Involvement of κ-Opioid and Endocannabinoid System on Salvinorin A-Induced Reward

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Background: The recreational drug, Salvinorin A, derived from the plant of Salvia divinorum, is a potent and selective κ-opioid receptor agonist. The abuse of selective k-agonists is a novel phenomenon, the mechanism of which is not fully understood.

Methods: We investigated salvinorin A given SC on the conditioned place preference (.05–160 μg/kg) and intracerebroventricular (ICV) self-administration (.01–1 μg/infusion) paradigms, in Wistar rats.

Results: The present results demonstrate the rewarding effects of Salvinorin A in a range of doses between .1 and 40 μg/kg SC for conditioned place preference test and .1–.5 μg/infusion for ICV self-administration. Highest doses (160 μg/kg for conditioned place preference test and 1 μg/infusion for ICV self-administration) were aversive. The rewarding effect was antagonized by intraperitoneal (IP) pretreatment with the cannabinoid CB_1 receptor antagonist, rimonabant [N-piperidino-5-(4-chlorophenyl)1-(2,4-dichloro phenyl)-4 methyl pyrazole 3-carboxamide] (1 mg/kg), and the κ-opioid receptor antagonist, nor-binaltorphimine (nor-BNI) (10 mg/kg). In the shell of nucleus accumbens, dopamine extracellular levels were increased after administration of salvinorin A (40 μg/kg SC), reaching a maximum value of about 150%.

Conclusions: These data provide the demonstration of the rewarding effects of Salvinorin A through an interaction between κ-opioid and (endo)cannabinoid system in rats.

Key Words: Conditioned place preference, dopamine, κ-opioid receptor, microdialysis, self-administration, SR 141716A

Salvinorin A is the only non-nitrogenous naturally occurring κ-opioid receptor agonist (1–5) known at present. It is the major active ingredient of the *Salvia divinorum* leaves in producing hallucinations in humans but distinct from those of the classical hallucinogens (lysergic acid diethylamide [LSD], psilocybin, and mescaline) (6). Salvinorin A induces in humans an intense, short-lived hallucinogenic and positive effects (changes in depth perception [i.e., the ability to perceive spatial relationships, especially distances between objects, in three dimensions]; increase in sensual and aesthetic appreciation; creative dream-like experience) (7). However, depending on the dose and the route of administration, the effects are sometimes extremely negative (overly intense experiences, fear, terror, and panic; increased perspiration; and a possible difficulty integrating experiences), and people can desire not to repeat the "bad trip" (8). Users, particularly teenagers, claim the drug is not addictive, but there is little actual systematic data available on patterns of use and abuse and on the frequency of repeated use.

Even if salvinorin A has been found to allosterically modulate the μ-opioid receptor in Chinese hamster ovary cells expressing the cloned human opioid receptor (9), it has been clearly shown to be a potent, selective, and efficacious κ-opioid receptor agonist in binding assays (1,2,3) and to reduce in vitro electrically induced contractions in guinea pig ileum (10).

Salvinorin A showed a low toxicity in rodents (11), even at doses many times greater than those that humans are exposed to. When given intraperitoneally (IP) (.5 and 4.0 mg/kg) (12) or intracerebroventricularly (ICV) (1 and 30 μg/rat) (13), it displays antinociceptive activity, whereas when systemically injected, it disrupted climbing behavior in mice, indicating a state of sedation and/or motor incoordination. Similar effects were observed with the μ-opioid agonist remifentanil and the κ-opioid agonist U69, 593 (14). Furthermore, a pro–depressant-like response after salvinorin A (.5–2 mg/kg) in the forced swimming test has been reported (15).

Even if the recreational use of salvinorin A in humans reveals a probable misuse, no data is available about its abuse potential in animals. A dose-dependent decrease of dopamine (DA) levels in the caudate putamen and a conditioned place aversion at doses of 1.0 mg/kg and 3.2 mg/kg in C57BL/6J mice (16) was obtained. Butelman *et al.* (17) demonstrated a discriminative stimulus effects in rhesus monkeys similar to that obtained with the κ-opioid agonist U69, 593.

In view of the fact that conditioned place aversion was found in animals by Zhang *et al.* (16) at doses higher (1–3.2 mg/kg) than those effective in the *Salvia divinorum* smoked by humans (200–500 μg [i.e., about 3–7.5 μg/kg]) and a bi-directional interaction between opioid and cannabinoid systems has been widely reported (18,19), the aim of the present study was to investigate the effect of low doses of salvinorin A (.01–160 μg/kg) in the conditioned place preference and ICV self-administration paradigms, in Wistar rats. To better clarify salvinorin A mechanism of action, we investigated the effect of the κ-opioid nor-binaltorphimine (nor-BNI) and the CB_1 cannabinoid rimonabant receptor antagonists on salvinorin A-induced behavioral effect. Furthermore, with the in vivo microdialysis technique in freely moving rats, the effect of salvinorin A on DA levels in the shell portion of nucleus accumbens was studied.

