ORIGINAL INVESTIGATION

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The plant-derived hallucinogen, salvinorin A, produces κ-opioid agonist-like discriminative effects in rhesus monkeys

Abstract *Rationale:* Salvinorin A is the active component of the hallucinogenic plant *Salvia divinorum*. The potential mode of action of this hallucinogen was unknown until recently. A recent in vitro study detected high affinity and efficacy of salvinorin A at κ-opioid receptors. It was postulated that salvinorin A would produce discriminative stimulus effects similar to those of a high efficacy κ-agonist (U69,593) in rhesus monkeys. *Methods:* Monkeys were previously trained to discriminate U69,593 (0.0056 or 0.013 mg/kg; SC) from vehicle in a food-reinforced FR20 (fixed ratio 20) operant conditioning procedure (n=3). The ability of salvinorin A to cause generalization ($\geq$90% U69,593-appropriate responding) was examined in time course and cumulative dose-effect curve studies. *Results:* All subjects dose-dependently emitted full U69,593-appropriate responding after salvinorin A (0.001–0.032 mg/kg, SC). Salvinorin A-induced generalization started 5–15 min after injection, and dissipated by 120 min. The opioid antagonist quadazocine (0.32 mg/kg) fully blocked the effects of salvinorin A. The κ-selective antagonist GNTI (1 mg/kg; 24 h pretreatment) did not cause significant antagonism of the effects of salvinorin A (GNTI, under these conditions, was only effective as an antagonist in two of three monkeys). The NMDA antagonist ketamine (0.1–3.2 mg/kg) was not generalized by any subject, indicating that not all compounds that produce hallucinogenic or psychotomimetic effects in humans are generalized by subjects trained to discriminate U69,593. *Conclusions:* The naturally occurring hallucinogen salvinorin A produces discriminative stimulus effects similar to those of a high efficacy κ-agonist in non-human primates.

Keywords κ-Opioid · Salvinorin A · Drug discrimination · Hallucinogen

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Introduction

Salvinorin A is the main pharmacologically active component of the ethnopharmacological hallucinogenic plant, *Salvia divinorum* (Ortega et al. 1982; Valdes 1994). *Salvia divinorum* was administered in traditional ethnopharmacological practice by the Mazatec indigenous people of Oaxaca, Mexico (Valdes et al. 1993; Valdes 1994). More recently, there have been reports of *Salvia divinorum* self-administration in non-ethnopharmacological settings, presumably due to its hallucinogenic effects. *Salvia divinorum* leaf preparations (occasionally fortified with extracted salvinorin A) are widely available in Western Europe and the USA, notably on Internet sites (Drug Enforcement Administration 2002; Observateur français des drogues et des toxicomanies 2002).

The potential mode of action of salvinorin A was unknown until recently, since salvinorin A does not bind to receptor sites known to be the targets of many psychotomimetic/hallucinogenic compounds (e.g. serotonergic, NMDA, PCP sites) (Siebert 1994; Roth et al. 2002). A recent pivotal study reported that salvinorin A was a selective, high efficacy κ-agonist in cloned human κ-receptors, and in guinea pig brain (Roth et al. 2002). Synthetic κ-agonists administered systemically to humans are known to produce a variety of dose-dependent and reversible subjective/interoceptive effects, including sedation, dysphoria and "psychotomimetic" effects (Pfeiffer et al. 1986; Ur et al. 1997; Walsh et al. 2001). Interestingly, potential κ-agonist effects of *Salvia divinorum* may underlie its voluntary use (self-administration) as a hallucinogen in non-ethnopharmacological practice in humans (Giroud et al. 2000; Drug Enforcement Administration 2002; Sheffler and Roth 2003).

There is at present no available evidence that focuses on the potential κ-agonist effects of salvinorin A in primates. In the present studies, we therefore characterized the effects of systemically administered salvinorin A in rhesus monkeys trained to discriminate the high-efficacy, centrally penetrating synthetic κ-agonist, U69,593 (Remmers et al. 1999; Butelman et al. 2002).

