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The 2-methoxy methyl analogue of salvinorin A attenuates cocaine-induced drug seeking and sucrose reinforcements in rats

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ABSTRACT

κ Opioid receptor activation by traditional arylacetamide agonists and the novel neoclerodane diterpene κ opioid receptor agonist Salvinorin A (Sal A) results in attenuation of cocaine-seeking behavior in pre-clinical models of addiction. However, adverse effects such as sedation, depression and aversion limit their clinical utility. The Sal A analogue, 2-methoxy-methyl salvinorin B (MOM Sal B) is a longer acting Sal A analogue with high affinity for κ opioid receptors. In this study, we tested MOM Sal B for its ability to modulate cocaine-seeking behavior in rats. MOM Sal B (0.3 mg/kg) successfully attenuated cocaine-seeking but also attenuated sucrose reinforcement. No change in activity was observed in either cocaine-induced hyperactivity or spontaneous open field activity tests but increased immobility and decreased swimming times in the forced swim test were observed. This study indicates that κ opioid receptor activation by more potent Sal A analogues modulates cocaine-seeking behavior non-selectively without causing sedation, suggesting an improved side effects profile. However, pro-depressive effects are seen, which may limit the therapeutic potential of this compound. Future studies with Sal A analogues having affinities at other opioid receptors are warranted as they have the potential to identify compounds having effective anti-addiction properties.

1. Introduction

Attenuation of drug self-administration by acute κ opioid receptor activation has been shown in rodents (Glick et al., 1995) and non-human primates (Mello and Negus, 1998). Behavioral effects of cocaine (Heidbreder et al., 1993, 1995), amphetamine (Gray et al., 1999), and nicotine (Hahn et al., 2000) have also been antagonized by pre-treatment with κ opioid receptor agonists. These findings have prompted further studies into the potential development of these compounds as anti-addiction pharmacotherapies (Prisinzano et al., 2005; Shippenberg et al., 2001, 2007), particularly in the binge or intoxication phase where κ opioid agonists antagonise the rewarding effects of drugs of abuse, for recent reviews see (Butelman et al., 2012; Shippenberg et al., 2007; Wee and Koob, 2010). However, adverse side effects including sedation (Butelman et al., 2007, 2009), dysphoria (Pfeiffer et al., 1986; Walsh et al., 2001) and depression (Todtenkopf et al., 2004) have limited the clinical development of traditional acrylacetamide κ opioid agonists. Antagonists of κ opioid receptors have been suggested to have therapeutic use later in the addiction cycle as they have been shown to modulate stress pathways in addiction (Chavkin, 2011).

Sal A, a neoclerodane diterpene derived from *Salvia divinorum*, was shown to selectively bind with high affinity to κ opioid receptors (Roth et al., 2002). Therefore, Sal A, a non-nitrogenous κ opioid receptor agonist has characterized a novel class of κ opioid receptor ligand. Sal A binds to κ opioid receptors with greater potency than U69593 or U50488H (Chavkin et al., 2004) and is 40-fold less potent than U50488H in its ability to internalize κ opioid receptors (Wang et al., 2005). These findings suggest that although it is a potent κ opioid receptor agonist, Sal A might differ pharmacologically and behaviorally from traditional arylacetamide κ opioid receptor agonists (Beguin et al., 2012; Fantegrossi et al., 2005; Prisinzano, 2005).

In vivo studies with Sal A have shown that it has a rapid onset of action (approx 5 min) and an elimination half-life of approximately 50 min (Butelman et al., 2009; Hooker et al., 2008; Schmidt et al., 2005). The short duration of action is thought to be due to its rapid metabolism at position C-2 to a pharmacologically inactive metabolite, salvinorin B (Beguin et al., 2005; Chavkin et al., 2004). However, other mechanisms are likely involved as well (Butelman et al., 2012). Structure activity relationships have revealed the

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importance of the C-2 acetoxy group for binding to and activation of κ opioid receptors (Beguin et al., 2008; Prisinzano and Rothman, 2008; Prevatt-Smith et al., 2011). Sal A analogues with C-2 substitution such as MOM Sal B have been shown to have a longer half-life *in vivo* (Baker et al., 2009; Wang et al., 2008). *In vitro* studies indicate that it is five- and seven-fold more potent at κ opioid receptors than U50488H and Sal A, respectively (Wang et al., 2008). Behavioral pharmacology studies with MOM Sal B have shown antinociception, motor suppression, hypothermia and diuresis (Inan et al., 2009; Wang et al., 2008). Recent reports show that MOM Sal B completely substituted for U50488H and Sal A in discriminative stimulus tests in rats (Peet and Baker, 2011). Work has been reported with Sal A analogues characterizing their anti-addiction profiles (Chartoff et al., 2008; Morani et al., 2009, 2012). Therefore, we tested the effect of MOM Sal B on cocaine-seeking using a cocaine prime induced reinstatement paradigm in rats (De Wit and Stewart, 1981, 1983). We also tested the effect of MOM Sal B on motor function (spontaneous locomotion and cocaine induced hyperactivity), reward reinforcement (sucrose reinforcement) and depression (forced swim test, FST).