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Methods and Materials

Animals

Male Wistar rats (Charles River; Calco, Como, Italy) weighing 200–300 g were housed in individual cages in a climatically controlled colony room under a 12/12-hour light/dark cycle (lights on at 8:00 AM). Food and water were continuously available, and each animal was handled daily through the 1st week. Then they were randomly assigned to different experimental groups of 10 each. All experiments were performed between 8:00 AM and 4:00 PM. Experimental protocols were approved by the local Ethical Committee and performed in strict accordance with the guidelines for Care and Use of Experimental Animals of the European Economic Community (86/609; DL27/01/92, number 116). All efforts were made to minimize the number of animals used and their discomfort.

Spontaneous Motor Activity

Spontaneous motor activity was evaluated as previously described (20) in an activity cage (43 cm long × 43 cm wide × 32 cm high; Ugo Basile, Varese, Italy) placed in a sound-attenuating room. The cage was fitted with two parallel horizontal infrared beams located 2 cm from the floor. Cumulative horizontal and vertical movement counts were recorded for 30 min, every 5 min. Salvinorin A (.05–160 μg/kg) or vehicle (Tween 80; ethanol; saline; 1:1:8) were given SC 5 min before testing began.

Conditioned Place Preference

Apparatus. Conditioned place preference was tested in a shuttle box as described elsewhere (21) with slight modifications. Briefly, the apparatus was divided into two equal-sized compartments separated by a guillotine door. Each compartment had different visual and textured cues in the form of brown and white horizontal lines or circles and rough or smooth wooden floor. The visual and tactile cues were balanced such that no evident preference was exhibited before conditioning.

Procedure. Conditioned place preference consisted of three phases: preconditioning, conditioning, and postconditioning.

Preconditioning: On Days 1–2, the rats were allowed to explore the two compartments for 15 min each day. To check for any initial unconditioned preference for either of the two sites, the time spent by each animal in the two compartments on the 3rd day was recorded.

Conditioning: Conditioning sessions (four for salvinorin A and four for vehicle) were conducted once daily (9:00 AM) for 8 days. Five minutes after the SC injection of salvinorin A (.05–160 μg/kg), animals were confined in the conditioned compartment for 30 min, with the door closed. On alternate days, animals receiving vehicle (Tween 80; ethanol; saline; 1:1:8) were confined in the opposite compartment for 30 min. For the antagonism studies, rats received, before each drug pairing, an IP injection of rimonabant (1 mg/kg) or nor-BNI (10 mg/kg) or the appropriate vehicle 20 min or 120 min before the maximal rewarding dose of salvinorin A or vehicle, respectively. After treatment, animals were placed in the conditioned compartment for 30 min. On alternate days, animals receiving double injection of appropriate vehicle were confined to the opposite compartment for 30 min. Drug–texture pairings were always counterbalanced.

Postconditioning: On the test day, neither drug nor vehicle was injected. Each rat was put at the intersection of the two compartments, with access to both sides, and the time spent in each of the two compartments was measured over a 15-min period as an indicator of rewarding properties.

ICV Self-Administration

Surgical Procedure. Animals were anesthetized with chloral hydrate (450 mg/kg IP) and implanted with ICV double-guide stainless steel cannulas (22 gauge), anchored to a pedestal as described elsewhere (22). Each rat was allowed to recover for approximately 1 week. To ascertain the accuracy of the ICV injections, at the end of the experiments, the rats were injected by the same route with 10 μL of a saturated solution of Evans blue (Merck, Whitehouse Station New Jersey) and killed immediately; mascroscopic examination of the brain confirmed that only the area around the lateral ventricles was stained.

Apparatus. An operant chamber (Coulbourn Instruments, Sevenoaks, Kent, England) was housed in a sound-attenuating cubicle. The chamber was equipped, as previously described (22), with two response levers. A response on either lever resulted in the illumination of a cue light fitted in each dispenser and the delivery of .1 mL of water over a period of 8 sec. During the daily experimental session, a bilateral injection cannula (28 gauge) was placed inside the double guide cannula. The distal ends of the injection cannula were connected to two Silastic flexible coiled spring tubes that, in turn, were connected to a flow-through swivel. The swivel was connected by tubing to two infusion pumps (Mod. A-99; Razel, Stamford, Connecticut), for drug delivery, outside the sound attenuated cubicle. The perfusion tubes easily rotated the liquid swivel. Each infusion delivered a volume of 2 μL/8 sec. If the rat pressed the lever twice within the 8-sec timeout period, the event was recorded as not reinforced. The chamber was connected to a Basilink Data Acquisition System (Ugo Basile, Comerio, Varese, Italy), which controlled reinforcement schedules. A microprocessor assembler (Ugo Basile) gathered, listed, and every 5 min printed the total number of bar pressings and the total number of reinforced bar pressings for each lever.

