Salvinorin B derivatives, EOM-Sal B and MOM-Sal B, produce stimulus generalization in male Sprague-Dawley rats trained to discriminate salvinorin A

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Salvinorin A, the main active component of *Salvia divinorum*, is a potent and selective κ opioid receptor agonist. Synthetic derivatives of this substance may be useful in the development of medicinal treatments for pain, mood disorders, and drug dependence. Such developments require extensive preclinical screening of these compounds. The drug discrimination assay is a valuable method for exploring potential similarities between novel compounds and known drugs of abuse with respect to their interoceptive stimulus properties, and can be used to investigate the potency of salvinorin A and its derivatives *in vivo.* This study used drug discrimination methods to compare two synthetic derivatives of salvinorin B, the ethoxymethyl ether (EOM-Sal B) and methoxymethyl ether (MOM-Sal B) with salvinorin A. Male Sprague-Dawley rats were trained to discriminate 2.0 mg/kg of salvinorin A from its vehicle (75% dimethylsulfoxide/25% water) in a fixed ratio 20 food-reinforced drug discrimination procedure, and were tested for stimulus generalization with EOM-Sal B and MOM-Sal B. For comparison, substitution tests were also conducted with a μ agonist, morphine, a dissociative hallucinogen, ketamine, and two serotonergic hallucinogens, D-lysergic diethylamide (LSD) and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane. Time-course tests were also conducted with salvinorin A and EOM-Sal B. Both EOM-Sal B and MOM-Sal B substituted fully for salvinorin A and displayed greater potency than salvinorin A. EOM-Sal B was discriminated at longer postinjection intervals than salvinorin A. Morphine and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane failed to substitute for salvinorin A, although ketamine and LSD produced significant drug-appropriate responding. The current findings are consistent with previous reports that salvinorin A produces detectable stimulus effects that are distinct from those of other drug classes and, for the first time, establish that synthetic derivatives of this substance produce similar discriminative stimulus effects. The unexpected partial substitution with LSD and ketamine indicate that further preclinical studies of these novel κ opioid receptor agonists may be warranted. *Behavioural Pharmacology* 22:450–457 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Salvinorin A, the main active component of *Salvia divinorum*, is the only known naturally occurring substance with high affinity and selectivity for κ opioid receptors (KOR; Roth *et al.*, 2002; Vortherms and Roth, 2006). This plant and its chemical derivatives are currently unscheduled under the US Federal Drug Laws, although several states have recently passed legislation banning its sale and distribution, mainly in response to the increasing popularity of this substance as a recreational drug. An unfortunate consequence of the increased media attention and legal restrictions on this substance is the potential hindrance of scientific progress toward the development of pharmacotherapeutic agents derived from this plant.

Indeed, there is a growing body of literature on the potential medicinal benefits of salvinorin A derivatives. In a recent literature review, Prisinzano and Rothman 2008 noted the potential medicinal benefits of salvinorin A and related compounds for a wide range of conditions including mood disorders, stimulant dependence, opioid abuse, obesity, and opioid-induced constipation. Moreover, Prisinzano *et al.* (2005) noted that KOR agonists may offer an indirect approach to the modulation of some abuse-related effects of central nervous system stimulants. For example, KORs are known to be involved indirectly in modulating synaptic dopamine levels (Zhang *et al.*, 2005; Carlezon *et al.*, 2006), and may play a critical role in the development of relapse and drug seeking behavior (Morani *et al.*, 2009).

With the recreational use of salvinorin A on the rise and the Drug Enforcement Administration's identification of salvinorin A as a drug of concern, preclinical investigations of novel compounds similar in structure to salvinorin A are of considerable importance. New chemical entities submitted to the Food and Drug Administration for

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approval, which produce stimulant, depressant, or hallucinogenic effects similar to those of a known controlled substance, require preclinical testing for abuse liability.

Drug discrimination procedures are widely used in behavioral pharmacology as a preclinical assay of the central nervous system-mediated actions of psychoactive compounds. This assay also provides a useful tool in drug discovery research to screen novel compounds for similarities to drugs with established abuse liability (Moser *et al.*, 2011). As noted by Colpaert (1999), the drug discrimination assay can contribute to our understanding of the mechanisms of drugs at the molecular, pharmacological, and behavioral level in whole organisms. For example, the discovery of the drug loperamide for the treatment of diarrhea was inspired by investigations involving drug discrimination assays (Colpaert, 1999).

