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COCAINE-INDUCED PROLIFERATION OF DENDRITIC SPINES IN NUCLEUS ACCUMBENS IS DEPENDENT ON THE ACTIVITY OF CYCLIN-DEPENDENT KINASE-5

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Abstract

Repeated exposure to cocaine produces an enduring increase in dendritic spine density in adult rat nucleus accumbens. It has been shown previously that chronic cocaine administration increases the expression of cyclin-dependent kinase-5 in this brain region and that this neuronal protein kinase regulates cocaine-induced locomotor activity. Moreover, cyclin-dependent kinase-5 has been implicated in neuronal function and synaptic plasticity. Therefore, we studied the involvement of this enzyme in cocaine's effect on dendritic spine density. Adult male rats, receiving intra-accumbens infusion of the cyclin-dependent kinase-5 inhibitor roscovitine or saline, were administered a 28-day cocaine treatment regimen. Animals were killed 24–48 h after the final cocaine injection and their brains removed and processed for Golgi–Cox impregnation. Our findings demonstrate that roscovitine attenuates cocaine-induced dendritic spine outgrowth in nucleus accumbens core and shell and such inhibition reduces spine density in nucleus accumbens shell of control animals. These data indicate that cyclin-dependent kinase-5 is involved in regulation of, as well as cocaine-induced changes in, dendritic spine density.

Keywords

psychostimulant abuse; dendritic morphology; synaptic plasticity

Long-term exposure to psychostimulant drugs produces enduring neuronal alterations in intracellular signaling pathways and structural changes in dendritic morphology (Nestler, 2001). For example, repeated exposure to cocaine increases spine density on dendrites of dopaminergic medium spiny neurons in the shell (but not core) division of nucleus accumbens (NAc) (Robinson and Kolb, 1999; Robinson et al., 2001) and increases the expression of several transcription factors that mediate gene expression in these neurons (Nestler, 2001). Recently, cyclin-dependent kinase-5 (Cdk5) was identified as a downstream target of the transcription factor FosB which is persistently expressed in NAc of mice repeatedly treated with cocaine. Overexpression of FosB increases Cdk5 expression and activity (Kelz et al., 1999; Bibb et al., 2001). Cdk5 is a protein serine/threonine kinase that regulates neurite outgrowth (Dhavan and Tsai, 2001) and modulates dopamine signaling (Bibb et al., 1999a). Interestingly, acute inhibition of Cdk5 activity in NAc potentiates the locomotor effects of chronic cocaine (Bibb et al., 2001). The purpose of the present study was to determine if this neuronal protein kinase is involved in cocaine-induced dendritic spine proliferation in rat NAc.

The potent Cdk5 inhibitor roscovitine (or vehicle) was bilaterally infused into NAc shell via ALZET mini-pump. The effect of a 4-week regimen of repeated i.p. injections of cocaine (or saline) on dendritic spine density was assessed by a modified Golgi staining technique. Detailed anatomical features along dendrites were easily discernible and dendritic spines were readily resolved (Fig. 1). Repeated cocaine administration increased dendritic spine density on medium spiny neurons in the NAc shell (16.2%, $P=0.001$) and core (16.7%, $P=0.037$) in control animals receiving ALZET mini-pump infusions of phosphate-buffered saline (PBS) (Fig. 2 panel a vs. b; panels e and f). This increase was blocked in both shell ($P<0.001$; Fig. 2 panel b vs. d) and core ($P=0.011$) when roscovitine, a potent Cdk5 inhibitor, was infused by ALZET mini-pump (Fig. 2, panels e and f). Interestingly, intra-accumbens infusion of roscovitine in animals not receiving i.p. cocaine injections reduced spine density in the shell (12.4%, $P=0.014$) (Fig. 2 panel a vs. c; panel e). There were no significant differences in mean dendritic length in any of the experimental conditions; this indicates that the observed changes in spine density do not result from dendritic lengthening or shortening. Together, these data demonstrate that the ability of chronic cocaine to increase NAc spine density is dependent on the activity of Cdk5.