Training Procedure. Before surgery, rats previously deprived of water for 23 hours were individually trained for 1 hour daily to press both active levers to obtain water as reinforcer for 1 week. One week after surgery, single rats were again placed in the operant chamber, in the same schedule. Two microliters of sterile vehicle (cremophor, ethanol, and cerebrospinal fluid, 1:1:8) were obtained each time the rats pressed either lever. During training, water was delivered after each lever pressing. This procedure was repeated daily for 1 hour until baselines were judged to be stable (5 days at least). Lever pressing was usually acquired within four/five sessions, and a stable pattern of responding developed within 2 weeks.

Testing Procedure. The drug sessions were carried out on the basis of individual preference for one of the levers, the preferred one always being associated with the vehicle (2 μL/infusion) and the nonpreferred one with salvinorin A (.01–1 μg/2 μL). Then, each rat, already checked during training for its preference for one of the two levers, was evaluated in a continuous reinforcement schedule for operant responding after the self-administration of the different concentrations of salvinorin A during a 1-hour daily session. Each unit dose was given in a counterbalanced order and only when the baseline response for the unit dose was stable. For the antagonism studies, further different groups of rats received an IP injection of rimonabant (1 mg/kg) or nor-BNI (10 mg/kg) or the appropriate vehicle 20 min or 120 min before each daily session, respectively, during which the maximally self-administered unit dose of salvinorin A was

available. Daily pretreatment with the antagonists lasted until the self-administration response for salvinorin A was stable (5–10 days). To avoid carryover effects, animals treated in such a way were only studied with one dose of salvinorin A.

Microdialysis Assay

Rats were anesthetized with Equithesin (5 mL/kg IP) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, California). A concentric self-made microdialysis probe (2 mm dialyzing surface, length) (AN 69AF; Hospal-Dasco, Bologna, Italy; cut-off 40,000 daltons, in vitro recovery approximately 30%) was inserted vertically into the shell of the nucleus accumbens (coordinates from bregma AP +1.7, L ± .7, V −8.2) according to the atlas of Paxinos and Watson (23). Starting 24 hours from implantation of the dialysis probe, artificial cerebrospinal fluid (aCSF) (147 mmol/L sodium chloride, 4 mmol/L potassium chloride, 1.5 mmol/L calcium chloride, pH 6–6.5) was pumped through the dialysis membrane at a constant rate of 2.5 μL/min with a CMA/100 microinjection pump (Carnegie Medicine, Stockholm, Sweden). Dialysate samples (50 μL) were collected every 20 min and directly injected into a high-performance liquid chromatography (HPLC) system in order to quantify DA. The system consisted of an isocratic pump (ESA model 580; ESA, Chelmsford, Massachusetts), a 7125 Rheodyne injector connected to a Hewlett Packard (Waldbronn, Germany) series 1100 column thermostat with a reverse phase column (LC18 DBSupelco, 5 μm, 4.6 × 150 mm), and an ESA Coulochem II detector. The first electrode of the detector analytical cell was set at 400 mV and the second at −180 mV; column temperature was set at 30°C. The mobile phase consisted of 50 mmol/L sodium acetate, .073 mmol/L Na2 ethylenediaminetetraacetic acid (EDTA), .35 mmol/L 1-octanesulfonic acid, 12% methanol, pH 4.21 with acetic acid, at a flow rate of 1.0 mL/min. In this condition the sensitivity of the assay for DA was 2 fmol/sample. Only results deriving from rats with correctly positioned dialysis probes were included in statistical analysis of data. The location of the probe was determined histologically at the end of each experiment as previously described by Bert *et al.* (24) (Figure 1).

Drugs

The drugs were: salvinorin A (Daniel Siebert, The *Salvia Divinorum* Research and Information Center, Malibu, California) injected SC in a range of doses between .05 and 160 μg/kg for spontaneous motor activity, conditioned place preference, and microdialysis experiments. For ICV self-administration, salvinorin A was given between .01 and 1 μg/2 μL/infusion. Salvinorin A was dissolved in Tween 80, ethanol, saline (1:1:8). Rimonabant [N – piperidino – 5 – (4- chlorophenyl) 1 – (2,4 – dichlorophenyl) – 4 methyl pyrazole 3-carboxamide] (kindly supplied by Synthelabo-Sanofi Recherche, Montpellier, France) (1 mg/kg) was dissolved in ethanol, cremophor, saline (1:1:18) and given IP. Nor-binaltorphimine HCl (nor-BNI) (10 mg/kg) (Sigma Aldrich, St. Louis, Missouri) was dissolved in saline and given IP. The doses of the drugs were calculated as salt. The κ-opioid receptor antagonist, nor-BNI (10 mg/kg), was given before salvinorin A at a dose that reversed salvinorin A-induced aversion and decreased DA extracellular levels in the caudate putamen (16). The dose of the CB_1 cannabinoid receptor antagonist (1 mg/kg) was chosen on the basis of its ability to antagonize Δ^9-tetrahydrocannabinol (THC)-induced conditioned place preference and self-administration (25).