To date, only seven published studies have used drug discrimination procedures to examine the interoceptive stimulus properties of salvinorin A in laboratory subjects (Butelman *et al.*, 2004, 2010; Willmore-Fordham *et al.*, 2007; Li *et al.*, 2008; Baker *et al.*, 2009; Killinger *et al.*, 2010; Walentiny *et al.*, 2010). Collectively, these investigations have demonstrated that salvinorin A substitutes reliably for other KOR agonists (Butelman *et al.*, 2004; Willmore-Fordham *et al.*, 2007; Baker *et al.*, 2009), but not for serotonergic hallucinogens (Li *et al.*, 2008; Killinger *et al.*, 2010), the noncompetitive NMDA antagonist, ketamine (Killinger *et al.*, 2010), or the cannabinoid, δ-9-tetrahydrocannabinol (Walentiny *et al.*, 2010).

Only two of the above-mentioned published studies involved training subjects to discriminate salvinorin A (Baker *et al.*, 2009; Butelman *et al.*, 2010). We reported full substitution with the KOR agonists, U69593 and U50488, in male Sprague-Dawley rats trained to discriminate 1.0 mg/kg of salvinorin A (Baker *et al.*, 2009). The extensive training required to establish a reliable discrimination with this dose of salvinorin A precluded testing additional compounds for substitution in these subjects. Butelman *et al.* (2010) reported that monkeys trained to discriminate salvinorin A generalized to other KOR agonists (bremazocine, U69593, and U50488). Full substitution for salvinorin A was not observed with any other test compound evaluated, including psilocybin, ketamine, fentanyl, or the δ opioid agonist, SNC80. These investigators also reported that discriminative effects of salvinorin A were blocked by the opioid antagonist quadazocine, but not by the $5HT_2$ antagonist ketanserin.

Modifications of salvinorin A have primarily centered on the modification of the C-2 acetyl group. The conversion of salvinorin A to various ethers at the C-2 position has resulted in the discovery of two synthetic derivatives, salvinorin B ethoxymethyl (EOM-Sal B) ether and salvinorin B methoxymethyl (MOM-Sal B) ether. Both derivatives have been shown *in vitro* to have greater binding affinity to KORs than salvinorin A (Munro *et al.*, 2008). Detailed information on the synthesis of EOM-Sal B and MOM-Sal B is described by Munro *et al.* (2008).

Few studies have assessed the effects of these salvinorin B derivatives *in vivo*. Wang *et al.* (2008) compared MOM-Sal B with salvinorin A and U50488 in tests of mobility, hypothermia, and nociception in rodents. Their findings indicate MOM-Sal B is a potent and efficacious KOR agonist with longer lasting in-vivo effects compared with salvinorin A. Hooker *et al.* (2009) investigated the pharmacokinetics and metabolism of salvinorin A, salvinorin B, and EOM-Sal B in baboons and rats. They reported that salvinorin B and EOM-Sal B are metabolized more slowly than salvinorin A in baboons after intravenous administration, whereas rapid uptake and clearance in the brain were comparable for all compounds. The brain kinetics of both compounds were similar in rats after intraperitoneal injection; however, EOM-Sal B concentrations in the whole brain were higher than that of salvinorin A. Both studies suggest a prolonged duration of action for salvinorin B derivatives, MOM-Sal B and EOM- Sal B, compared with salvinorin A.

In the only drug discrimination study to assess salvinorin B derivatives to date, EOM-Sal B and MOM-Sal B produced full substitution in rats trained to discriminate U50488 (Baker *et al.*, 2009). The aim of this study was to further examine these salvinorin B derivatives in rats trained to discriminate salvinorin A. The time course of EOM-Sal B was also compared with salvinorin A. For comparison, two serotonergic hallucinogens [D-lysergic diethylamide (LSD) and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM)], an NMDA antagonist (ketamine), and a μ opioid agonist (morphine) were also assessed for substitution.

Methods

Subjects

Eight adult drug-naïve male Sasco Sprague-Dawley rats (Charles River Laboratories, Portage, Michigan, USA) served as subjects. Subjects were 5–8 months old and weighed between 350 and 400 g at study initiation. They were individually housed in polycarbonate cages with corncob bedding in a temperature-controlled ($20 \pm 2^{\circ}C$) and humidity-controlled ($50 \pm 5\%$) room with a 12 : 12 h light/dark cycle (fluorescent lighting provided from 7 a.m. to 7 p.m.). Free access to water was available in the home cages and food access (LabDiet, Rodent Diet 5001, a constant nutrition formula; PMI Nutrition International Inc., Brentwood, Missouri, USA) was restricted to maintain animals at 80–85% of free-feeding body weights. Subjects were maintained in accordance with the National Research Council (1996). The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Western Michigan University.

Apparatus

Eight sound-attenuated, operant test chambers (Med-Associates Inc., Georgia, Vermont, USA) were equipped with three retractable levers (left, center, and right) on the front panel, a food dispenser above the center lever, and a 28-V house light located on the opposite wall. Experimental procedures were controlled and data were recorded with MED-PC (version 4.0 for Windows) software (St Albans, Vermont, USA). Lever pressing was reinforced with Dustless Precision Pellets (45 mg; Rodent Purified Diet BioServ, Frenchtown, New York, USA).

