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Sigma$_2$ (σ_2) receptors as a target for cocaine action in the rat striatum

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Abstract

Studies from our laboratory have shown that agonists at sigma$_1$ and sigma$_2$ receptors inhibit *N*-methyl-D-aspartate (NMDA)-stimulated dopamine release from motor and limbic areas of rat brain. In the current study, we examined the effects of cocaine on *N*-methyl-D-aspartate (NMDA)-stimulated [^{3}H]dopamine release in rat striatal slices. Cocaine inhibited *N*-methyl-D-aspartate-stimulated [^{3}H]dopamine release in a concentration-dependent manner with a K_i of approximately 10 μM, under conditions in which the dopamine transporter (DAT) was blocked by 10 μM nomifensine. The inhibition seen by cocaine was reversed by the selective σ_2 antagonist 1′-[4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]-spiro[isobenzofuran-1(3*H*), 4′piperidine] (Lu28-179). Inhibition of release by cocaine and (+)pentazocine, under conditions in which sigma$_1$ receptors were blocked, was also reversed by the conventional PKC inhibitor 3-[1-[3-(dimethylamino)propyl-1*H*-indole-3-yl]-1-*H*-pyrpole-2-5′-dione. These results suggest that cocaine or other agonists, acting through the σ_2 receptor, require an intact conventional PKC (cPKC), most likely PKCα or PKCγ in order to inhibit dopamine release.

Keywords: σ receptor; Cocaine; Dopamine release; Dopamine transporter; Protein kinase C

1. Introduction

Cocaine is known to interact with dopaminergic systems, and as such be profoundly reinforcing. Although the well-characterized target for cocaine is the dopamine transporter (DAT), its ability to block reuptake of dopamine is unlikely to completely explain all actions of cocaine, including its high abuse potential, relapse rate, and toxicity. For instance, Rocha et al. (1998) showed that DAT knockout mice continue to self-administer cocaine suggesting that other targets of cocaine contribute to its addictive properties. Recent studies have demonstrated the effect of cocaine at several sites in addition to the DAT. At the neurochemical level, cocaine use produces a decrease in the density of the vesicular monoamine transporter, VMAT$_2$ (Little et al., 2003), but an increase in the density and function of the DAT (Mash et al., 2002). Cocaine also binds at several loci with K_i values comparable to its K_i at the DAT. Among these other binding sites are the sigma (σ) receptors, which are localized to dopaminergic brain areas. The affinity of cocaine for the σ_1 subtype (K_i of 2.9 μM, Matsumoto et al., 2001) is very close to its affinity for the DAT (IC_{50} 1.7 μM, Koe, 1976), while its affinity for the σ_2 receptor is about ten fold lower (K_i, 29 μM, Matsumoto et al., 2001). The concentration of cocaine achieved in the brain of a user significantly occupies both σ_1 and σ_2 receptors. Matsumoto et al. (2004) have identified the σ_1 receptor as critical to several features of cocaine toxicity. They have shown that several antagonists with high affinity for σ_1, as well as antisense oligonucleotides reverse cocaine toxicity.

In addition to its rewarding effects, cocaine also causes permanent changes that may underlie craving and relapse. Among long term changes identified as a result of cocaine exposure are those mediated by the σ_1 receptor. Several studies have shown that antagonists at σ_1 receptors alter the neurochemical and behavioral responses to cocaine (Maurice et al., 2002). The action of cocaine specifically at the σ_2 receptor has been less well-studied.

We have previously shown that agonists at σ_1 and σ_2 receptors inhibit *N*-methyl-D-aspartate (NMDA)-stimulated dopamine release from motor and limbic areas of rat brain. Other groups have also demonstrated a relationship between sigma receptors and dopaminergic systems (e.g. Thompson et al.,

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2001). Further, we have demonstrated that the components attributable to the two σ receptor subtypes could be distinguished based on sensitivity to selective antagonists; inhibition of release by σ agonist that is blocked by 100 nM 1-(*cyclo*-propylmethyl)-4-(2′-(4″-fluorophenyl)-2′-oxyethyl)piperdine HBr (DuP734), but not by a 1 nM concentration of 1′-[4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]-spiro[isobenzofuran-1(3*H*), 4′piperidine] (Lu28-179) is identified as mediated by σ_1 receptors. In contrast, inhibition of release by σ agonist that is blocked by 1 nM Lu28-179, but not by 100 nM DuP734 is identified as mediated by σ_2 receptors. In the current study, we examine the ability of cocaine to similarly regulate release under conditions in which the contribution of the DAT is prevented. We examine cocaine regulation of dopamine release, and test its pharmacological profile with regard to actions via a σ receptor subtypes. Furthermore, we test signaling mechanisms involved in the actions of cocaine via σ receptors.

