Neuroscience 328 (2016) 147–156

METHAMPHETAMINE REDUCES EXPRESSION OF CAVEOLIN-1 IN THE DORSAL STRIATUM: IMPLICATION FOR DYSREGULATION OF NEURONAL FUNCTION

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Abstract—Role of striatal dopamine D1 receptors (D1Rs) in methamphetamine (Meth) taking and seeking is recognized from contingent Meth self-administration studies. For example, Meth increases levels of D1Rs in the dorsal striatum in animal models of Meth addiction, and blockade of striatal D1Rs decreased responding for Meth and reduced Meth priming-induced drug seeking. However, the mechanism underlying enhanced expression of striatal D1Rs in animals self-administering Meth is unknown and is hypothesized to involve maladaptive intracellular signal transduction mechanism via hyperphosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2). D1Rs are predominantly localized to detergent-resistant membrane/lipid raft fractions (MLR fraction), and *in vitro* studies indicate that D1R signaling and recycling is regulated by the MLR-resident protein caveolin-1 (Cav-1), in an endocytotic-dependent manner. Notably, expression of Cav-1 is inversely regulated by ERK1/2 activation, suggesting a signaling interplay among D1Rs, ERK1/2 and Cav-1. We therefore evaluated the effects of extended access Meth self-administration on expression of striatal D1Rs, activated ERK1/2 and Cav-1. We first report that Cav-1 is heavily expressed in neurons located in the dorsal striatum. We also report that extended access Meth produces compulsive-like unregulated intake of the drug, and these behavioral outcomes are associated with enhanced expression of D1Rs, increased activity of ERK1/2, and reduced Cav-1 expression in the dorsal striatum. These data suggest a possible cellular mechanism that involves Cav-1 regulation of D1R expression in response to escalated Meth intake, and how this response of altered D1Rs and enhanced ERK1/2 activation to Meth self-administration contributes to contingent-related processes such as addiction.

Key words: psychostimulant, animal models, neurotoxicity, dopamine D1 receptor, caveolin, ERK.

INTRODUCTION

Dopamine D1 receptors (D1Rs) are most abundant in the striatum (dorsal and ventral striatum) and D1Rs in the dorsal striatum are expressed in the medium-sized spiny striatonigral neurons (MSNs; (Baik, 2013)). Pharmacological studies indicate that Meth (via noncontingent administration) acts as an indirect D1R agonist and produces its rewarding effects through D1Rs in the striatum. For example, D1R antagonism (via SCH23390, a potent D1R antagonist (Millan et al., 2001)) prevented Meth-induced maladaptive intracellular signal transduction mechanism via hyperphosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), and inhibition of ERK1/2 activity with 2'-amino-3'-methoxyflavone (PD98059; MAPK kinase inhibitor) reduced rewarding properties of Meth (Mizoguchi et al., 2004). These pharmacological studies implicate a strong role of striatal D1Rs in rewarding properties of Meth (Gross et al., 2011), suggesting that alterations in D1Rs could also accelerate the development of Meth addiction-like behavior (Ares-Santos et al., 2012; Ares-Santos et al., 2013; Toriumi et al., 2014). In support of this hypothesis, contingent Meth via self-administration increases levels of D1R in the dorsal striatum in animal models and human subjects (Worsley et al., 2000; Segal et al., 2005), and blockade of D1Rs in animal models decreased Meth self-administration and reduced Meth priming-induced drug seeking (Bardo et al., 1999; Brennan et al., 2009; Carati and Schenk, 2011). Therefore, it appears that systemic D1R antagonism is neuroprotective, which may correlate with reduced motivation to seek Meth. However, the cellular mechanisms regulating the expression of D1Rs in the dorsal striatum in Meth self-administering animals are unknown. Novel evidence suggests a non-canonical role of membrane lipid raft (MLR) proteins, such as caveolin-1 (Cav-1), in the regulation D1R function (Kong et al., 2007; Voulalas et al., 2011).

In the context of the above hypothesis, Cavs are cholesterol-binding and scaffolding proteins within

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Abbreviations: ANOVA, analysis of variance; Cav-1, caveolin-1; D1Rs, dopamine D1 receptors; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; ERK1/2, extracellular signal-regulated kinase 1/2; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; Meth, methamphetamine; MLR, membrane/lipid raft; MSNs, medium-sized spiny striatonigral neurons.

