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Nucleus accumbens shell excitability is decreased by methamphetamine self-administration and increased by 5-HT_{2C} receptor inverse agonism and agonism

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ABSTRACT

Methamphetamine profoundly increases brain monoamines and is a widely abused psychostimulant. The effects of methamphetamine self-administration on neuron function are not known for the nucleus accumbens, a brain region involved in addictive behaviors, including drug-seeking. One therapeutic target showing preclinical promise at attenuating psychostimulant-seeking is 5-HT_{2C} receptors; however, the effects of 5-HT_{2C} receptor ligands on neuronal physiology are unclear. 5-HT_{2C} receptor agonism decreases psychostimulant-mediated behaviors, and the putative 5-HT_{2C} receptor inverse agonist, SB 206553, attenuates methamphetamine-seeking in rats. To ascertain the effects of methamphetamine, and 5-HT_{2C} receptor inverse agonism and agonism, on neuronal function in the nucleus accumbens, we evaluated methamphetamine, SB 206553, and the 5-HT_{2C} receptor agonist and Ro 60-0175, on neuronal excitability within the accumbens shell subregion using whole-cell current-clamp recordings in forebrain slices *ex vivo*. We reveal that methamphetamine self-administration decreased generation of evoked action potentials. In contrast, SB 206553 and Ro 60-0175 increased evoked spiking, effects that were prevented by the 5-HT_{2C} receptor antagonist, SB 242084. We also assessed signaling mechanisms engaged by 5-HT_{2C} receptors, and determined that accumbal 5-HT_{2C} receptors stimulated G_q, but not $G_{i/o}$. These findings demonstrate that methamphetamine-induced decreases in excitability of neurons within the nucleus accumbens shell were abrogated by both 5-HT_{2C} inverse agonism and agonism, and this effect likely involved activation of G_q–mediated signaling pathways.

1. Introduction

Psychostimulants increase synaptic concentrations of monoamines and neuronal maladaptations can be a persistent consequence of repeated administration (for review, see (Kauer and Malenka, 2007)). Some maladaptations reflect changes in serotonin (5-HT) systems (Napier and Istre, 2008; Krasnova et al., 2010; Reichel et al., 2012) and 5-HT_{2C} receptors (Rs) are likely involved. For example, chronic methamphetamine (meth) increases neuronal sensitivity in the limbic (ventral) pallidum to systemic administration of a 5-$HT_{2A/2C}$R agonist (Napier and Istre, 2008). Moreover, agonists at these receptors attenuate cocaine-associated reinstatement (Grottick et al., 2000; Fletcher et al., 2002; Neisewander and Acosta, 2007; Burbassi and Cervo, 2008; Cunningham et al., 2011), self-administration (Grottick et al., 2000; Fletcher et al., 2002; Cunningham et al., 2011), as well as behavioral hyperactivity and sensitization (Grottick et al., 2000; Filip et al., 2004). 5-HT_{2C}Rs are constitutively active in rat brain (De Deurwaerdere et al., 2004), antagonists or inverse agonists with high affinity for 5-$HT_{2A/2C}$Rs nullify meth-induced neuronal sensitization (McDaid et al., 2007), and the putative 5-HT_{2C}R inverse agonist SB 206553 (SB206) reduces meth-seeking and meth-evoked motor activity (Graves and Napier, 2012).

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5-HT_{2C}R inverse agonists and agonists exhibit similar behavioral effects in rats (Graves and Napier, 2012; Navailles et al., 2013). In contrast, neurochemical studies show that inverse agonists *increase* accumbal and striatal dopamine (De Deurwaerdere et al., 2004), whereas agonists have *no effect, or decrease* dopamine (Di Matteo et al., 1998; Willins and Meltzer, 1998; Di Matteo et al., 2000; Gobert et al., 2000). Discord also exists regarding 5-HT_{2C}R-mediated function at the cellular level; inverse agonists *decrease* 5-HT_{2C}R-linked second messengers (e.g., basal phospholipase (PL) PLC, PLA2 and $G_{i/o}$ activity) (Berg et al., 1999; De Deurwaerdere et al., 2004; Berg et al., 2006,, 2008a; 2008b; Labasque et al., 2010), but *increase* surface expression of 5-HT_{2C}Rs (Marion et al., 2004; Chanrion et al., 2008). However, after incubation with an inverse agonist, responses to serotonin are *enhanced* (Berg et al., 1998a; Marion et al., 2004; Chanrion et al., 2008), presumably due to the increased surface expression. Pleiotropic signaling further complicates 5-HT_{2C}R function. The canonical pathway involves G_q stimulation (Cussac et al., 2002), yet the receptors are also reported to signal *via* $G_{i/o}$ and G_{13} proteins as well as non-G protein coupled pathways (Berg et al., 1998b; Cussac et al., 2002; McGrew et al., 2002; De Deurwaerdere et al., 2004; Werry et al., 2005; Labasque et al., 2008, 2010).

