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The role of adenylyl cyclase in the medial prefrontal cortex in cocaine-induced behavioral sensitization in rats

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ABSTRACT

Repeated exposure to cocaine progressively increases drug-induced locomotor activity, which is termed behavioral sensitization. Previous research has demonstrated that in the medial prefrontal cortex (mPFC) modulation of cocaine-induced motor activity by agonists of G_i-coupled receptors, such as dopamine D_2, $GABA_B$ and Group II metabotropic glutamate receptors, is reduced in sensitized animals, suggesting a loss in receptor function. Stimulation of each of these receptors acts in part to inhibit adenylyl cyclase activity, and thus, the formation of cAMP. The present studies tested the hypothesis that intra-mPFC inhibition of adenylyl cyclase by infusion of an inhibitor, SQ22536, could bypass the loss of inhibitory receptor function seen in this region, and thereby inhibit the expression of cocaine sensitization. Additional studies determined whether activation of mPFC adenylyl cyclase with NKH 477 could enhance the motor-stimulant response to cocaine. Initial studies demonstrated that cocaine-induced (15 mg/kg, i.p.) motor activity was dose-dependently reduced by injection of SQ22536 (5-75 nmol/side) into the mPFC, whereas NKH 477 (1.25–40 nmol/side) produced no significant effects. Additional studies showed that intra-mPFC injection of SQ22536 (50 nmol/side) attenuated the initiation of cocaine-induced behavioral sensitization and blocked the expression of sensitization following 1, 7 or 30 days of abstinence from cocaine. Also, intra-mPFC injection of NKH 477 enhanced cocaine-induced behavioral sensitization following 21 days of abstinence from cocaine. The results of the present study suggest modulation of adenylyl cyclase in the medial prefrontal cortex plays a key role in the expression of cocaine sensitization.

1. Introduction

Repeated administration of psychostimulants such as cocaine can induce a progressive enhancement in locomotor activity, which is termed behavioral sensitization (Downs and Eddy, 1932; Steketee, 2005). Numerous studies have demonstrated that sensitization, which is a long-lasting phenomenon, shares similar neuroadaptations that underlie relapse to compulsive drug-seeking and drug-taking behavior (Steketee and Kalivas, 2011). Thus, much research has been undertaken to elucidate the neural mechanism underlying cocaine-induced sensitization. It has been shown that the mesocorticolimbic dopamine system plays an important role in development of cocaine-induced sensitization (Steketee, 2005). The mesolimbic and mesocortical divisions of this system are each comprised of dopaminergic cell bodies within the ventral tegmental area (VTA) that project to several limbic and cortical regions, including the nucleus accumbens and medial prefrontal cortex (mPFC), respectively (Oades and Halliday, 1987). It is suggested that the VTA is the site of early and transient neuroadaptations associated with the initiation of behavioral sensitization while the nucleus accumbens is the site of persistent drug-induced changes that may underlie the expression of sensitization (Kalivas et al., 1993; Kalivas and Stewart, 1991). In addition to the VTA and nucleus accumbens, the role of the mPFC in sensitization has also received attention because of its intricate involvement in the oversight of the mesolimbic system (Steketee, 2003, 2005).

The mPFC receives dopaminergic projections from the VTA (Lindvall et al., 1978) and glutamatergic projections from the VTA and other principal components of the mesocorticolimbic system including the amygdala and hippocampus (Bacon et al., 1996; Jay et al., 1996; Yamaguchi et al., 2011). In addition, the mPFC provides reciprocal excitatory glutamatergic output to the regions discussed above along with and nucleus accumbens (Carr and

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Sesack, 2000; Omelchenko and Sesack, 2007; Sesack et al., 1989). In addition to afferent and efferent connections of the mPFC this region also contains GABAergic local circuit neurons (Retaux et al. 1992, 1993). Previous studies demonstrated that dopamine D_2, $GABA_B$ and Group II metabotropic glutamate (mGluR) receptors within the mPFC are capable of modulating cocaine-induced locomotion (Beyer and Steketee, 2000, 2002; Steketee and Beyer, 2005; Xie and Steketee, 2009). However, in animals with a history of repeated cocaine exposure the ability of agonists for these receptors to inhibit cocaine-induced motor activity is reduced (Beyer and Steketee, 2002; Steketee and Beyer, 2005; Xie and Steketee, 2009). Taken with data demonstrating a reduction in receptor coupling to G proteins, these data suggest that cocaine sensitization is associated with an attenuation of inhibitory receptor function in the mPFC (Bowers et al., 2004; Febo et al., 2003).