Preliminary training

Subjects were initially acclimated to the operant test chambers and the location and sound of the food pellets during a single 60-min session. During this session, all levers were retracted and the delivery of food pellets was programmed according to a fixed-time (60 s) schedule. Subsequently, two to four 20-min training sessions were conducted with the center lever extended and lever presses were reinforced under a continuous reinforcement schedule. Once subjects were reliably responding on the center lever, a series of 20-min errorless training sessions were conducted during which only the left or the right lever was extended.

The number of errorless training sessions varied among subjects depending on individual subject performance and ranged from 14 to 38 sessions. This extended period of errorless training was conducted in an effort to establish reliable stimulus control, as previous difficulties were encountered maintaining reliable discrimination with salvinorin A (Baker *et al.*, 2009). Twenty minutes before errorless training sessions, subjects received an intraperioneal injection of either 2.0 mg/kg of salvinorin A or vehicle. Using a pseudorandom schedule, drug and vehicle sessions were alternated such that for every six consecutive sessions, drug was administered for three sessions and vehicle was administered for three sessions, but neither condition was administered for more than two sessions in a row (e.g. VVDVDD, DDVVDV, or VDVVDD). Sessions were conducted for 5–7 days per week. For half of the subjects, responses on the left lever were reinforced after salvinorin A injections and responses on the right lever were reinforced after vehicle injections. Conditions were reversed for the remaining subjects. Responding was initially reinforced under a fixed ratio 1 (FR 1) schedule of reinforcement and then gradually incremented up to a maximum FR 20 schedule of reinforcement (e.g. FR 1, FR 2, FR 5, FR 10, FR 15, and FR 20). The FR was programmed to increment by one, two, or five every fifth reinforcer depending on each individual subject's performance. The number of reinforcers that could be earned during any given training session was only limited by the schedule of reinforcement and the duration of the session. When subjects had progressed to the FR 20 schedule under both the salvinorin A and vehicle conditions, discrimination training began.

Discrimination training

Both left and right levers were extended during discrimination training sessions. Drug and vehicle sessions were alternated using a pseudorandom schedule as noted above during preliminary training. The FR schedule of reinforcement was programmed so that only a fixed number of consecutive responses on the drug-appropriate or vehicle-appropriate lever would result in pellet delivery and incorrect responses reset the response counter. Responding was initially reinforced under a FR 5 schedule, which was programmed to increment by five after every fifth reinforcer until the final FR 20 schedule was in effect. Under both drug and vehicle stimulus conditions, the FR schedule was incremented within sessions as follows: FR 5, FR 10, FR 15, and FR 20. Once responding was maintained under a FR 20 schedule of reinforcement for both drug and vehicle stimulus conditions, this reinforcement schedule remained in effect for all subsequent training sessions.

Discrimination accuracy was determined by calculating the percentage of responses on the correct lever before delivery of the first food pellet in each session. The criteria for discrimination acquisition were met when accuracy for any individual subject was more than or equal to 80% correct lever responses for at least eight of 10 consecutive discrimination training sessions.

Stimulus generalization tests

Once a subject met the above-mentioned criteria for stimulus discrimination, test sessions were conducted to determine substitution with a range of salvinorin A doses (0, 0.25, 0.50, 1.0, and 2.0 mg/kg). Substitution tests were then conducted with the following test compounds: EOM-Sal B (0.003, 0.01, 0.03, 0.10, 0.30, and 0.60 mg/kg); MOM-Sal B (0.003, 0.01, 0.03, 0.10, 0.30, and 0.60 mg/kg); morphine (0.56, 1.0, 3.2, 5.6, and 10 mg/kg); ketamine (2.0, 4.0, 6.0, 8.0, and 10 mg/kg); LSD (0.02, 0.04, 0.08, and 0.16 mg/kg); and DOM (0.032, 0.10, 0.32, 1.0, and 2.0 mg/kg). Salvinorn A (20 min, 40 min, 1 h, 2 h, 4 h) and EOM-Sal B (20 min, 1 h, 2 h, 4 h) were also assessed for substitution at several postinjection intervals. These postinjection times were selected based on a previous unpublished study in our laboratory of these compounds in subjects trained to discriminate U69593. One subject was euthanized due to poor health and was not included in most tests. MOM-Sal B and DOM were only tested in five subjects due to low supplies of these compounds. For each compound tested, doses were administered in a randomized order among subjects. For each test dose examined, approximately half of the subjects were tested after a discrimination training session in which salvinorin A was administered, and the other half were tested after a discrimination training

session in which vehicle was administered. Test sessions were conducted according to an individual subject's performance during training sessions. Once testing began, all subjects received at least one salvinorin A training session and one vehicle training session between test sessions, allowing for a minimum of 72 h between substitution tests with different test compounds. Test sessions were only conducted when discrimination performance during the preceding training sessions for both vehicle and training drug was greater than or equal to 80%. Test sessions were conducted similarly to training sessions, with the exception that no food pellets were delivered and the session ended when the first 20 consecutive responses on either lever was completed. Subjects were removed from the chambers immediately after the completion of each test session.