Previous studies have demonstrated that Cdk5 co-localizes with presynaptic proteins involved in exocytosis and synaptic transmission (Shuang et al., 1998; Fletcher et al., 1999) as well as post-synaptic D1 receptor-mediated second messenger cascades (Bibb et al., 1999a). Cdk5 co-localizes with DARPP-32 (Dopamine and cyclic AMP [cAMP] Regulated Phosphoprotein, Mr 32 kDa) in cell bodies and dendritic shafts in NAc (Bibb et al., 2001). We also examined whether Cdk5 is expressed presynaptically in NAc using laser confocal microscopy and fluorescently labeled secondary antibodies. Cdk5 and synapsin 1 were found to be co-localized to axon terminals in NAc (Fig. 3) indicating that Cdk5 is expressed both pre- and post-synaptically.

The data presented here demonstrate that infusion of the Cdk5 inhibitor roscovitine into NAc shell: (1) attenuates cocaine-induced elevations in dendritic spine density in NAc and (2) reduces spine density in the shell when administered to saline-injected controls. These

two findings demonstrate a role for Cdk5 in increasing and maintaining dendritic spine density. Previous research has shown that the activity of Cdk5 is necessary for axonal migration and dendritic outgrowth during neurogenesis (Nikolic et al., 1996). This is the first evidence, however, suggesting that Cdk5 activity regulates the outgrowth and maintenance of spines in adult neurons *in vivo*. In addition, we report increased spine density in both NAc core and shell 1–2 days after 28-day cocaine exposure, whereas at 24–25 days post-treatment this effect was seen in the shell only (Robinson and Kolb, 1999; Robinson et al., 2001).

The observed effects of the Cdk5 inhibitor roscovitine on spine density may reflect Cdk5 inhibition of several synaptic proteins expressed pre- and post-synaptically in NAc (Dhavan and Tsai, 2001). It is possible that Cdk5 is locally involved in spine outgrowth through an interaction with actin and its associated cytoskeletal proteins as in Cdk5-mediated axonal migration and dendritic morphogenesis (Humbert et al., 2000; Smith et al., 2001; Dhavan and Tsai, 2001). Presynaptically, Cdk5 binds to or phosphorylates several proteins that mediate vesicular exocytosis and synaptic transmission such as Munc-18, syntaxin 1A, and synapsin I (Matsubara et al., 1996; Fletcher et al., 1999). Thus, changes in Cdk5 activity within nerve terminals of NAc could alter neurotransmitter release that, in turn, could change spine density. Dendritic spines are highly responsive to fluctuations in dopaminergic and glutamatergic transmission (Meredith et al., 1995; Segal and Andersen, 2000; Robinson et al., 2001). Postsynaptically, Cdk5 can modulate D1/cAMP/PKA/DARPP-32 intracellular signaling and regulate NMDA receptor function (Bibb et al., 1999a,b; Li et al., 2001). Roscovitine may therefore impact cocaine-induced spine proliferation and normal spine maintenance by altering spine actin dynamics, intracellular signaling cascades, or glutamatergic and/or dopaminergic neurotransmission.

EXPERIMENTAL PROCEDURES

General methods were according to published procedures (Horger et al., 1999) and were performed in accordance with international guidelines for the ethical use of laboratory animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals used. Male Sprague–Dawley (Camm, NJ, USA) rats with food and water available *ad libitum* were housed in pairs in a climate-controlled (12-h light/dark cycle) colony. Osmotic mini-pumps (ALZET model #2004, 0.25 μ l/h) were surgically placed s.c. between the scapulae and were connected via plastic tubing (PE 60) to bilateral L-shaped cannulae (Plastics One, #3220PD) terminating in NAc. Stereotaxic coordinates for the NAc were anterior-posterior +1.7 mm from bregma, medial-lateral $\pm$ 1.0 mm and dorsoventral –7.4 mm from dura (Paxinos and Watson 1986). Pumps were loaded with roscovitine (gift from Dr Laurent Meijer, Centre National de la Recherche Scientifique) or vehicle solution (10 mM sodium phosphate, pH 7.4, 0.9% NaCl, 50% dimethyl sulfoxide) and delivered 40 nM/day/side (microinfused in 0.5 μ l over a 2-min period) into NAc shell for 28 days. The roscovitine dosage schedule was based on previous studies using intracerebral infusions of roscovitine and cAMP analogues (Punch et al., 1997; Bibb et al., 2001). Cocaine hydrochloride (15 mg/kg i.p., dissolved in 0.9% saline) was given daily.

