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Muscarinic receptor M_4 positive allosteric modulators attenuate central effects of cocaine

Camilla Dall[a], Pia Weikop[a], Ditte Dencker[a], Anna C. Molander[a], Gitta Wörtwein[a], P. Jeffrey Conn[c], Anders Fink-Jensen[a], Morgane Thomsen[a,b,*]

[a] Laboratory of Neuropsychiatry, Psychiatric Centre Copenhagen and University of Copenhagen, Copenhagen, Denmark
[b] Alcohol and Drug Abuse Research Center, McLean Hospital/Harvard Medical School, Belmont, MA, USA
[c] Vanderbilt Program in Drug Discovery, Vanderbilt Specialized Chemistry Center (Molecular Libraries Probe Production Centers Network; MLPCN), Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA

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ABSTRACT

Background: Cocaine addiction is a chronic brain disease affecting neurotransmission. Muscarinic cholinergic receptors modulate dopaminergic signaling in the reward system, and muscarinic receptor stimulation can block direct reinforcing effects of cocaine. Here, we tested the hypothesis that specific muscarinic M_4 receptor stimulation can attenuate the discriminative stimulus effects and conditioned rewarding effects of cocaine, measures believed to predict the ability of cocaine and cocaine-associated cues to elicit relapse to drug taking.
Methods: We tested the M_4-selective positive allosteric modulators VU0152100 and VU0467154 in a drug discrimination assay and a conditioned place preference assay, including extinction and reinstatement of place preference. Specificity of the cocaine discrimination effect was verified using knockout mice lacking either M_1 or M_4 receptors ($M_1^{-/-}$, $M_4^{-/-}$). We also replicated previous findings in cocaine-induced locomotor hyperactivity and striatal dopamine microdialysis assays.
Results: VU0152100 attenuated the discriminative stimulus effect of cocaine in wild-type mice and $M_1^{-/-}$ mice, but not in $M_4^{-/-}$ mice, without affecting rates of responding. As previously shown with VU0152100, VU0467154 almost eliminated cocaine-induced hyperactivity and striatal dopamine efflux. VU0467154 failed to attenuate acquisition of cocaine-conditioned place preference, but facilitated extinction and prevented reinstatement of the conditioned place preference.
Conclusions: These findings further support the notion that M_4 receptors are promising targets for the treatment of cocaine addiction, by showing that results can be replicated using distinct ligands, and that in addition to blocking reinforcing effects of cocaine relevant to ongoing drug taking, M_4 positive allosteric modulators can also attenuate subjective and conditioned effects relevant to relapse.

1. Introduction

Cocaine addiction remains a serious public health problem for which no effective treatments are available. Muscarinic cholinergic systems have been shown to modulate the effects of drugs of abuse including cocaine, thereby emerging as potential targets for the development of treatments (Kruse et al., 2014). The orthosteric binding site on muscarinic acetylcholine receptors is highly conserved between subtypes, but in the last decade, highly subtype-selective ligands have been developed by targeting allosteric binding sites, making it possible to investigate the functions of muscarinic receptor subtypes (Nickols and Conn, 2014; Thal et al., 2016).

We previously reported that activation of the M_4 subtype by a positive allosteric modulator (PAM) inhibited behavioral and neurochemical effects of cocaine in rodents. Specifically, the M_4-selective PAM VU0152100 decreased the reinforcing effects of cocaine in a single-session (acquisition) self-administration procedure, cocaine-induced locomotor activation, and cocaine-stimulated increases in extracellular striatal dopamine (Dencker et al., 2012). Combined M_1/M_4 receptor stimulation also decreased cocaine self-administration in rats and mice, after both acute and chronic treatment (Thomsen et al., 2014, 2012). Conversely, knockout mice lacking M_4 receptors ($M_4^{-/-}$ mice) self-administered more cocaine, and showed higher cocaine-induced increases in extracellular dopamine levels, relative to wild-type mice (Schmidt et al., 2011). Taken together, those findings indicate that M_4 receptors exert an inhibitory effect on the reinforcing effects of cocaine.

* Corresponding author at: Laboratory of Neuropsychiatry, Psychiatric Centre Copenhagen and University of Copenhagen, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark.
E-mail address: mthomsen@mclean.harvard.edu (M. Thomsen).

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Although candidate medications that reduce the direct reinforcing effects of cocaine, and maintain this effect when administered chronically, have shown the most promise in terms of reducing cocaine use clinically (Czoty et al., 2016; Haney and Spealman, 2008), there is also an interest in treatments that reduce the ability of drug-associated stimuli to trigger relapse to drug use. Medications that attenuate both the reinforcing and conditioned effects of cocaine may be the most effective. Therefore, we here extended our previous studies to test the hypothesis that selective M_4 receptor stimulation can also attenuate conditioned or "subjective" effects of cocaine in addition to attenuating its reinforcing effects, using a drug discrimination assay and a conditioned place preference (CPP) assay in mice, including extinction and reinstatement tests. We previously found that combined stimulation of M_1 and M_4 receptors decreased the discriminative stimulus effects of cocaine, an effect that was diminished in $M_1^{-/-}$ mice and $M_4^{-/-}$ mice and was absent in $M_1^{-/-}M_4^{-/-}$ double-knockout mice, indicating the involvement of both receptor subtypes (Thomsen et al., 2012, 2010). Here, we again verified the specificity of the drug discrimination effect using knockout mice lacking either the M_4 receptor or the M_1 receptor. In order to further strengthen confidence in the generality of the findings, we also verified that we could replicate previous results – reduction of cocaine-induced locomotor activation and dopamine efflux – using a newer, longer-acting, M_4 receptor-selective PAM, VU0467154, which was then applied in the CPP studies. Since both PAMs were expected to produce comparable effects, it was not a goal of the investigation to compare the two ligands across endpoints, e.g., for drug medication development lead compound optimization or other purposes.