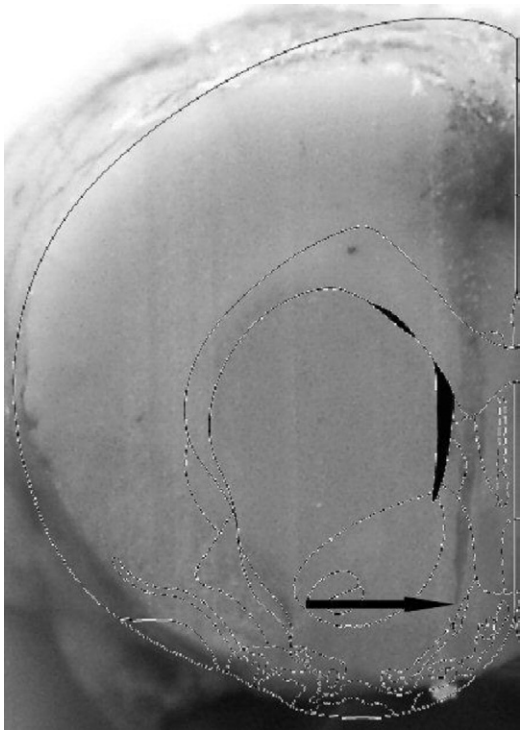

Figure 1. Photomicrograph of a coronal section of the whole rat brain showing representative location of dialysis probe in the shell portion of the nucleus accumbens. The coronal diagram (Paxinos and Watson, 1986) has been fitted and superimposed on the photomicrograph. The arrow indicates the tip of the dialysis probe.

Statistical Analysis

The data were expressed as mean ± SEM and analyzed by one- or two-way analyses of variance (ANOVAs) for multiple comparisons followed by Tukey or Bonferroni's post hoc comparisons. Owing to the individual animal's baseline in the self-administration experiments, different numbers of sessions (from 15 to 20) were needed to reach a stable baseline of lever pressing (no more than approximately 15% difference across the sessions) with each drug unit dose. Thus, statistical analyses involved only the last 5 days of stable baseline. During this period of stable baseline, the mean total daily intake (μg) of salvinorin A was plotted against the log of the self-administered unit doses and was adapted to sigmoidal dose-response curve. The accepted level of significance was $p < .05$. All statistical analyses were done with software Prism, version 4 (GraphPad, San Diego, California).

Results

Behavioral Studies

Spontaneous Motor Activity. Salvinorin A did not affect the mean number of horizontal and vertical counts at any of the doses tested, when compared with vehicle (Figure 2).

Conditioned Place Preference. The development of a conditioned place preference induced by salvinorin A at different doses is shown in Figure 3. The ANOVA revealed significant treatment effect between subjects when comparing the time during the pre- and post-conditioning period in the drug-paired compartment [$F(6,49) = 3.48$; $p < .005$]. Thus, post hoc analysis showed that salvinorin A induced an increase in the time spent in the drug-paired compartment on the post-conditioning day,

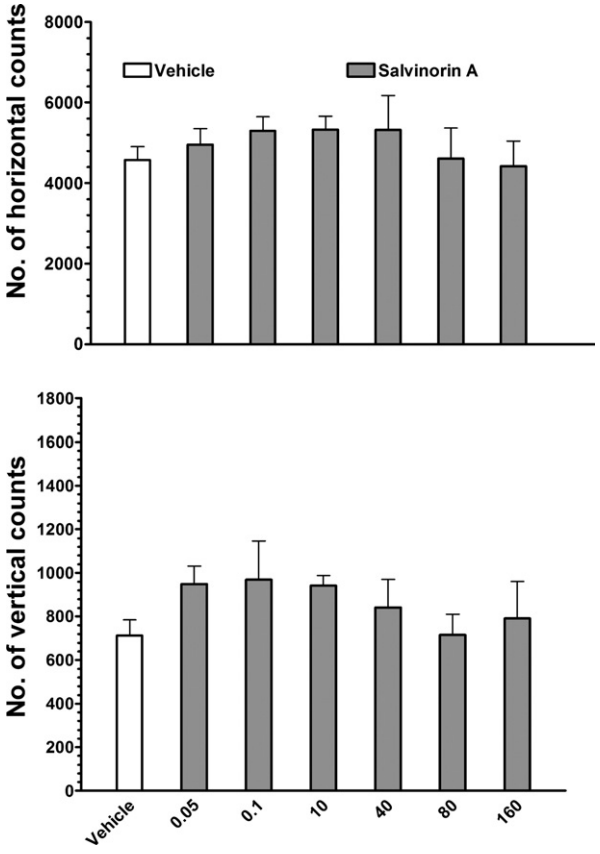

Figure 2. Salvinorin A dose-response. Locomotor response to SC administration of vehicle or increasing doses (μg/kg) of salvinorin A. Animals were treated with vehicle or the drug and 5 min after were placed for 30 min in an activity cage. Their 30-min cumulated activity is reported. There was no difference in locomotor response between vehicle and different doses of salvinorin A in terms of horizontal (top) and vertical (bottom) counts. Results are expressed as mean ± SEM. The *p* values were >.05 (analysis of variance followed by post hoc Tukey's test).

