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Interactions between the mGluR2/3 agonist, LY379268, and cocaine on *in vivo* neurochemistry and behavior in squirrel monkeys

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ABSTRACT

Recent evidence indicates that group II metabotropic glutamate receptors (mGluR2 and mGluR3) may play a role in the pathology of cocaine addiction. The purpose of the current study was to determine the effects of the mGluR2/3 agonist, LY379268, on cocaine-induced changes in DA neurochemistry in nonhuman primates. Furthermore, the current study aimed to determine if changes in DA neurochemistry would correlate with LY379268-induced changes in the behavioral effects of cocaine. *In vivo* microdialysis was conducted in conscious squirrel monkeys ($n=4$) in order to monitor cocaine-induced changes in extracellular DA in the caudate nucleus. Separate groups of subjects were trained on a fixed-interval schedule of stimulus termination ($n=4$) or a second-order schedule of cocaine self-administration ($n=5$) to characterize the behavioral-stimulant and reinforcing effects, respectively. LY379268 significantly attenuated cocaine-induced increases in DA. LY379268 also significantly attenuated cocaine-induced behavioral-stimulant effects following a short pretreatment time, but not following a longer pretreatment time. Cocaine self-administration was significantly attenuated but only at an intermediate pretreatment dose of LY379268. Moreover, reinstatement of previously extinguished cocaine self-administration was not significantly attenuated by LY379268. Hence, drug interactions on neurochemistry did not correlate well with behavioral measures.

1. Introduction

Glutamate is the major excitatory neurotransmitter and essential for many functions in the mammalian central nervous system (Watkins, 2000). Glutamate receptors can be divided into two classes: ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs) (Conn and Pin, 1997; Nakanishi, 1994; Nakanishi et al., 1998). The G-protein coupled mGluRs have the capacity to modulate the actions of the ionotropic glutamate receptors as well as other neurotransmitter receptors, which make them good candidates for pharmacotherapeutic targets (Pin and Duvoisin, 1995). Eight mGluR subtypes have been identified and classified into three distinct groups based on pharmacology and sequence homology. Group I mGluRs (mGluR1 and 5) are positively coupled to phospholipase C. Group II mGluRs (mGluR2 and 3) and III (mGluR4, 6, 7 and 8) are negatively coupled to adenyl cyclase. Group II mGluRs are primarily localized perisynaptically on presynaptic neurons and function as autoreceptors to regulate neurotransmitter release. Group II mGluRs have been associated with several neurological and psychiatric disorders including anxiety (Grillon et al., 2003; Muly et al., 2007), schizophrenia and PCP-induced psychosis (Gupta et al., 2005; Moghaddam, 2004), and psychostimulant abuse (Peters and Kalivas, 2006; Xi et al., 2002a).

Chronic administration of cocaine can alter glutamate neurotransmission. In rats chronically treated with cocaine, there is a reported decrease in extracellular glutamate concentrations in the nucleus accumbens (Baker et al., 2003; Pierce et al., 1996). The reduction in basal levels of glutamate is associated with enhanced release of glutamate by an acute infusion of cocaine and may play an important role in cocaine-induced reinstatement of previously extinguished self-administration behavior (Baker et al., 2003). A possible mechanism for the observed augmentation of glutamate release may be the decreased stimulation of mGluR2/3 autoreceptors by extracellular glutamate. Therefore, it may be therapeutically beneficial to reduce glutamatergic signaling by stimulating these receptors.

In rats, mGluR2/3s are highly expressed in many regions of the brain including (but not restricted to) the prefrontal cortex, ventral tegmental

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area, nucleus accumbens and striatum, areas which have been shown to play a pivotal role in addiction (Ohishi et al., 1994; Ohishi et al., 1993; Testa et al., 1998; Xi et al., 2002b). Studies have demonstrated a significant glutamatergic tone on mGluR2/3 receptors to suppress dopaminergic release in the nucleus accumbens (Hu et al., 1999). Furthermore, activation of mGluR2/3 receptors decreased basal DA and the magnitude of DA response to systemic phencyclidine administration (Greenslade and Mitchell, 2004). Activation of mGluR2/3 receptors also attenuated behavioral responses believed to be mediated via dopaminergic pathways. Amphetamine-induced increases in locomotor activity were attenuated by mGluR2/3 agonist treatment (David and Abraini, 2003), while locomotor activity was enhanced by selective mGluR2/3 antagonists (O'Neill et al., 2003). Furthermore, mGluR2/3 agonists attenuated amphetamine self-administration in rats (Kim et al., 2005) and cocaine self-administration in nonhuman primates (Adewale et al., 2006), and blocked cue-induced reinstatement of extinguished heroin self-administration in rats (Bossert et al., 2005) and drug-primed reinstatement of extinguished cocaine self-administration in rats (Peters and Kalivas, 2006) and nonhuman primates (Adewale et al., 2006). The current studies characterized the effects of the mGluR2/3 agonist, LY379268, on cocaine-induced increases in extracelluar DA in conscious nonhuman primates, as well as on the behavioral-stimulant and reinforcing effects of cocaine. It was hypothesized that pharmacological activation of mGluR2/3 would result in an attenuation of cocaine-induced increases in DA and this attenuation would be associated with decreased behavioral-stimulant and reinforcing properties of cocaine.