Focused antagonist studies with quadazocine and with the κ-selective antagonist GNTI were instituted, in order to study the receptor mediation of the discriminative effects of salvinorin A (Butelman et al. 1999; Negus et al. 2002). The effectiveness of ketamine (a non-competitive NMDA antagonist) was also studied in this assay, in order to determine whether pharmacologically diverse hallucinogenic psychotomimetic compounds may produce U69,593-like discriminative effects (Krystal et al. 1994; Bowdle et al. 1998).

Materials and methods

Subjects

Intact, adult captive-bred rhesus monkeys (*M. mulatta;* one male and two females) were singly housed in a room maintained at 20–22ºC with controlled humidity, and a 12:12 hour light: dark cycle (lights on at 0730 hours). They were fed approximately 10 jumbo primate chow biscuits (PMI Feeds, Brentwood, MO, USA) daily, supplemented by fruit, nuts and multivitamins. Water was available ad libitum in home cages. These studies were reviewed by the Institutional Animal Care and Use Committee of the Rockefeller University, and were in accordance with Guidelines of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare, Publication ISBN 0-309-05377-3, revised 1996).

Procedures

Three subjects were previously trained to discriminate U69,593 (0.0056 or 0.013 mg/kg, SC) from vehicle in a two-key (fixed ratio) FR 20 task, in operant conditioning chambers (Med Associates, St Albans, Vt., USA). The chambers were connected to a computer, via a Med Associates interface. Subjects were not exposed to chronic drug administration at any time and had received principally opioid compounds intermittently as part of discrimination training and testing.

Training

Subjects were trained in multiple-cycle sessions; each cycle consisted of an initial SC injection followed by a 15-min time out period (time-out responses had no consequence). At the end of the time-out period, a 5-min response period started (signaled by stimulus lights above each lever), during which monkeys could receive up to 10 sucrose pellets (300 mg; PJ Noyes/Research Diets; New Brunswick, N.J., USA), by responding on the injection-appropriate lever. A maximum of 4 cycles occurred within each training session. When multiple training cycles occurred, the first cycle was always a vehicle cycle. If drug (U69,593) was administered, it would always be on the last cycle within a training session. Monkeys were previously trained and had attained criterion (e.g. at least 90% injection-appropriate responses in four consecutive sessions, and no more than 20 responses on the incorrect lever before the first food presentation in any cycle, with response rates of at least 0.8 responses/s).

Testing

Test sessions were identical to that described above, except that 20 responses on either lever resulted in pellet delivery. Tests followed either a time course or a cumulative dosing procedure. In time course tests, a response period would commence at standard time points (e.g. 5–120 min) after a single SC injection. In cumulative dosing tests, doses of the compounds were increased in 0.5 log unit steps (each cycle commenced with a 15-min time-out period, followed by a 5-min response period, as described above). Agonist doses were increased in each subject until either a) generalization (i.e. at least 90% U69,593-appropriate responding), b) rates of responding were decreased by more than 50%, or c) in the presence of effects which interfered with behavioral testing. Tests in the same subject were separated by at least 2 consecutive training days at criterion performance (i.e. at least 90% injection-appropriate responding and no more than 20 responses on the incorrect lever before the first food presentation in any cycle, with responses rates of at least 0.8 responses/s).

Design

Salvinorin A (0.001–0.032 mg/kg), its vehicle (0.16 ml/kg) and U69,593 (0.001–0.01 mg/kg) were compared in time course and cumulative dose-effect curve studies. Selected time course experiments for salvinorin A were re-determined 60 min following pretreatment with quadazocine (0.32 mg/kg). A quadazocine control study (i.e. for the effects of quadazocine alone over the relevant time period) was also completed. The cumulative salvinorin A dose-effect curve was re-determined after the same quadazocine pretreatment, and after GNTI (1 mg/kg; 24 h) pretreatment. Due to the long duration of action of GNTI, training and testing was suspended for 3 weeks after the GNTI test (Negus et al. 2002). A cumulative dose-effect curve with ketamine (0.1–3.2 mg/kg) was also completed. Each of the above experiments was carried out as one or two determinations per subject.