between .1 and 40 μg/kg, in comparison with vehicle group. A dose of 80 μg/kg had no effect, whereas the highest (160 μg/kg) provoked aversion. In antagonism studies, a significant treatment effect was found when comparing the time in the drug-paired compartment, during pre-and post-conditioning periods, in rats given rimonabant or nor-BNI before each drug pairing [$F(5,42) = 13.76$; $p < .0001$, ANOVA] (Table 1). Post hoc analysis showed that the two antagonists, alone, did not change the mean time spent in the drug-paired side between the pre- and post-conditioning periods. However, when combined with the maximally effective dose of salvinorin A (40 μg/kg), they completely reduced the time spent in the drug-paired compartment during post-conditioning, in comparison with salvinorin A alone.

ICV Self-Administration. During the training phase the operant responding of all rats did not change before or after surgery (data not shown). The intake of water, delivered after each lever pressing, did not vary during the training or testing procedure, and food intake and body weight were not modified throughout the experiment (data not shown). Figure 4A illustrates the time course of one representative rat during the training and testing procedure. For the sake of brevity, only the last 10 sessions are shown. Starting from .1 μg/infusion of salvinorin A, a progressive increase in number of pressings of the less-preferred lever was found. The highest concentration produced a gradual decrease in the number of pressings delivering salvinorin A paralleled by an increase of those delivering vehicle. The mean number of pressings on the lever delivering vehicle or increasing concentrations of salvinorin A is depicted in Figure 4B. Self-administration of different unit doses significantly changed operant responding [$F(9,50) = 9.67$; $p < .0001$]. Post hoc comparison indicated that self-administration of salvinorin A (.1 and .5 μg/2 μL) significantly increased the mean number of drug-associated lever pressings in comparison with corresponding vehicle. A significant reduction in the number of drug-associated lever pressings was observed only at the unit dose of 1 μg/2 μL. The mean number of pressings are shown in Figure 5A, where a statistically symmetrical parabola was obtained for each lever ($R^2_{drug} = .51$; $p < .05$, $R^2_{vehicle} = .47$; $p < .05$). The mean daily intake was fitted by a dose-response sigmoidal curve of the self-administered unit doses ($R^2 = .84$; $p < .01$) between a range of .01 and 1 μg/2 μL as depicted in figure 5B. In Figure 6 the mean number of lever pressings under salvinorin A or vehicle self-administration in combination with vehicle, the CB_1 antagonist rimonabant (1 mg/kg) or the k-agonist nor-BNI (10 mg/kg), is shown. The mean number of lever pressings significantly changed between groups [$F(11,60) = 7.75$; $p < .0001$, ANOVA]. Post hoc comparison indicated that pre-treatment with rimonabant or nor-BNI per se did not affect the mean number of pressings on the levers delivering vehicle, in comparison with that obtained during training. When combined with salvinorin A, pre-treatment with both the antagonists significantly reduced the number of drug-associated lever pressings compared with salvinorin A alone.

Microdialysis Assay

Basal dialysate DA concentrations during the pre-session period did not differ significantly between vehicle and salvinorin A groups (data not shown).

Salvinorin A (40 μg/kg SC) significantly increased extracellular DA levels in the shell of the nucleus accumbens within 20 min, remaining elevated until 160 min (Bonferroni post-test), whereas vehicle administration did not modify DA release over

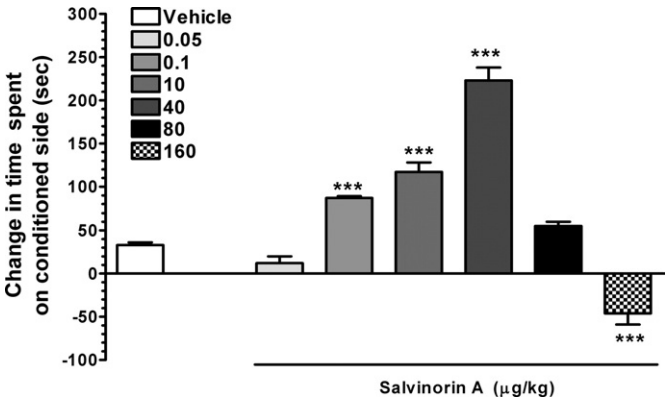

Figure 3. Effect of increasing doses of salvinorin A given SC on conditioned place preference test. A two-compartment box, separated by a guillotine door, with different-textured floors was used for conditioned place preference: place preference was established if the rats spent more time in the compartment associated with salvinorin A injection. Preference was calculated by subtracting the time spent in the salvinorin A–paired compartment before drug conditioning from the time spent after drug conditioning. Place preference was evaluated as the time (mean ± SEM) spent in the drug-paired compartment before and after conditioning on the test day, during which neither drug nor vehicle was injected. ***$p < .001$ as compared with vehicle group during postconditioning (analysis of variance followed by post hoc Tukey's test).