Animals were perfused transcardially with 4% formaldehyde under deep anesthesia with sodium pentobarbital (60 mg/kg) 24–48 h after the last cocaine injection. Brains were prepared for Golgi impregnation according to previous methods (Norrholm and Ouimet, 2000).

Dendritic spines were counted on 1250× camera lucida images that included all spines observable in each focal plane occupied by the dendrite. Dendritic spines were counted along dendritic processes extending from the soma of fully impregnated medium spiny neurons in both shell and core of NAc (Fig. 1). Twenty dendritic segments (10 in each cerebral hemisphere; 50–100 µm in length) were examined in each NAc division for each rat (thus, a total of 100 dendrites were analyzed per NAc division per treatment condition). The 20 dendritic segments analyzed in each region of each animal represented three to five different neurons per cerebral hemisphere. When significant changes in dendritic spine density were observed, camera lucida images and the Zeiss CLSM measurement program were used to quantify dendritic length; this analysis was necessary to rule out changes in spine density resulting from increased or decreased dendritic length. Photomicrographs were obtained with helium-neon 633 laser and Zeiss 410 confocal laser scanning microscope. Experimenters were blind to treatment conditions as tissue slides were coded throughout the entire process of cell identification, counting, and subsequent data analysis. Data were analyzed using two-factor analysis of variance and Tukey post-hoc pairwise comparison tests ($P < 0.05$).

Immunocytochemistry was performed as previously described (Bibb et al., 2001) with a monoclonal antibody against synapsin I and a polyclonal antibody against Cdk5 (C-8, Santa Cruz Biotechnologies, Inc., Santa Cruz, CA, USA).

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Abbreviations

Cdk5	cyclin-dependent kinase-5
Coc	cocaine
DARPP-32	dopamine and cyclic AMP [cAMP] regulated phosphoprotein, Mr 32 kDa
NAc	nucleus accumbens
PBS	phosphate buffered saline
ROSC	roscovitine
Sal	saline

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Fig. 1.
Representative laser confocal photomicrograph of Golgi-impregnated medium spiny neuron from nucleus accumbens shell. Scale bar=10 μ m.

Fig. 2.

Representative laser confocal photomicrographs of dendritic processes from NAc shell of rats administered 4 week regimen of daily cocaine or saline injections while receiving intra-accumbens infusion of Cdk5 inhibitor roscovitine or PBS. Daily i.p. drug treatment is indicated above the panels and mini-pump perfusate is indicated to the left of each row in the figure (ROSC=roscovitine). Panels a–d illustrate dendritic segments from NAc shell after treatment with (a) saline + PBS, (b) cocaine + PBS, (c) saline + ROSC, or (d) cocaine + ROSC. Scale bar=10 μm . Panels e and f illustrate mean dendritic spine density in NAc (e)

shell and (f) core of animals treated with chronic cocaine (Coc) or saline (Sal) while receiving intra-accumbens infusion of ROSC or PBS. $*P<0.05$ vs. PBS/Saline, $^+P<0.05$ vs. PBS/Cocaine, $n=5$ animals per treatment group.

Fig. 3. NAc of adult male rat that has been double-immunostained for Cdk5 (red) and synapsin I (green). The distribution of Cdk5 is broader than that of synapsin I but most synapsin I-containing puncta also contain Cdk5 and are labeled yellow (arrows). Prominent colocalization (yellow) observed in many axon terminals demonstrates presynaptic expression of Cdk5. Scale bar=1 μ m.