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats weighing 325–350 g (for cocaine self-administration), 250–300 g (for sucrose reinforcement and spontaneous locomotion tests) and 350–400 g (for forced swim test) were used. Self-administering rats were housed individually in hanging polycarbonate cages in a temperature and humidity (55% relative humidity, 19–21 °C) controlled room. Rats used for sucrose reinforcement, spontaneous locomotion and forced swim tests were housed in groups of 2–3 per cage. The lights were maintained on a 12/12 h cycle with lights on at 0700 h. Animals used for reinstatement tests, locomotion experiments and forced swim tests had free access to food and water except during testing. Animals tested for sucrose reinforcements had free access to water within their home cages and were maintained at approximately 85% of their initial feeding weights during the experiments by restricting access to food. The animal colonies were maintained at the School of Psychology, Victoria University of Wellington. All experimental procedures were reviewed and approved by the Animal Ethics Committee of Victoria University of Wellington, New Zealand.

2.2. Surgery

Under deep anesthesia produced by ketamine/xylacine (90/9 mg/kg, intraperitoneal, i.p.), the right external jugular vein was isolated and a silastic catheter was inserted. The distal end (22 gauge stainless steel tubing) was passed subcutaneously (s.c.) to an exposed portion of the skull where it was fixed to embedded jeweler's screws with dental acrylic. Rats received carprofen (5 mg/kg, s.c.) at 0, 24, and 48 h post-surgery. In order to prevent infection and clot formation, the catheters were daily infused with 0.1 ml of a sterile saline solution containing heparin (30.0 U/ml), penicillin G Potassium (250,000 U/ml) and streptokinase (8000 U/ml). Testing began five days post-surgery.

2.3. Apparatus

2.3.1. Cocaine self administration

Self-administration training and reinstatement tests were carried out in humidity (55% relative humidity) and temperature (19–21 °C) controlled rooms containing standard operant chambers equipped with two levers (Med Associates, ENV-001, VT, USA). Depression of the active lever led to a programmed intravenous (i.v.) infusion of a 0.l ml solution of cocaine hydrochloride dissolved in sterile physiological saline containing heparin (3.0 U/ml) in order to maintain the patency of the catheter. The infusions were of 12 s duration and were accompanied by the illumination of a stimulus light located directly above the active lever. This stimulus light remained illuminated throughout each 12 s cocaine infusion. Depression of the other lever (the inactive lever) was without programmed consequence. Prior to each training and testing session, the catheter lines were infused with 0.1 ml of the heparin–penicillin–streptokinase solution. The stainless steel catheter was connected to the syringe by a length of microbore tubing. After completion of each session, the lines were again infused with 0.1 ml of the heparin–penicillin–streptokinase solution, the stainless steel tubing was plugged, and the animal was returned to its home cage. Drug delivery and data acquisition were controlled by Med Associates software. Cocaine deliveries were made via mechanical pumps (Razel Scientific, Model A with 1.0 rpm motor equipped with 20 ml syringe; VT, USA).

2.3.2. Sucrose self-administration apparatus

Eight standard operant chambers (Med Associates, ENV-008) placed in a light and sound attenuating room were used for training and testing. Each operant chamber had two retractable levers with a bottle delivering 0.1 ml of 10% sucrose solution in a tray placed on the chamber wall. The sucrose was delivered according to the imposed schedule of reinforcement. Sucrose delivery and data acquisition were controlled by Med Associates software. All experiments were conducted between 0900 and 1600 h.

2.3.3. Locomotion activity tests

Eight Open field chambers (Med Associates ENV-520) were used for this test. Each chamber was equipped with two banks of 16 photocells on each wall to measure horizontal and vertical locomotion. The open field boxes were interfaced with a microcomputer located in an adjacent laboratory. Testing was conducted in the dark between 1000 and 1600 h. White noise was present throughout the experiment.

2.3.4. Forced swim tests (FST)

The FST chamber was a cylindrical chamber of 44 cm in height and a diameter of 20 cm with water level up to a depth of 30 cm. Water temperatures was maintained at 25 ± 1 °C.

2.4. Procedure

2.4.1. Cocaine self-administration training and reinstatement test

Rats were trained to self-administer cocaine during daily 2 h sessions. During each session the animals received an experimenter-delivered infusion of cocaine (0.5 mg/kg/infusion (cocaine hydrochloride dissolved in sterile physiological saline containing heparin (3.0 U/ml)). Thereafter, depression of the active lever produced automated cocaine infusions. Initially the animals were maintained on FR-1 schedule of reinforcement (single infusion following a single lever press). The criterion for acquisition of cocaine self-administration consisted of three consecutive days during which there were at least 20 reinforced responses (10 mg/kg during a single session) and a ratio of active: inactive lever responses of at least 2:1. Following acquisition, the reinforcement schedule was increased to FR-5 (five lever presses resulted in a single infusion). Daily 2 h sessions were conducted until there was less than 20% variation in responding for three consecutive days. Once responding under the FR-5 schedule was stable, the effect of