Table 1. Effects Induced by Rimonabant and Nor-BNI on the Place Preference Induced by Salvinorin A in Rats

Pre-Treatment	Dose mg/kg	Treatment	Dose μg/kg	Pre-	Post-	Δ (Pre-Post)
Veh	—	Saline	—	369.90 ± 50.11	402.00 ± 14.09	+42.10 ± 13.00
Nor-BNI	10	Saline	—	376.00 ± 25.71	414.00 ± 72.99	+38.00 ± 28.00
Rim	1	Saline	—	333.00 ± 22.21	355.00 ± 37.34	+22.00 ± 12.00
Veh	—	Salv-A	40	342.70 ± 32.75	565.00 ± 33.53	+222.30 ± 15.00[a]
Nor-BNI	10	Salv-A	40	393.50 ± 12.50	316.00 ± 2.00	+15.00 ± 14.50[b]
Rim	1	Salv-A	40	340.30 ± 31.53	421.12 ± 112.21	+80.82 ± 35.00[b]

Time spent in the drug-paired compartment during pre- and post-conditioning on the test day, in the conditioned place preference test. A two-compartment box, separated by a guillotine door, with different-textured floors was used. Place preference was established if the rats spent more time in the compartment associated with salvinorin A (Salv-A) injection. Preference was calculated by subtracting the time spent in the salvinorin A–paired compartment before drug conditioning from the time spent drug conditioning thereafter. Values represent time (mean ± SEM) in seconds spent in each compartment during the pre-conditioning and the post-conditioning test day measurements. Rimonabant (Rim) and nor-binaltorphimine (nor-BNI) were injected IP 20 or 120 min before salvinorin A given SC, respectively. Vehicle (Veh) = pool of 10 rats, 5 receiving saline and 5 receiving cremophor, ethanol, saline, 1:1:18.

[a] $p < .001$ versus vehicle, nor-BNI, and rimonabant groups.

[b] $p < .001$ versus salvinorin A alone (analysis of variance followed by *post-hoc* Tukey's test).

time, in comparison with baseline (Figure 7). Two-way ANOVA revealed significant treatment effect [$F(1,98) = 34.03$; $p < .0001$] but not time effect [$F(9,98) = 5.86$; $p = .22$] or treatment × time interaction [$F(9,98) = 6.95$; $p = .12$].

Discussion

Our results demonstrate for the first time the abuse potential of the naturally occurring hallucinogen salvinorin A. Indeed when given at very low dose it is able to induce place preference in rats. These findings likely agree with the rewarding effects recently obtained with salvinorin A in the zebrafish model (26). In accordance with Zhang *et al.* (16), we also reported a conditioned place aversion at higher doses (1 and 3.2 mg/kg). Differences in the species used and in the route of administration might account for the non-complete overlapping of this dosage. Thus, we focused our attention only on the rewarding doses of salvinorin A. The low dosages of salvinorin A to give rewarding effects in rats were similar to those ingested by humans. In fact, it has been reported that the amount of salvinorin A contained in the leaves of *Salvia Divinorum* to give psychotropic effects is about 200–500 μg (6,27), which corresponds to 3–7.5 μg/kg. This regimen is in the range of doses found to provoke conditioned place preference in our experiments. Furthermore, the rewarding effect was not accompanied by motor impairment. In fact, the mean number of horizontal and vertical movements of treated rats was not modified when compared with control subjects. The rewarding results obtained by means of the conditioned place preference are confirmed by data found in self-administration task. Salvinorin A (.1–1 μg/infusion) sustained ICV self-administration behavior in naïve rats with an inverted "U" dose-effect curve. A similar pattern was also reported for other drugs such as Δ^9-tetrahydrocannabinol or CP 55,940 (25,28) and 3,4-methylenedioxymethamphetamine (29), with the same task.

The daily total drug intake seemed to be a sigmoidal function against unit doses, where the maximal amount did not exceed 50 μg/session. Rats self-administering drugs at stable levels tend to adjust the dose during sessions by modifying the response frequency (30): responding rate usually drops when the reinforcer unit dose is increased and vice versa. That the observed bar pressing decrease on drug-associated lever obtained with the unit dose of 1 μg/infusion might be the result of motor impairment can be ruled out, because the total number of self-injections/session did not decrease.