Drugs

Salvinorin A, EOM-Sal B, and MOM-Sal B were generously provided by Harvard McLean Hospital (Belmont, Massachusetts, USA). These test compounds were prepared fresh daily in a 75% dimethylsulfoxide solution. They were first dissolved in dimethylsulfoxide and then diluted with sterile water. This vehicle has been used in previous studies (Carlezon *et al.*, 2006; Willmore-Fordham *et al.*, 2007; Baker *et al.*, 2009; Nemeth *et al.*, 2010) and was well tolerated by the subjects in this study. Morphine sulfate, LSD, and DOM were obtained from the National Institute on Drug Abuse (Bethesda, Maryland, USA) and ketamine-hydrochloride was purchased from Sigma-Aldrich Chemical Company (St. Louis, Missouri, USA). These test compounds were dissolved in sterile 0.9% saline with the exception of ketamine, which was dissolved in sterile water. All drugs were administered through intraperitoneal injection at a dose volume of 1 ml/kg. Pretreatment times were 20 min for all drugs with the exception of LSD, which was 15 min. Dose calculations were determined from the weight of the salts.

Data analysis

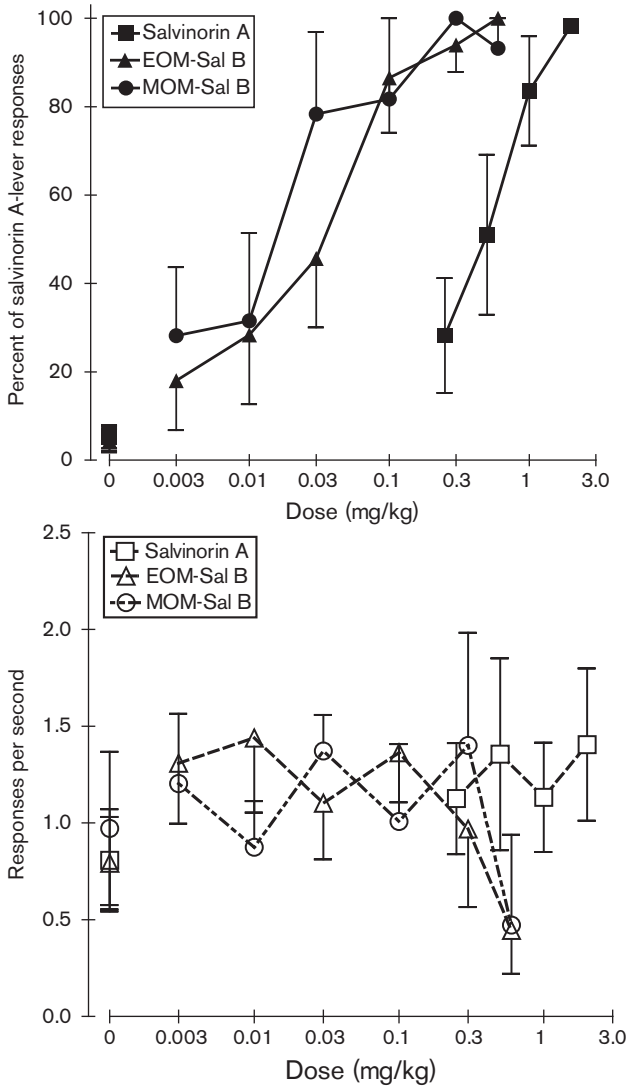
The mean [± standard error of the mean (SEM)] number of sessions to criteria ($\geq$ 80% correct lever responses for eight of 10 sessions) was calculated. From the results of drug substitution tests, the mean (± SEM) percentage of responses on the salvinorin A lever and the mean (± SEM) response rate (responses per second) were calculated and dose response curves were plotted for each test compound. For each test compound, a one-way repeated-measures analysis of variance (ANOVA) and Dunnett's multiple comparison tests were conducted to compare individual dose levels with vehicle. Statistical tests on percent drug–lever responses excluded subjects that failed to make at least 10 total responses during test sessions at all dose levels of a particular test compound. For test compounds that produced full substitution, a nonlinear regression analysis was also performed to estimate the median effective dose (ED_{50}) and 95% confidence intervals. Full substitution was established if the test compound produced more than or equal to 80% responses on the salvinorin A-appropriate lever. Partial substitution was established if drug appropriate responding was less than 80% but significantly different from vehicle. The results of time-course tests with salvinorin A and EOM-Sal B were analyzed by a repeated-measures two-factor (drug, time) ANOVA. Statistical analyses were performed using GraphPad Prism (version 4.0) software (GraphPad Software Inc., San Diego, California) and Minitab 15 software (Minitab, Inc., State College, Pennsylvania, USA).