Dopamine D_2, $GABA_B$ and Group II mGluR receptors all couple to adenylyl cyclase via G proteins to inhibit the formation of cAMP (Bettler and Tiao, 2006; Jackson and Westlind-Danielsson, 1994; Kew and Kemp, 2005). As mentioned above, repeated cocaine is known to produce a decrease in $GABA_B$, D_2 and Group II mGluR receptor coupling in the mPFC. Thus, while receptor-G protein coupling is reduced the remainder of the second messenger signaling pathway may remain intact in cocaine sensitized animals. The present report tested this hypothesis by examining the effects of intra-mPFC injections of SQ22536, an adenylyl cyclase inhibitor, on the acute locomotor response to cocaine, as well as on the initiation and expression of cocaine-induced sensitization. Since it is hypothesized that sensitization is associated with increased excitability of pyramidal output neurons in the mPFC, follow-up studies were conducted to determine whether stimulation of adenylyl cyclase activity in this region could enhance the locomotor response to cocaine using NKH 477.

2. Materials and methods

2.1. Animals and surgery

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by The University of Tennessee Health Science Center Animal Resources Advisory Committee. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) that weighed 275–300 g at the time of surgery were housed under 12-h light/dark cycle and had free access to food and water. Rats were housed in groups of four before surgery and were individually housed after surgery. All experimental procedures were conducted during the light phase of the light/dark cycle. Rats were anesthetized with ketamine hydrochloride and xylazine (80 and 6.0 mg/kg, respectively, i.p.) and their heads were mounted in a stereotaxic frame (Kopf Instruments) with bregma and lambda aligned in the same horizontal plane. Cannulae for microinjections (25 gauge, 14 mm) were bilaterally implanted 1.0 mm above the ventral mPFC (+3.2 mm posterior to bregma, ±0.6 lateral to the midline, and −3.5 mm ventral from dura, (Paxinos and Watson, 1986). Cannulae were anchored with three stainless steel screws and dental acrylic. Obturators (32 gauge, 14 mm) were inserted into the cannulae in order to prevent their occlusion. Animals were allowed at least 7 days to recover from surgery.

2.2. Behavior and microinjections

Locomotor activity was monitored using a Digiscan system (Accuscan, Columbus, OH, USA) as previously described (Xie and Steketee, 2009). Following a 60-min adaption to activity boxes, animals received intra-mPFC injections 5 min before systemic injections. Intracranial injections (0.5 μl/min, 0.5 μl/side) were made using stainless steel injectors (15 mm, 32 gauge) attached to 1 μl syringes via PE 20 tubing mounted in a Sage syringe pump as described previously (Beyer and Steketee, 2002). Injectors were left in place for 20 s to allow for diffusion of the infused solution and obturators were reinserted into guide cannulae after injections. Motor activity was monitored for 2 h in 15-min intervals following injection.

2.3. Experimental design

2.3.1. Acute cocaine studies

The effects of intra-mPFC injections of the adenylyl cyclase inhibitor, SQ22536 (5, 15, 50 and 75 nmol/side) or the adenylyl cyclase activator, NKH 477 (1.25 and 40 nmol/side), on cocaine (15 mg/kg, ip)-induced motor activity were studied using separate groups of animals for each drug and dose tested. For each group of rats tested each animal received each of the four possible treatment combinations (saline/saline, saline/cocaine, SQ22536 or NKH 477/saline, SQ22536 or NKH 477/cocaine) using a Latin Square design with a minimum 3-day inter-trial interval.

2.3.2. Sensitization studies

The basic design of the sensitization experiments included an initiation phase of once daily injections of saline (1.0 ml/kg, ip) or cocaine (15 mg/kg, ip) over four consecutive days, followed by an expression phase that involved a challenge injection of cocaine in all animals. A drug free period of 1, 7 or 30 days separated the initiation and expression phases. Each animal received the same treatment on each injection day during the initiation phase. Motor activity was monitored following injections on test day, but not during the pretreatment (i.e initiation phase) days. The impact of inhibiting cortical adenylyl cyclase on the initiation of sensitization was determined by injecting saline or SQ22536 (50 nmol/side) into the mPFC before saline or cocaine injections on each of the four pretreatment days. Intracranial injections were not administered on test day, which occurred 7 days after the last of the daily injections. This drug free period was based on previous experiments in our laboratory in which sensitization was clearly and reliably expressed at this time point (Beyer and Steketee, 2002; Xie and Steketee, 2009). The effects of inhibiting cortical adenylyl cyclase on the expression of sensitization were determined by injecting saline or SQ22536 into the mPFC before the test day cocaine injections that occurred 1, 7 or 30 days after the daily injections, which did not include intracranial injections during the initiation phase. The effects of stimulating cortical adenylyl cyclase on the expression of sensitization was determined by injecting saline or NKH 477 (40 nmol/side) into the mPFC before the test day cocaine injections that occurred 21 days after the initiation phase. As a control for potential nonspecific drug effects animals received saline injections with or without intracranial injections the day before the onset of initiation treatment regimen and the test for sensitization. Test day time points for sensitization studies were based on previous studies (Xie and Steketee, 2009).

2.4. Histology

After completion of studies, animals were deeply anesthetized with sodium pentothal (333 mg/kg, ip) and were perfused by intracardiac infusion of phosphate-buffered saline (50 ml) and 10% formaldehyde (50 ml). Brains were sectioned (100 μm) and sections were mounted onto gelatin-coated slides and stained with cresyl violet. Injection sites were visualized by light microscopy.