Data analysis

The main dependent variable was percent drug (U69)-appropriate responding (%DAR; presented graphically as mean±SEM). The subjects were considered to fully generalize if they emitted at least 90% of the responses within a response period on the U69,593-appropriate lever. Cumulative dose-effect curves for salvinorin A (0.001–0.032 mg/kg) alone or after quadazocine (0.32 mg/kg) or GNTI (1 mg/kg) pretreatment were analyzed in a two-way (salvinorin A dose×pretreatment condition) repeated measures ANOVA, followed by Duncan's test for pretreatment condition (Sigmastat- SPSS, Chicago, Ill., USA). In two individual cases in which 100% U69,593-appropriate responding occurred at a non-maximal salvinorin A dose (e.g. 0.01 mg/kg), a value of 100% was assigned for the maximal dose for this analysis. Significance (α) was set at the 0.05 level.

Drugs

U69,593 (Pharmacia, Kalamazoo, Mich., USA) and quadazocine mesylate (Sanofi-Winthrop, Malvern, Pa., USA) were dissolved in sterile water with the addition of 2 drops of lactic acid (pH approximately 6; further dilutions were made with sterile water). GNTI dihydrochloride (5'-guanidinonaltrindole; Tocris, Ellisville, Mo., USA) was dissolved in sterile water. Salvinorin A (Biosearch, Novato, Calif., USA) was dissolved in ethanol: Tween 80: sterile water vehicle (1:1:8 proportion, by volume). Ketamine hydrochloride (Fort Dodge, Iowa, USA) was diluted with sterile water, from stock solution (100 mg/ml). All drugs were injected SC in volumes of 0.05–0.16 ml/kg. Drug doses are expressed as the salt or base forms of each compound described above.
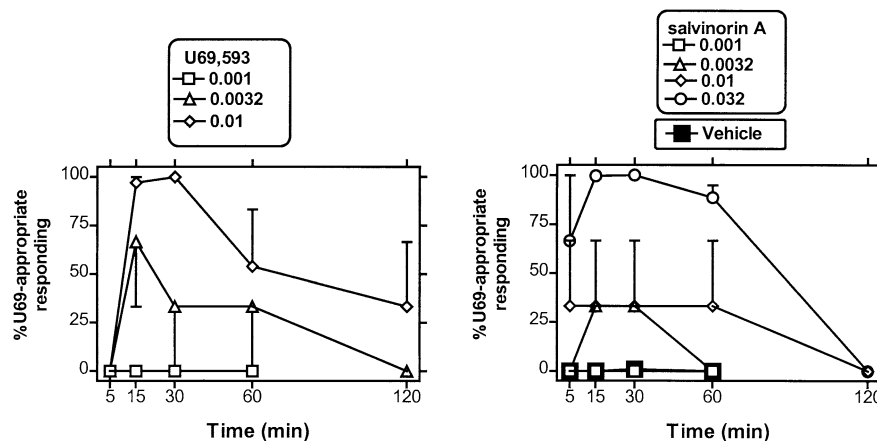
Results

Baseline performance

Monkeys were previously trained in the discrimination and had achieved criterion performance prior to the beginning of these studies. Stimulus control was stable in these subjects in the period prior to these studies. Thus, in the 5 consecutive training days before the first salvinorin A test, the following mean training values were obtained: vehicle training cycles mean (SEM): %DAR=0.67% (SEM 0.6); rate of responding=2.76 responses/s (SEM 0.84); U69,593 training cycles mean (SEM): %DAR= 99.8% (SEM 0.17); rate of responding=2.62 responses/s (SEM 0.12). None of the experimental conditions studied with salvinorin A or U69,593 resulted in robust changes in rates of responding; rate of responding data are therefore not presented herein.