Results

Stimulus control was established with 2.0 mg/kg of salvinorin A in all eight subjects, within an average of 34.5 (± 6.2) training sessions (range: 19–64). Results of stimulus generalization tests with salvinorin A ($n = 8$), EOM-Sal B ($n = 8$), and MOM-Sal B ($n = 5$) are shown in Figure 1. Only five subjects were tested on MOM-Sal B due to insufficient supplies of this test compound. Group means (± SEM) at each dose level are shown for the percentage of salvinorin A-associated lever responses in the upper panel and for response rate in the lower panel. All three of these drugs produced dose-dependent increases in salvinorin A-lever responding and full substitution for salvinorin A after at least two doses. A repeated measures one-way ANOVA revealed a statistically significant effect of salvinorin A on percent drug-appropriate responding [$F(4,39) = 11.15$, $P < 0.001$] and a Dunnett's multiple comparison test indicated that the effects at 0.50, 1.0, and 2.0 mg/kg were all significantly different from vehicle responding ($P < 0.01$). Statistically significant effects of EOM-Sal B and MOM-Sal B on percent salvinorin A responding were also evident. Two highest doses of EOM-Sal B disrupted responding in three subjects and were excluded from statistical analysis. However, a repeated measures ANOVA including only five dose levels (0, 0.003, 0.01, 0.03, and 0.10 mg/kg) was highly significant [$F(4,39) = 8.41$, $P < 0.001$] and Dunnett's tests indicated that the percentage of salvinorin A lever responses after 0.03 and 0.10 mg/kg was significantly different from that after vehicle injections ($P < 0.01$). A repeated measures ANOVA on five dose levels of MOM-Sal B (0, 0.003, 0.01, 0.03, and 0.10 mg/kg) was also significant [$F(4,24) = 4.23$, $P < 0.05$] and the effects of 0.03 and 0.10 mg/kg were significantly different from those of vehicle ($P < 0.05$). It is also evident from the dose–response curves plotted in Figure 1 that EOM-Sal B and MOM-Sal B exhibited greater potency compared with salvinorin A. The ED_{50} for salvinorin A was 0.62 mg/kg (95% confidence limits: 0.24–1.55 mg/kg). The ED_{50} for EOM-Sal B was 0.043 mg/kg (0.01–0.20), and the ED_{50} for MOM-Sal B was 0.02 mg/kg (0.001–0.23).

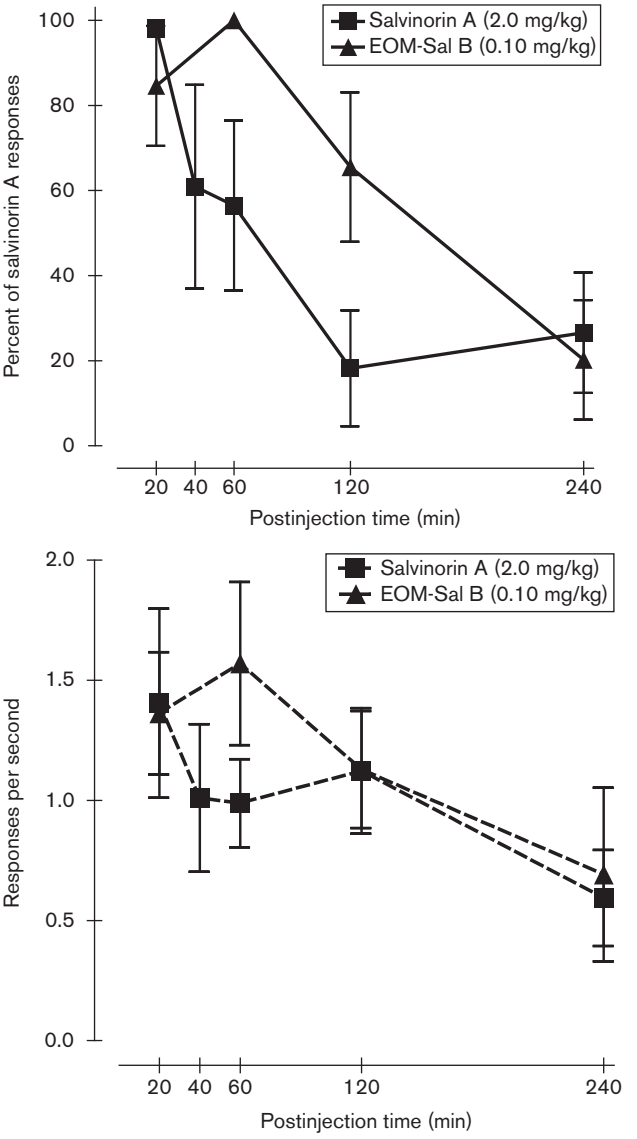
Salvinorin A (2.0 mg/kg) and EOM-Sal B (0.10 mg/kg) were also assessed for stimulus generalization after a