2. Methods

2.1. Subjects

Fourteen male adult squirrel monkeys (*Saimiri sciureus*) (5–15 years) weighing between 800 and 1200 g served as subjects. Between experimental sessions, subjects were individually housed and allowed access to food twice daily (Harlan Teklad monkey chow; Harlan Teklad, Madison, WI; fresh fruit and vegetables) and had access to water ad libitum. All subjects had served in previous experiments involving acute administration of cocaine and monoaminergic drugs. These studies were conducted in strict accordance with the NIH "Guidelines for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Emory University.

2.2. Apparatus

Experimental sessions were conducted daily in a ventilated, sound-attenuating chamber in which each monkey was seated comfortably in a commercially available primate chair (Modular Primate Chair, Med Associates Inc, St. Albans, VT). The front wall of the chair facing the monkey was equipped with a response lever and red and white lights. The response lever registered responses monitored by computers and integrated circuitry interfaced with the chambers. Typically, operant behavioral sessions lasted 1 h and were conducted five days a week. Drug time-course determinations lasted one and a half hours. *In vivo* microdialysis sessions were limited to 5 h and were conducted no more frequently than once per week in individual animals.

2.3. Surgical procedures

For *in vivo* microdialysis experiments, subjects (s159, s168, s175, and s181) were implanted with bilateral guide cannulae (CMA/11, CMA/Microdialysis, North Chelmsford, MA) using stereotaxic procedures to target the caudate nuclei utilizing sterile surgical procedures (Czoty et al., 2000). Coordinates for the stereotaxic procedures were obtained from a standard squirrel monkey atlas (Gergen et al., 1962) (from the earbar: A/P + 15.0, L/M ± 3.0). Subjects were initially sedated using a cocktail of 0.1 mg/kg atropine, 20 mg/kg ketamine, and 2 mg/kg Telazol to prepare the animal for surgery and position the subject's head in the stereotaxic frame. Anesthesia was maintained with isoflurane (1–2%) during the surgery. Nylon screws (2.4 mm, Plastics One, Inc., Roanoke, VA) were implanted surrounding the guide cannulae and the preparation was secured in place with dental cement (Plastics One, Inc., Roanoke, VA). After the surgery, a stainless steel stylet was placed in each guide cannulae to protect the site when not in use. Subjects were allowed two to three weeks to recover before the experiments were initiated.

Subjects involved in behavioral protocols in which drugs were administered i.v. were surgically implanted with a chronic indwelling PVC catheter (0.38 mm ID; 0.76 mm OD) as described previously (Howell and Wilcox, 2001). Subjects were anesthetized with 0.1 mg/kg atropine, 20 mg/kg ketamine, and 2 mg/kg Telazol, and anesthesia was maintained by ketamine supplements during the surgery. The catheter was implanted in either a right or left femoral or external jugular vein to the level of the right atrium using sterile surgical techniques. The catheter was routed subcutaneously to the interscapular region and exited the skin under a protective nylon-mesh jacket. The catheter was filled with heparinized saline and sealed with a stainless-steel obturator when not in use. Subjects were allowed to recover for one week before beginning any experiments. If the catheter became occluded or damaged, the catheter was removed and another catheter was implanted in the same vein if possible, or another vein.

2.4. Microdialysis procedures

Teflon tubing passed through the ceiling of the chamber to connect the probe implanted in the monkey to the perfusion pump. In order to prevent the subject from disturbing the probe or tubing, a Lexan plate was positioned perpendicularly to the medial plane of the body just above the shoulders. Commercially available probes (CMA/11) with a shaft length of 14 mm and active dialysis membrane measuring 4 × 0.24 mm were inserted into the guide cannulae. A Harvard PicoPlus microinfusion pump continuously flushed artificial cerebrospinal fluid (1.0 mM Na_2HPO_4, 150 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 0.15 mM ascorbic acid) through the probes via FEP Teflon tubing to the probe at a flow rate of 2.0 µL/min for the duration of the experiment. Following a 60-min equilibration, four consecutive 10-min samples were collected for determination of baseline DA concentration. Following collection of baseline samples, saline or LY379268 was administered i.m. Three 10-min samples were collected after the pretreatment, allowing for a 30-min pretreatment before administering saline or cocaine. After cocaine or saline was administered i.m., samples were collected every 10 min for 2 h. All samples were collected in microcentrifuge tubes and immediately refrigerated. Probes were tested *in vitro* to determine the suitability of probe efficiency and performance before and after each experiment. Subjects were tested no more frequently than once per week and each site was accessed no more frequently than once every 2 weeks. Under the conditions described, consistent responses to drug treatments have been observed following repeated access to the caudate without significant gliosis (Czoty et al., 2000).

Small-bore, high-performance liquid chromatography (HPLC) and electrochemical detection quantified levels of DA according to previously established protocols (Kimmel et al., 2005; Kimmel et al., 2007). The HPLC system consisted of a small-bore (3 mm i.d. × 100 mm) column (5 µm C_{18} stationary phase; Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA) with a commercially available mobile phase (ESA, Inc., Chelmsford, MA) delivered by an ESA 582 solvent delivery pump at a flow rate of 0.6 mL/min. After loading onto the refrigerated sample tray, samples (20 µl) were automatically mixed with 3 µl of ascorbate oxidase, and 5 µl of the mixture was injected into the HPLC system by an ESA model 542 autosampler. Samples were