http://dx.doi.org/10.1016/j.neuroscience.2016.04.039
0306-4522/

MLRs in neurons, and function as organizers of plasmalemmal signaling molecules including G-protein-coupled receptors (assist with receptor internalization and trafficking to the recycling endosomes) (Head et al., 2014; Egawa et al., 2015). Cavs are present in three isoforms (Williams and Lisanti, 2004). Cav-1, -2 and -3 are expressed in the central nervous system (Shin et al., 2005), and Cav-1 regulates neuronal signaling (Head et al., 2008; Head et al., 2011). Cavs can inhibit signaling via protein–protein interactions (Feron et al., 1998). When up-regulated or overexpressed, Cavs promote signaling via enhanced receptor-effector coupling or enhanced receptor affinity (Feron and Balligand, 2006; Head et al., 2011). Cav-1 is involved in multiple cellular processes, including vesicular transport, cholesterol and calcium homeostasis, and signal transduction (Yamamoto et al., 1998). Cav-1 also provides temporal and spatial regulation of signal transduction of D1Rs (Kong et al., 2007; Voulalas et al., 2011). For example, D1Rs are predominantly localized to detergent-resistant membrane fractions (MLR fraction) demonstrating characteristics of proteins associated with Cavs (Voulalas et al., 2011). Furthermore, D1R function (signaling and turnover) is regulated by Cav-1-dependent endocytosis (Kong et al., 2007). Cav-1-dependent D1R internalization is dependent on the integrity of MLRs, because disruption of MLRs (through Cav-1 deficiency) reduces agonist (dopamine)-mediated sequestration of D1R (Kong et al., 2007). However, whether Cav-1 is expressed in the dorsal striatum in neuronal cells is unknown and could assist with defining the role of Cav-1 in regulating Meth-induced dysregulation of D1Rs in the dorsal striatum.

In vitro and *in vivo* studies have begun elucidating the signaling pathways that govern Cav-1 expression. Mechanistic studies conducted *in vitro* in non-neuronal cells demonstrate that Cav-1 gene and protein expression are regulated by ERK1/2 activity, where hyperphosphorylation of ERK1/2 downregulated Cav-1 gene and protein expression by inhibiting Cav-1 promoter activity (Engelman et al., 1999). Notably, the effects of ERK1/2 were abolished by pretreatment with PD98059, suggesting that there is negative regulation between expression of Cav-1 and hyperphosphorylation of ERK1/2 (Engelman et al., 1999). In support of this negative regulation, subsequent *in vivo* study using loss-of-function approaches demonstrated that ERK1/2 is hyperphosphorylated in Cav-1 knockout mice (Cohen et al., 2003), suggesting that Cav-1 can act as an inhibitor of signaling cascades involving ERK (Engelman et al., 1998; Galbiati et al., 1998). Furthermore, Cav-binding motifs are present within the kinase domains of ERKs and several other kinases involved in G-protein-coupled signaling (Couet et al., 1997), suggesting a general pattern of negative regulation between Cav-1 and receptor kinases, particularly, ERK1/2 (Okamoto et al., 1998). Therefore, the current study investigated the expression profiles of Cav-1, in the dorsal striatum and examined the alterations in the expression of D1Rs and Cav-1 and activation of ERK1/2 in the dorsal striatum in rats that self-administered Meth via an extended access schedule of reinforcement.

EXPERIMENTAL PROCEDURES

Animals

Surgical and experimental procedures were carried out in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85–23, revised 1996) and approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. Thirty-eight adult, male Wistar rats (Charles River), weighing 200–250 g at the start of the experiment, were housed two per cage in a temperature-controlled vivarium under a reverse light/dark cycle (lights off 8:00 am–8:00 pm) and completed the study.

Neurotoxic regimen of Meth (noncontingent Meth)

Rats received systemic injections of Meth [Meth hydrochloride, generously provided by the National Institute on Drug Abuse; (4 mg/kg, s.c.; in saline; $n = 4$)], four times at 2-h intervals (Gross et al., 2011). The room where the rats were injected was maintained at an ambient room temperature of 23 ± 1 °C. Forty-five minutes after the last injection, rats were euthanized by rapid decapitation and brain tissue (right hemisphere) was processed for Western blotting.

Surgery for implanting jugular vein catheters

Nineteen rats underwent surgery for catheter implantation for intravenous self-administration. Rats were anesthetized with 2–3% of isofluorane mixed in oxygen and implanted with a sterilized silastic catheter into the right jugular vein under aseptic conditions. The distal end of the catheter was threaded under the skin to the back of the rat and exited the skin via a metal guide cannula (Galinato et al., 2014). Post surgery care was provided with analgesics (Flunixin) and antibiotics (Cefazolin (Sobieraj et al., 2014)). Catheters were flushed daily with heparinized saline and tested for patency using methohexital sodium (Brevital; (Galinato et al., 2014)).

Training and maintenance on an extended access schedule (contingent Meth)

Four to five days after surgery rats ($n = 19$) were trained to press a lever according to an FR1 schedule of Meth reinforcement (0.05 mg/kg/injection of Meth for every correct response) in operant boxes (Med Associates, St. Albans, VT, USA) under extended access conditions (6 h access per day for 17 days). During daily sessions, a response on the active lever resulted in a 4-s infusion (90–100 μl of Meth), followed by a 20-s time-out period to prevent overdose. Each infusion was paired for 4 s with white stimulus light over the active lever (conditioned stimulus [CS]). Response during the time-out or on the inactive lever was recorded but resulted in no programed consequences. All animals were housed