Fig. 1



Dose–response curves for salvinorin A ($n=8$), ethoxymethyl ether salvinorin B (EOM-Sal B, $n=8$) and methoxymethyl ether salvinorin B (MOM-Sal B, $n=5$) in salvinorin A-trained (2.0 mg/kg, intraperitoneally, 20 min) rats. Upper panel: percentage of salvinorin A responses; lower panel: response rate. Each point represents the mean (± standard error of the mean).

Fig. 2

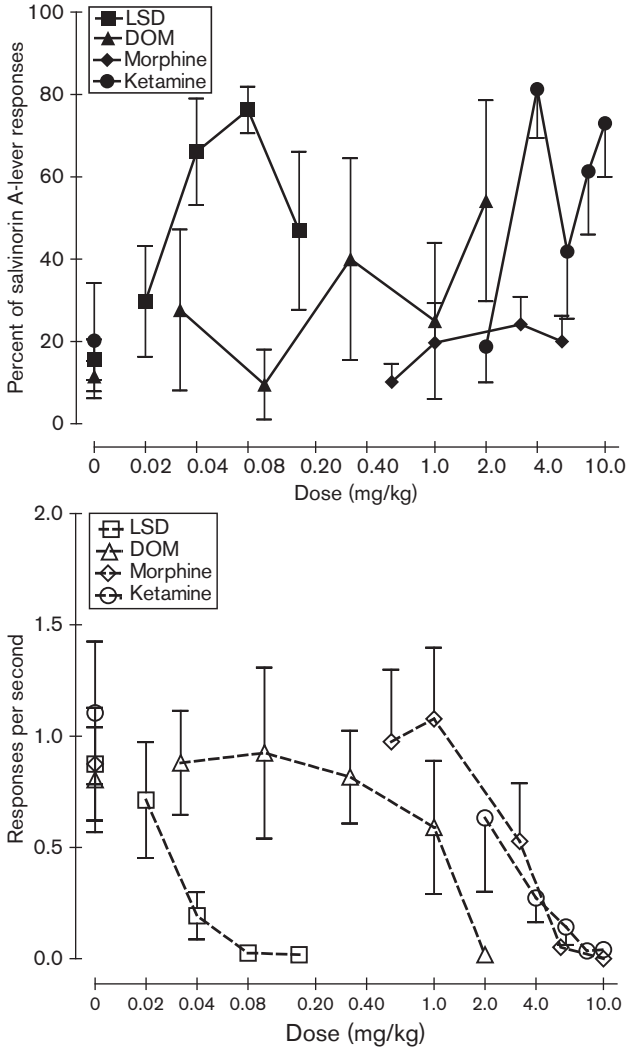


Time course of 2.0 mg/kg of salvinorin A ($n=7$) and 0.10 mg/kg ethoxymethyl ether salvinorin B (EOM-Sal B, $n=7$) in salvinorin A- trained rats. Upper panel: percentage of salvinorin A responses; lower panel: response rate. Each point represents the mean (± standard error of the mean).

range of postinjection times to assess differences in their duration of action. As shown in Figure 2, the time course of the discriminative effects of EOM-Sal B seem to be slightly longer than that of salvinorin A. Salvinorin A failed to produce substitution at all postinjection times greater than 20 min, whereas EOM-Sal B still produced full substitution when tested 60 min after injection, but not when tested 120 or 240 min after injection. At the 60-min postinjection time, only four of the seven subjects responded on the salvinorin A-associated lever, whereas all seven subjects emitted 100% of their responses on the salvinorin A-associated lever 60 min after EOM-Sal B administration. A repeated-measures two-way ANOVA comparing the time-dependent effects of salvinorin A and EOM-Sal B indicated a significant main effect of postinjection time [$F(3,33) = 12.12$, $P < 0.001$] but not a significant difference between these two test compounds, although there was a significant interaction between drug and postinjection time [$F(3,33) = 3.53$, $P < 0.05$].

Figure 3 illustrates the results of stimulus generalization tests administered with morphine ($n = 7$), ketamine ($n = 7$), LSD ($n = 7$), and DOM ($n = 5$). Percentage of salvinorin A-lever responses is shown in the upper figure and response rate is shown in the lower figure. All doses

Fig. 3



Dose–response curves for D-lysergic diethylamide (LSD, $n=7$), 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM, $n=5$), morphine ($n=7$), and ketamine ($n=7$) in salvinorin A-trained (2.0 mg/kg, intraperitoneally, 20 min) rats. Upper panel: percentage of salvinorin A responses; lower panel: response rate. Each point represents the mean (± standard error of the mean).

of morphine failed to substitute for salvinorin A and the highest dose tested (10 mg/kg) completely abolished responding in all subjects. The effects of morphine on the percentage of salvinorin A-lever responding were not statistically significant. Response rates were significantly reduced by morphine [$F(5,41) = 6.30$, $P < 0.001$] with 5.6 and 10 mg/kg significantly different from vehicle ($P < 0.05$).