It might be surprising that a κ-opioid agonist as salvinorin A produced conditioned place preference. κ-opioid agonists (U-50488H, U-69593, and TRK-820) induce conditioned place aversion in rats (31–34) even if in one report the opioid peptide dynorphin A (1-17) showed significant place preference when given ICV (35). However, RU51599, a selective κ-opioid agonist, is self-administered by rodents (36) and causes euphoria in human patients (37). Under different conditions κ-opioid activation can enhance or decrease cocaine reward in mice, suggesting a biphasic effect of κ-opioid ligands to reward (38).

Salvinorin A has been reported to be an unusual κ-opioid receptor agonist. It is similarly potent as U50,488H in stimulating [^{35}S]GTPγS binding but 40-fold less potent in promoting internalization of the human κ-opioid receptors, causing less down-regulation of surface receptors than U50,488H and showing little antiscratching and no abdominal constriction activities in mice (39). In addition, an antidepressant effect has been reported in a single human case report (40), in contrast with the feature of κ-opioid agonists to cause depressive-like behaviors in laboratory animals (15).

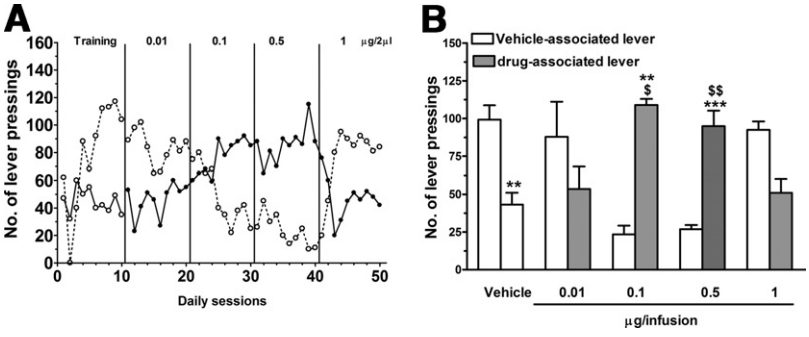

Figure 4. Salvinorin A–induced intracerebroventricular (ICV) self-administration. **(A)** Number of pressings, in a free-choice situation, by one representative rat during a 1-hour daily session on the preferred and non-preferred lever. Vehicle was delivered ICV by pressing the lever found preferred during training. Salvinorin A was delivered ICV by pressing the lever found non-preferred during training. **(B)** Number of pressings on the drug- or vehicle-associated lever. Results are mean ± SEM of the last five daily sessions after 15 ± 20 days of acquisition. $^{**}p < .01$, $^{***}p < .01$ versus the corresponding vehicle group; $^{\$}p < .05$, $^{\$\$}p < .01$ versus the corresponding vehicle-associated lever pressing (analysis of variance followed by post hoc Tukey's test).

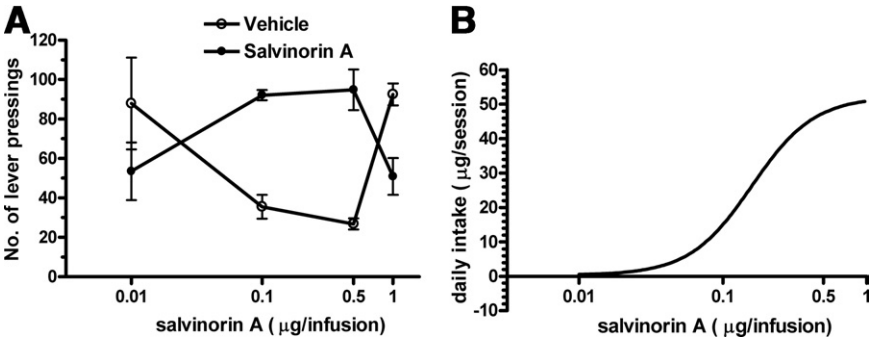

Figure 5. Effect of Salvinorin A on mean (± SEM) lever pressings associated with drug or vehicle **(A)** and daily intake **(B)** in rats submitted to operant responding, in a free-choice situation. Each symbol reflects the averages of 5 days of stable self-administration for each animal at each unit dose.

The rewarding effect of salvinorin A was significantly blocked by pretreatment with the κ-opioid antagonist nor-BNI and cannabinoid CB_1 receptor antagonist rimonabant, suggesting that both κ-opioid and cannabinoid CB_1 receptors are involved in these rewarding effects. However, further binding studies on CB1 cannabinoid and κ-opioid receptors or on mutant mice will be necessary to prove the interaction of salvinorin A with a cannabinoid and opioid system.

The possibility that nonspecific effects could be responsible for the observed antagonism might be ruled out, because the two antagonists per se had no effect on reward. The κ-opioid antagonism observed in the conditioned place preference experiment agrees with findings of Zhang *et al.* (16) where preinjection of nor-BNI blocked salvinorin A-induced place aversion and hypomotility. Furthermore, salvinorin A significantly decreased DA levels in the caudate putamen but not in the nucleus accumbens, and this effect was completely blocked by preinjection with nor-BNI.