Time course studies

The vehicle solution used for salvinorin A resulted in vehicle-appropriate responding over the course a 60-min time course test in all subjects (Fig. 1). U69,593 produced dose-and time-dependent generalization in all subjects (0.001–0.01 mg/kg). When tested under identical conditions, salvinorin A (0.001–0.032 mg/kg) also dose-dependently generalized in all subjects (one subject, JA, generalized initially at 0.0032–0.01 mg/kg, the two other subjects, FL and VE, generalized at 0.032 mg/kg). At the active doses, both U69,593 and salvinorin A displayed rapid onsets following bolus SC administration (i.e. within 5–15 min). At these doses, the two compounds did not cause a robust rate-decreasing effect (not shown). Quadazocine (0.32 mg/kg) was administered 60 min before the lowest dose of salvinorin A, which produced robust generalization in each subject (e.g. 0.01 mg/kg for subject JA and 0.032 mg/kg for the other two subjects). In each case, quadazocine fully blocked the discriminative effects of salvinorin A in each 60-min time course test. That is, all salvinorin A-treated subjects emitted less than
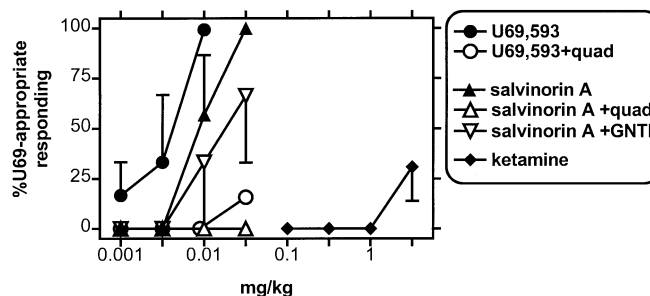

Fig. 2 Cumulative dose-effect curve studies of U69,593, salvinorin A and ketamine in monkeys trained to discriminate U69,593 from vehicle. *Abscissa*: dose (mg/kg); *ordinate*: percent drug-appropriate responding. The U69,593 dose-effect curve was re-determined after 60 min pretreatment with quadazocine (*quad*; 0.32 mg/kg; error bars for this data set omitted for clarity). The salvinorin A dose-effect curve was also re-determined after 60 min pretreatment with quadazocine (0.32 mg/kg), or 24 h pretreatment with 5'-guanidinonaltrindole (*GNTI*; 1 mg/kg). Other details as in Fig. 1

2% DAR in all time points, in the presence of quadazocine (not shown). Quadazocine (0.32 mg/kg) alone, tested over this same time period (i.e. 60–120 min, in the presence of bolus vehicle administration) produced 0% DAR in all subjects, throughout (not shown).

Cumulative dosing studies

A "cumulative" vehicle test (4 cycles) resulted in vehicle-appropriate responding in all subjects (i.e. 0% DAR was obtained in all subjects, throughout). Cumulative U69,593 and salvinorin A were dose-dependently generalized by all subjects, within a similar dose range (Fig. 2). Rates of responding were not robustly decreased over the studied dose ranges for U69,593 and salvinorin A (not shown).

Quadazocine pretreatment (0.32 mg/kg; 60 min before the test) fully blocked the effects of salvinorin A (0.001–0.032 mg/kg) in all subjects in these cumulative dosing determinations. For comparison, quadazocine (0.32 mg/ kg; under identical conditions) also caused a robust antagonism of the effects of U69,593 (0.001–0.032 mg/ kg). Complete surmountability experiments were not

attempted, due to solubility limits for salvinorin A in the present vehicle. The long-lasting κ-selective antagonist GNTI (1 mg/kg) was studied as a 24-h pretreatment to the cumulative salvinorin A dose-effect curve. GNTI caused a shift in the salvinorin A mean dose-effect curve, and this was not fully surmounted at the largest salvinorin A dose tested herein. However, it should be noted that GNTI did not block the salvinorin A dose-effect curve for subject JA, but did block those for subjects FL and VE.