To advance understanding of 5-HT_{2C}Rs on meth-induced neuronal function and to provide insights into signaling mechanisms engaged in medium spiny neurons (MSNs) of the nucleus accumbens by these receptors, we used electrophysiological and biochemical approaches to evaluate 5-HT_{2C}Rs from rats trained to self-administer meth. Based in part on our prior demonstration that SB206 attenuates meth-seeking behavior (Graves and Napier, 2012), we hypothesized that acute 5-HT_{2C}R inverse agonism opposes meth-induced adaptations in the nucleus accumbens shell and that 5-HT_{2C}Rs in the nucleus accumbens engage the canonical G_q pathway.

2. Materials and methods

2.1. Animals

Seventy-seven male Sprague–Dawley rats were purchased from Harlan (Indianapolis, IN). Subjects were housed in pairs, acclimated to the vivarium for 5 days, and handled a minimum of 3 times prior to the surgical procedures required for self-administration protocols. Food and water were provided *ad libitum* throughout the study. Rats were maintained in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and with approval of the Rush University Institutional Animal Care and Use Committee. All efforts were made to minimize suffering and reduce the number of animals used.

2.2. Test drugs

(+)-Methamphetamine HCl (meth; Sigma, St. Louis, MO) was dissolved in sterile saline. The stimulant was self-administered at 0.1 mg/kg/0.1 ml intravenously (iv). Ro 60-0175 (Ro), SB 206553 (SB206), and SB 242084 (SB242) were purchased from Tocris Bioscience (Ellisville, MO), dissolved in ddH_20 as 1.0 mM stock solutions, and added at appropriate concentrations to artificial cerebrospinal fluid (aCSF) for electrophysiological studies, or assay buffer for biochemical studies.

2.3. Intravenous catheter implantation

Isoflurane-anethetized rats ($n = 77$) were instrumented as previously described (Graves and Napier, 2011) with custom built catheters constructed using silastic tubing (0.3 mm i.d. × 0.64 mm o.d.; Dow Corning Co., Midland, MI) and implanted into the right jugular vein. The distal end of the catheter extended to the mid-scapular region with a metal guide canulae (22 gauge; Plastics One Inc., Roanoke, VA) and anchored to a plastic mesh. Rats were allowed to recover for a minimum of 5 days prior to initiating self-administration procedures.

2.4. Self-administration

Forty-one rats were trained to self-administer meth for 3hr/day for 14 days in operant chambers enclosed in ventilated, sound-attenuating cabinets (Med-Associates, St. Albans, VT). Each operant chamber contained two levers; the left lever was assigned as the "active" lever and the right lever was assigned as the "inactive" lever. Above each lever was a "cue" light, and located on the opposite wall was an "in-house" light. The cue light above the active lever was illuminated when the infusion pump was activated. The in-house light was subsequently illuminated for 20 s, indicating a "time-out" period during which responses had no programmed consequences. Responding on the inactive lever had no programmed consequences. On protocol days 1–7, rats self-administered meth for 3hr/day on a fixed ratio (FR) 1 schedule of reinforcement; on days 8–14 rats self-administered on a FR5 for 3hr/day. The described paradigm was chosen based on our prior studies demonstrating stable and consistent self-administration, as well as the ability of mirtazapine (Graves and Napier, 2011) and SB206 (Graves and Napier, 2012) to attenuate meth-seeking behavior.

Thirty-six saline-yoked rats were used as controls. These subjects were implanted with jugular vein catheters as described for the meth self-administration protocol; 0.1 ml infusions of saline were administered with cue and time out lights triggered according to the behavioral pattern of a meth self-administering rat (lever pressing had no programmed consequences). A total of 4 rats died after a behavioral test session (2 saline-yoked and 2 meth self-administering rats).

