocking cdk-5 activity on apomorphine-induced locomotor response.

Stress-induced locomotor activity

Nine-week-old lesioned and sham control rats ($n = 90$, each) were habituated for 60 min and their locomotor activity recorded for a further 30 min in an automated open-field activity chamber (San Diego Instruments, San Diego, CA, USA). The rats were then subjected to 6 min of tail pinch as a stressor (Scornaiencki et al., 2009), and their locomotor activity was recorded for an additional 30 min. Locomotor testing was done during the light cycle, between 10:00 am and 3:00 pm. Rats were re-tested at 19 weeks of age using the same paradigm.

Apomorphine-induced locomotor activity

Groups of lesioned and control rats at 19 weeks of age ($n = 40$, each) were anesthetized with intraperitoneal injections of sodium pentobarbital (65 mg/kg; MTC Pharmacueticals, Cambridge, ON, Canada) and were placed on a Kopf stereotaxic frame. They were implanted with 21-gauge guide cannulae 1 mm over the NA bilaterally using stereotaxic coordinates (AP $+1.4$ mm and ML ± 1.8 mm from the bregma, and DV -6.8 mm; Paxinos and