Rimonabant might produce antagonism on salvinorin A effects for several reasons: 1) the endocannabinoid system, through the CB_1 cannabinoid receptor, exerts an overall modulatory effect on the reward circuitry and might participate in the rewarding properties of salvinorin A, as already demonstrated for other drugs of abuse (19); 2) salvinorin A might act as a cannabinoid-like ligand; however, this hypothesis seems unlikely, because no appreciable affinity for CB_1 cannabinoid receptors by salvinorin A was found (3); and 3) rimonabant could be a weak κ-opioid antagonist, but this item still has to be demonstrated.

Our data on in vivo microdialysis showed an increment of DA extracellular levels in the shell of nucleus accumbens after injection of 40 μg/kg of salvinorin A, dose that induced a maximal rewarding effect in the conditioned place preference test. Given the extensive connections of the nucleus accumbens with limbic brain areas involved in emotion (41), the salvinorin A induction of DA levels in the nucleus accumbens might be involved in the modulation of affective and motivational properties. This finding is in line with the ability of the most common drugs of abuse to preferentially increase DA transmission in the same area leading to rewarding properties (42,43).

Our findings on salvinorin A as a potential substance of abuse are not in opposition with those found in the literature asserting that κ-opioid agonists have great promise in treating addiction to alcohol (44), opiates (45), cocaine (46), and nicotine (47). In fact, as described earlier, salvinorin A is an anomalous κ-opioid agonist, whereas another κ-opioid compound, spiradoline, does not exhibit rewarding properties even if tested at very low doses in the zebrafish (26).

In conclusion, the present study provides evidence, in the rat, for the rewarding effects of salvinorin A, obtained with doses smaller than those that caused place aversion, hypomotility decrease of extracellular DA (16), and depressant-like effects (15), suggesting an abuse potential in humans.

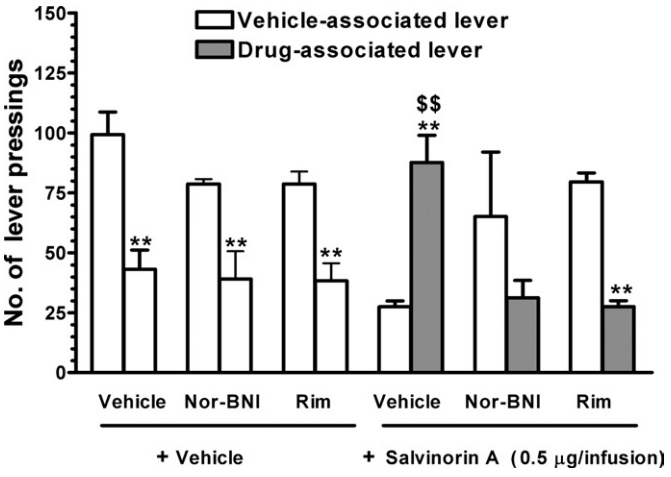

Figure 6. Block induced by CB_1 (rimonabant) (Rim) and k-opioid receptor (nor-binaltorphimine) (nor-BNI) antagonists on the established salvinorin A–induced ICV self-administration. Values are expressed as mean (± SEM) of operant responding, in a free-choice situation, to the drug- and vehicle-lever pressing during the last five stable daily sessions of 15 ± 20 days of acquisition. Drug-lever pressing delivered .5 μg/2 μL/infusion of salvinorin A. Vehicle and rimonabant (1 mg/kg) were given IP 15 min before each daily session, whereas nor-BNI (10 mg/kg) was given 2 hours before. $^{**}p < .01$ versus controlateral lever; $^{\$\$}p < .01$ versus the corresponding vehicle-associated lever-pressing (analysis of variance followed by post hoc Tukey's test).

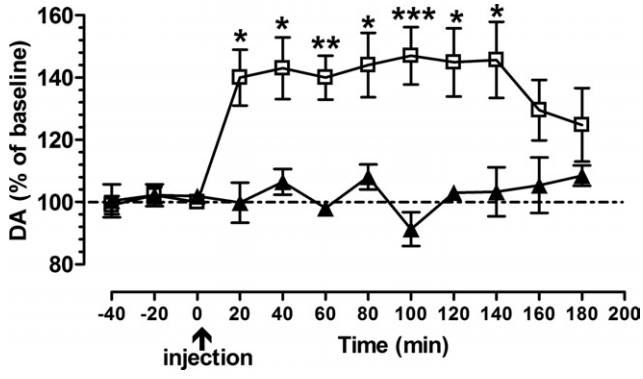

Figure 7. Salvinorin A and dopamine (DA) increase. The drug, given SC at a dose that induced a clear conditioned place preference (40 μg/kg), increased extracellular DA in the shell of the nucleus accumbens. Each point represents the mean ± SEM percent variation of basal levels. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ as compared with vehicle group (two-way analysis of variance followed by Bonferroni test).

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