Although none of the hallucinogens examined produced complete substitution for salvinorin A, both ketamine and LSD produced substantial salvinorin A-lever responding that was significantly different from vehicle at some doses. Five of the seven subjects tested with ketamine exhibited complete stimulus generalization to 4.0 mg/kg and the other two made 25 and 48% salvinorin A-lever responses after this dose. The highest dose of ketamine tested (10 mg/kg) produced full substitution in three subjects, 72% in one subject, 24% in one subject, and completely suppressed responding in the remaining two subjects. Data from three subjects were excluded from statistical analyses due to severe response disruption after a dose of 8 or 10 mg/kg of ketamine. Four subjects that made the minimum required responses on all six dose levels were included in a one-way repeated measures ANOVA, which indicated that the effects of ketamine on drug-appropriate responses were statistically significant [$F(5,23) = 6.06$, $P < 0.01$]. Dunnett's multiple comparison test indicated that responding on the salvinorin-A-associated lever after 4.0 ($P < 0.01$), 8.0, and 10 mg/kg ($P < 0.05$) was significantly different from salvinorin-A-lever responding after the vehicle. A repeated-measures ANOVA indicated that ketamine significantly reduced response rate [$F(5,41) = 6.10$, $P < 0.001$] and the effects of nearly all doses (4, 6, 8 and 10 mg/kg) on response rate were significantly different from those of vehicle ($P < 0.01$).

Among the seven subjects tested with LSD, 0.08 mg/kg produced greater than 80% salvinorin A-appropriate responding in three subjects, between 60 and 65% salvinorin A-lever responding in two subjects and completely disrupted responding in two subjects. A repeated-measures ANOVA was conducted on the data from five subjects that met the response requirement with all LSD test doses. This analysis indicated that LSD produced a significant amount of salvinorin A-associated responding [$F(4,24) = 7.21$, $P < 0.01$] and Dunnett's comparison tests indicated that both 0.04 and 0.08 mg/kg of LSD produced salvinorin A-lever responding that was significantly different from vehicle ($P < 0.01$). A repeated-measures one-way ANOVA indicated a significant effect of LSD dose on response rate [$F(4,34) = 7.2$, $P < 0.001$] and Dunnett's multiple comparison tests indicated that response rates after vehicle were significantly different from those after 0.04 ($P < 0.05$), 0.06 ($P < 0.01$), and 0.08 ($P < 0.01$) mg/kg of LSD.

DOM engendered less drug-lever responding than LSD; however, only five subjects were tested due to a low supply of this test compound. Two of the five subjects tested exhibited full stimulus generalization (100%) at two doses of DOM each (0.032 and 0.32 mg/kg and 0.32 and 1.0 mg/kg). Substitution was not present in three of five subjects at doses of less than or equal to 1.0 mg/kg. At 2.0 mg/kg, responding was disrupted in four of five subjects. Overall, no dose produced a group mean greater than 60%. A repeated-measures ANOVA on percentage of drug-lever responses was not significant and the effects DOM on response rates did not quite reach statistical significance [$F(5,29) = 2.54$, $P = 0.06$].

Discussion

Few studies have explored the discriminative stimulus effects of salvinorin A and only two published studies to date have implemented salvinorin A as the training stimulus (Baker *et al.*, 2009; Butelman *et al.*, 2010). This study confirms previous findings that salvinorin A can establish and maintain discriminative stimulus control in laboratory subjects. Moreover, this is the first study to establish that synthetic salvinorin B derivatives, EOM-Sal B and MOM-Sal B, produce similar discriminative stimulus effects to those of salvinorin A. These substances exhibited full substitution and were more potent than salvinorin A.

A limited number of in-vivo assessments have been conducted with salvinorin B derivatives. Wang *et al.* 2008 compared MOM-Sal B with salvinorin A and U50488 in tests of mobility, hypothermia, and nociception in rodents. Their findings indicate that MOM-Sal B is a potent and efficacious KOR agonist with longer-lasting in-vivo effects compared with salvinorin A. In rats, MOM-Sal B produced antinociceptive effects up to 120-min postinjection and hypothermic effects up to 90-min postinjection, whereas salvinorin A (10 mg/kg) elicited neither antinociception nor hypothermia 30 min after injection (Wang *et al.*, 2008). Although this study did not assess the time course of MOM-Sal B due to insufficient supplies of this test compound, our findings are consistent with those of Wang *et al.* (2008) regarding higher potency of MOM-Sal B compared with salvinorin A.