2. Materials and methods

2.1. Materials

Domperidone, nomifensine, thapsigargin, cocaine were purchased from Sigma/RBI (Natick, MA). L-ascorbic acid and GF109203× were purchased from Sigma-Aldrich (St. Louis, MO). KN-93 and methyl acachidonyl fluorophosphonate were purchased from BIOMOL (Plymouth Meeting, MA). DuP734 was a gift from DuPont Merck Pharmaceutical Co. (Wilmington, DE). Lu28-179 was a gift from H. Lundbeck (Copenhagen, Denmark). LY379196 was a gift from Dr. Robin Bowman, Eli Lilly and Co., (Indianapolis, IN). (+)Pentazocine was obtained from the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD). [^{3}H]Dopamine was purchased from Amersham Biosciences Inc. (Piscataway, NJ).

2.2. Measurement of stimulated [^{3}H]dopamine release from striatal slices

All experiments were carried out in accordance with the guidelines and the approval of the George Washington University Institutional Animal Use and Care Committee. Male Sprague–Dawley rats (Hilltop Laboratory Animals Inc., Scottdale, PA) weighing 200 to 225 g were decapitated and their brains removed to ice. Striata were dissected, chopped in two planes at right angles into 250×250 μm strips with a Sorvall T-2 sectioner, and suspended in modified Krebs-HEPES buffer (127 mM NaCl, 5 mM KCl, 1.3 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 15 mM HEPES, 10 mM glucose, pH adjusted to 7.4 with NaOH) by trituration through a plastic pipette. Buffers were oxygenated throughout the experiments and brain slices were kept at a constant temperature of 37 °C. After 3 washes in modified Krebs-HEPES buffer, tissue was resuspended in 20 ml of modified Krebs-HEPES buffer and incubated for 30 min with 0.1 mM ascorbic acid and 15 nM [^{3}H]dopamine. Under these conditions, we have found uptake of [^{3}H]dopamine to be almost exclusive via the DAT (Werling et al., 1989). Tissue was then washed twice with 20 ml of modified Krebs-HEPES buffer and once in 20 ml of modified Krebs-HEPES buffer containing 10 μM nomifensine and 1 μM domperidone. These drugs were included in all subsequent steps to prevent reuptake and feedback inhibition by the released [^{3}H]dopamine. Tissue was suspended a final time in 7.5 ml of modified Krebs-HEPES buffer, containing 10 μM nomifensine and 1 μM domperidone, and distributed in 275 μl aliquots between glass fiber filter discs into chambers of a superfusion apparatus (BRANDEL Inc., Gaithersburg, MD). Modified Krebs-HEPES buffer was superfused over tissue at a rate of 0.6 ml/min. Each experimental treatment was performed in triplicates within an individual experiment (*N*). A low stable baseline release of approximately 0.9%/min was established over a 30-min period. Tissue was then stimulated by exposure to 25 μM NMDA (S1) for 2 min. Inflow was then returned to a non-stimulating buffer during a 10-min interstimulus interval (ISI). If cocaine or (+)pentazocine was tested it was included at this time. Tissue was then exposed to a second stimulus (S2) identical to the first (S1) except in the presence of cocaine or (+)pentazocine. If a kinase inhibitor or calcium channel blocker was being tested it was present throughout S1, ISI and S2. We have previously shown that nitrendipine has no effect on NMDA-stimulated [^{3}H]dopamine release from striatum (Werling et al., 1994), in agreement with the lack of a role for L-type VDCC on stimulated release of neurotransmitters (reviewed by Barclay et al., 2005). If σ antagonists were being tested they were present in the ISI and S2 only. In the experiments testing thapsigargin, tissue was incubated for 5 min with 2 μM thapsigargin prior to loading the tissue into chambers.

The mean fractional release (%)±S.E.M. in S1 was 12.2±2.3 and the mean fractional release (%)±S.E.M in S2 was 7.34±1.4 (N=10). All data were statistically analyzed as ratios (S2/S1). In this way, differences in response between tissue samples are taken into account and therefore do not affect the comparison among treatments. The mean S2/S1 ratio for NMDA-stimulated release was 0.59±0.1 (N=10). An enhancement by test drug would result in a higher ratio and inhibition in a lower ratio. Under the experimental conditions used, the release radioactivity has been shown to be primarily dopamine (Werling et al., 1988). All statistical analyses were performed by one-way factorial analysis of variance with post hoc Dunnett's test. Statistical significance is considered at $P<0.05$.

3. Results

3.1. Cocaine-mediated inhibition of NMDA-stimulated [^{3}H]dopamine release

Previous data had established that 25 μM NMDA was on the ascending portion of the concentration–response curve for stimulation of [^{3}H]dopamine release from rat striatal slices, and that the σ receptor agonist (+)pentazocine inhibited NMDA-stimulated release with a biphasic concentration response curve attributable to contributions from σ_1 and σ_2 receptors (Gonzalez-Alvear and Werling, 1994). In the current study we tested cocaine for its effects on NMDA-stimulated [^{3}H]dopamine release from rat striatal slices. As seen in Fig. 1, cocaine also