2. Materials and methods

2.1. Animals

Drug discrimination studies were conducted at the McLean Hospital in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the McLean Hospital Institutional Animal Care and Use Committee. Male $M_1^{-/-}$ and $M_4^{-/-}$ mice bred at Taconic Farms (Germantown, NY) were generously provided by J. Wess (Gomeza et al., 1999; Miyakawa et al., 2001; backcrossed 11 generations to C57BL/6NTac), male age-matched C57BL/6NTac wild-type mice were purchased from Taconic Farms. The mice were acquired at 4–8 weeks of age and were acclimated to the housing facilities for at least 7 days before experiments began. Most mice had been tested previously with muscarinic receptor ligands (M_1 agonist, scopolamine and/or other antagonists) before the tests reported here. Mice were group housed in a 12-h light/dark cycle at ~22 °C. Water was accessible ad libitum and food (rodent diet 5001; PMI Feeds, Inc., St. Louis, MO) was provided daily after training/testing sessions, 4 g/mouse/day. Rodent "treats", nesting material, and exercise/nesting devices were provided for enrichment. All testing was conducted during the light phase of the circadian cycle to allow comparison with previous studies.

All other studies were conducted at the Laboratory of Neuropsychiatry in accordance with guidelines from the Animal Experimentation Inspectorate, Denmark and the European Communities Council Directive of 24 November 1986 (86/609/EEC). Experimentally naïve male C57BL/6 mice (Taconic, Denmark) were acquired at 10 weeks of age and were acclimated to the housing facilities for at least 7 days before experiments began. Mice were group housed in cages enriched with housing and nesting material, and were kept at 22–24 °C, on a reversed 12-h light/dark cycle (lights on at 7 pm), with free access to water and food. All testing was conducted during the dark phase of the circadian cycle, except the microdialysis experiment, which was conducted in the middle of the light cycle.

2.2. Apparatus

Behavioral studies were conducted in equipment from Med Associates (St Albans, VT, USA): mouse modular operant-conditioning chambers (ENV-307A) for drug discrimination studies (Thomsen et al., 2010, 2012), and open field activity arena (OFA 510) for locomotor activity and CPP studies. Both types of apparatus were individually enclosed in sound-attenuating cubicles equipped with a light, and, for the operant-conditioning chambers, a ventilation fan. The operant-conditioning chambers contained two nose-poke holes each fitted with a photocell and a yellow cue light, and a plate into which liquid food was delivered from a syringe pump.

The open field activity chambers (27 × 27 × 30 cm) were fitted with a beam-break movement detection system consisting of three 16 × 16 arrays of infrared photobeams, arranged to detect movement along X, Y, and Z dimensions. For locomotor activity studies, the chambers had white floors and clear walls. For CPP studies, a partition of clear red plastic (not transparent for mice) was used to create two compartments each measuring 27 × 13.5 × 30 cm. One compartment had white walls, a smooth white floor, and a clear plastic lid, and the other compartment had black and grey striped walls, a dark Lego® plate floor, and a dark plastic lid. During habituation, testing, extinction, and reinstatement phases, the partition had a 4 × 4 cm opening allowing the mice free movement between compartments. During conditioning, the partition had no opening, constraining the mouse to the compartment it was placed in.

2.3. Drug discrimination

The experimental procedure was as previously described (Thomsen et al., 2012, 2010; Thomsen and Caine, 2016). In brief, mice were trained to discriminate 10 mg/kg cocaine from saline, under an FR 10 schedule of food reinforcement. Stable discrimination was defined as at least 7 of 8 consecutive sessions satisfying the following criteria: (1) ≥10 reinforcers earned per session, (2) ≥80% correct responses for the first reinforcer, and (3) ≥90% correct total responses. Once criteria were met, mice were tested with saline and 0.32, 1.0, 3.2, 10, and 18 mg/kg cocaine to generate dose-effect functions. In pretreatment tests, VU0152100 (0.1–3.2 mg/kg) was administered 30 min before cocaine, in the same mice (within-subjects). Doses were tested within subjects in a pseudorandom order, counterbalanced between subjects and genotypes. At least one training session was interspersed between each test session, and tests were only performed when mice satisfied discrimination criteria.

2.4. Open field locomotor activity

Mice were habituated to the test room for at least 45 min before beginning a session. Each mouse was placed in an open field chamber and was allowed to habituate for 90 min before the first injection was given, either vehicle or VU0467154 (0.3, 1 or 3 mg/kg); 30 min later, saline or cocaine (30 mg/kg) was administered, and locomotor activity was recorded for an additional 120 min. For time course analysis of the locomotor activity data, ambulation was recorded as distance travelled in meters per 10 min bin over the 4-h session. Total activity data were calculated as the total distance travelled from the time of cocaine administration (t = 120 min) to the end of the experiment (t = 240 min). In addition, total activity recorded from the time of cocaine administration was obtained for vertical counts (breaks of elevated beams), small movements activity counts that may represent stereotypies (beam breaks within a predefined area surrounding the mouse), ambulatory episodes (number of periods with movement), resting time (periods in-between ambulatory episodes in seconds), and velocity (speed in cm/s averaged over ambulatory episodes).