The ED_{50} determined for MOM-Sal B in this study was slightly lower than that of EOM-Sal B, but this difference was not significant. These results are inconsistent with results of in-vitro studies indicating EOM-Sal B was approximately 10 times more potent than MOM-Sal B (Munro *et al.*, 2008). These contradictory findings may be due to differences in bioavailability of the two compounds. Further in-vivo studies are required to fully characterize the potential differences in potency and efficacy of EOM-Sal B and MOM-Sal B. Nonetheless, the present results that these compounds displayed a higher potency than salvinorin A *in vivo* are indeed comparable to previous in-vitro investigations.

The present study also compared the time course of EOM-Sal B to that of salvinorin A. Results suggest that the discriminative stimulus effects of 2.0 mg/kg salvinorin A are present 20 min postinjection, but responding no longer generalizes to salvinorin A by 40 min postinjection. In contrast, EOM-Sal B produced stimulus generalization to salvinorin A at 60-min postinjection. These findings are somewhat compatible with those of Hooker *et al.* 2009 who investigated the duration of action and metabolism of salvinorin A in comparison with EOM-Sal B. They determined that the kinetics in rat brain over 60 min were similar for both compounds, and whole brain concentrations of EOM-Sal B were almost 3-fold higher than salvinorin A at approximately 65-min postinjection, indicating that EOM-Sal B is metabolized more slowly than salvinorin A.

The current findings regarding the time course of salvinorin A are similar to results obtained in unconditioned behavioral tests of facial relaxation and ptosis in nonhuman primates (Butelman *et al.*, 2010) and the pharmacokinetics and brain distribution of salvinorin A in rats (Teskin *et al.* 2009). Butelman *et al.* (2010) reported that the effects of salvinorin A on facial relaxation and ptosis peaked between 5 and 15-min postsubcutaneous administration and within 1 to 2-min postintravenous administration. Teskin *et al.* (2009) examined the in-vivo pharmacokinetics and brain distribution of salvinorin A after a single intraperitoneal injection (10 mg/kg) in cohorts of adult male Sprague-Dawley rats. Salvinorin A was shown to have a rapid onset and short duration of action. T_{max} was observed within 10–15-min postinjection for both the plasma profile and brain uptake of salvinorin A. Salvinorin A was eliminated rather quickly as demonstrated by a half-life ($t_{1/2}$) of 75 min and a clearance (Cl/F) of 26 l/h/kg. The brain half-life ($t_{1/2}$) was only 36 min.

Recent findings indicate that monkeys trained to discriminate salvinorin A did not generalize to the serotonergic hallucinogen, psilocybin or the dissociative anesthetic, ketamine (Butelman *et al.*, 2010), nor did rats trained to discriminate ketamine or LSD generalize to salvinorin A (Killinger *et al.*, 2010). It was, therefore, somewhat surprising that both ketamine and LSD produced a significant amount of salvinorin-A-associated responding in this study. Earlier findings comparing dissociative hallucinogens with KOR agonists have been somewhat inconsistent. Shearman and Herz (1982) reported that phencyclidine and ketamine substituted in some rats trained to discriminate bremazocine, but not in rats trained to discriminate ethylketocyclazocine. In other studies, phencyclidine failed to substitute in rats trained to discriminate spiradoline (Holtzman et al., 1991) or U50488 (Picker *et al.*, 1990). However, a more recent study reported that noncompetitive NMDA antagonists (phencyclidine, ketamine, and MK-801) all produced full substitution for U50488 in rats, whereas competitive NMDA antagonists failed to do so (Mori *et al.*, 2006). These investigators suggest that similarities in the discriminative stimulus effects of U50488 and noncompetitive NMDA-receptor antagonists may be associated with their aversive effects. Nemeth *et al.* (2010) have recently noted that salvinorin A and ketamine share similar behavioral and pharmacological profiles that have been previously underappreciated. Furthermore, they reported that salvinorin A and ketamine produce similar patterns of disruption in behavioral tests of attention in rats and pretreatment with the KOR antagonist, JDTic, blocked the effects of

salvinorin A and some of the effects of ketamine (Nemeth *et al.*, 2010).

Although ketamine and LSD exhibited only partial substitution for salvinorin A and the dose–effect curves were not linear, the current findings are of particular interest in light of a recent report that smoked salvinorin A produced mystical-type effects similar to classic hallucinogens in humans with earlier hallucinogen experience (Johnson *et al.*, 2011). In consideration of these findings, the influence of an individual drug history on stimulus substitution between KOR agonists and other hallucinogens may also be of particular interest in future drug discrimination investigations with these substances.

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Conflicts of interest

There are no conflicts of interest.

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