2.5. Electrophysiological experiments

On protocol days 15–18 (1–4 days after last behavioral test), rats were anesthetized with chloral hydrate (400 mg/kg, ip) and transcardially perfused with 60 mL of an ice cold, modified aCSF containing (in mM): 248 sucrose, 2.9 KCl, 2.0 $MgSO_4$, 1.25 NaH_2PO_4, 10.0 glucose, 26.0 $NaHCO_3$, 0.1 $CaCl_2$, 3.0 kynurenic acid, and 1.0 ascorbic acid (225–235mOsm, pH = 7.4). Forebrain coronal slices (300 μm thick) containing the nucleus accumbens shell were cut with a vibrating microtome (Leica VT 1000S; Leica Microsystems Inc., Buffalo Grove, IL), and transferred to a holding chamber containing normal aCSF (in mM: 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 15 glucose, and 2 $CaCl_2$; 305–315mOsm, pH = 7.4) with 1 mM ascorbic acid at room temperature. A gas mixture of 95% O_2/5% CO_2 was constantly bubbled for a minimum of 45 min prior to beginning patch clamp experiments. The nucleus accumbens shell was selected for study based on evidence for constitutively active 5-HT_{2C}Rs (Navailles et al., 2006) and its involvement in addiction (Di Chiara, 2002; Di Chiara et al., 2004). An early period of withdrawal was selected for study based on the ability of mirtazapine (Graves and Napier, 2011) and SB206 (Graves and Napier, 2012) to attenuate meth-seeking, and presence of psychostimulant-induced plasticity (Kourrich and Thomas, 2009) during this period.

Slices containing the nucleus accumbens shell were moved from the holding to the recording chamber which was perfused at a rate of 2 ml/min by normal aCSF and held at 34 °C. Neurons were visualized using an Olympus BX51WI upright microscope (Olympus Tokyo, Japan) and targeted under a 40X water-immersion objective, differential interference contrast, and infrared filter. The image from the microscope was enhanced using an IR-1400 DAGE-MIT (Michigan City, IN) camera and displayed on a computer monitor. Electrodes were pulled with a P-97 or P-1000 micropipette puller (Sutter Instruments, Novato, CA) to a resistance of 4–6 MΩ and filled with a K^+/gluconate-based internal solution (in mM: 0.1 EGTA, 120.0 K^+ gluconate, 10.0 HEPES, 20.0 KCl, 2.0 $MgCl_2$, 3.0 Na_2ATP, and 0.3 NaGTP; 280–285mOsm, pH = 7.3). Whole-cell current clamp recordings were obtained using a Multiclamp 700B (Molecular Devices, Instruments, Sunnyvale, CA). Signals were digitized by a Digidata 1320 A/D converter (Axon Instruments) and stored on-line using pClamp 9 software (Axon Instruments). All neurons included in the analyses were medium spiny neurons (MSNs) that met the criteria of a resting membrane potential more negative than −70 mV and action potential peak greater than 60 mV under basal conditions. MSNs were readily identifiable by their mid-sized soma and electrophysiological characteristics including hyperpolarized resting membrane potentials, latency before the first action potential at rheobase, and absence of I_h current (Wilson and Groves, 1980; Dong et al., 2006). The recording protocol consisted of 500 ms pulses of current beginning at −0.5 nA with increment steps of 50 pA. Active and passive membrane properties were measured at the first action potential evoked by the rheobase (i.e., lowest current generating an action potential). Input resistance (R_{in}) was measured at −0.2 nA current injection; data to construct the current–voltage curves were obtained at 400 ms from the start of the current pulse.

MSN excitability was tested under one of the following conditions: (i) ascending concentration-response assessment of 0.1, 1.0, and 10.0 μM of the 5-HT_{2C} inverse agonist SB 206553 (SB206); (ii) 1.0 μM of the 5-HT_{2C} antagonist SB 242084 (SB242) followed by 1.0 μM SB242 + 10.0 μM SB206; (iii) ascending concentration-response assessment of 0.1, 1.0, and 10.0 μM of the 5-HT_{2C} agonist Ro 60-0175 (Ro); (iv) 1.0 μM SB242 followed by 1.0 μM SB242 + 10.0 μM Ro. Prior to treatment, neurons were recorded while normal aCSF was perfused to establish a baseline (basal). Each drug concentration tested was perfused for at least 5 min before collecting concentration-related data. Whole-cell pipette series resistance was less than 20 MΩ, and compensation for bridge resistance was monitored throughout experiments.

Pilot studies were conducted with accumbal shell slices from untreated naïve rats to determine the following: (i) Baseline stability. This was accomplished by monitoring the active and passive membrane properties during 30 min of aCSF perfusion. For example, in neurons from naïve rats the resting membrane potential varied by less than 1% over a 30 min period with continual aCSF perfusion (data not shown). (ii) Stability of responses to 5-HT_{2C} ligands. This was accomplished by monitoring recordings during continual perfusion of 10.0 μM of Ro or SB206. For example, in naïve rats the resting membrane potential varied by less than 1% with continual Ro perfusion (data not shown).