Cumulative dose-effect curves for salvinorin A (0.001–0.032 mg/kg) alone or after quadazocine (0.32 mg/kg) or GNTI (1 mg/kg) pretreatment were analyzed using a two-way (salvinorin A dose×pretreatment condition) repeated measures ANOVA. This ANOVA was significant for salvinorin A dose [$F(3,6)=7.32$] and for pretreatment condition [$F(2,4)=7.41$], as well as for their interaction [$F(6,12)=3.16$]. A Duncan's test for pretreatment condition revealed that the quadazocine ($q=5.38$), but not the GNTI ($q=1.96$) pretreatment condition was different from salvinorin A alone.

The ketamine cumulative dose-effect curve (0.1–3.2 mg/kg), was determined under identical conditions. Up to ketamine doses that suppressed response rates by more than 50% from mean vehicle baseline values, none of the subjects generalized ketamine.

Discussion

Salvinorin A produces discriminative stimulus effects similar to those of the centrally penetrating κ-agonist U69,593, following SC administration. Salvinorin A was approximately equipotent with U69,593 in the present studies. Salvinorin A is also approximately equipotent with U69,593 at κ-receptors in vitro (Roth et al. 2002). That is, EC_{50} values in the adenylyl cyclase and GTPγS assays were within 0.25 log units of each other, for these two agonists. Time course studies with the larger doses of SC salvinorin A revealed a fast onset and moderate duration of action (e.g. 5 and 60 min, respectively). A rapid onset for interoceptive effects of smoked salvinorin A has been reported in humans (Siebert 1994); no data are available for any systemic route in humans, to our knowledge.

The largest salvinorin A dose used herein (0.032 mg/kg; SC) caused only slight overt behavioral effects (sedation-like) over the 60 min following administration (pilot observational studies). This is consistent with previous studies indicating that discriminable doses of κ-agonists in this procedure (e.g. U69,593 0.01 mg/kg) are accompanied by only slight sedative effects (Butelman and Kreek 2001; Butelman et al. 2003). All subjects returned to baseline behavior after the present salvinorin A tests. No emesis or motor effects (e.g. tremors) were observed during or after any of the present salvinorin A tests.

The discriminative effects of salvinorin A were blocked by quadazocine (0.32 mg/kg) in all subjects. This quadazocine dose is sufficient to block the behav-

ioral and neuroendocrine effects of the selective κ-agonist U69,593 in this species (Butelman et al. 1999). The long-lasting κ-selective antagonist GNTI (Negus et al. 2002) antagonized the effects of cumulative salvinorin A in only two of the three subjects, under the present conditions. In recent studies, these U69,593-discriminating subjects generalized structurally diverse κ-agonists, but not μ- or δ-opioid agonists (Butelman et al. 2002). These findings, taken together with salvinorin A's high and selective affinity at κ-receptors in vitro (Roth et al. 2002), indicate that salvinorin A produces κ-agonist-like discriminative effects following SC administration in non-human primates.

In a control study, the NMDA antagonist ketamine was not generalized by any of the subjects, up to doses that produced robust rate-decreasing effects. The presently studied ketamine dose range (0.1–3.2 mg/kg) includes doses that produce discriminative effects in ketamine-trained monkeys (France et al. 1989). Ketamine has also been reported to produce psychotomimetic or hallucinatory effects in humans (Krystal et al. 1994; Bowdle et al. 1998). The present ketamine experiment therefore suggests that not all compounds with psychotomimetic or hallucinatory effects in humans produce U69,593-like effects in rhesus monkeys.

These are the first studies on the behavioral effects of salvinorin A in non-human primates, and the first drug discrimination studies with salvinorin A in any species, to our knowledge. The findings are consistent with the in vitro characterization of salvinorin A as a κ-agonist (Roth et al. 2002). The present studies are also consistent with the hypothesis that *Salvia divinorum*'s self-administration in humans (presumably for its interoceptive/hallucinogenic effects) is due to the κ-agonist effects of salvinorin A.

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