

# Hallucinatory and rewarding effect of salvinorin A in zebrafish: $\kappa$ -opioid and CB<sub>1</sub>-cannabinoid receptor involvement

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## Abstract

**Rationale** The hallucinatory effect and potential abuse of salvinorin A, the major ingredient of *Salvia divinorum*, has not been documented in animals.

**Objective** The effects of salvinorin A on the zebrafish (*Danio rerio*) model, through its swimming behavior and conditioned place preference (CPP) task, was studied.

**Materials and methods** Swimming activity was determined in a squared observational chamber after an i.m. treatment of salvinorin A (0.1–10  $\mu\text{g}/\text{kg}$ ). For the CPP test, zebrafish were given salvinorin A (0.2 and 1  $\mu\text{g}/\text{kg}$ ) or vehicle and evaluated in a two-compartment chamber.

**Results** Salvinorin A (0.1 and 0.2  $\mu\text{g}/\text{kg}$ ) induced accelerated swimming behavior in comparison with vehicle, whereas a “trance-like” effect, at doses as 5 and 10  $\mu\text{g}/\text{kg}$ , was obtained. Pretreatment with the  $\kappa$ -opioid antagonist, nor-binalthorphimine (nor-BNI; 10 mg/kg) and the cannabinoid type 1 (CB<sub>1</sub>) antagonist, rimonabant (1 mg/kg), blocked salvinorin A-induced both stimulating and depressive effects obtained at a dose of 0.2 and 10  $\mu\text{g}/\text{kg}$ , respectively. In the CPP test, salvinorin A (0.2 and 0.5  $\mu\text{g}/\text{kg}$ ) produced an increase in the time spent in the drug-associated compartment. A dose of 1  $\mu\text{g}/\text{kg}$  produced no effect, whereas a dose of 80  $\mu\text{g}/\text{kg}$  induced aversion.

Pretreatment with nor-BNI or rimonabant fully reversed the reinforcing properties of salvinorin A (0.5  $\mu\text{g}/\text{kg}$ ).

**Conclusions** Taken together, these results indicate that salvinorin A, as is sometimes reported in humans, exhibits rewarding effects, independently from its motor activity, suggesting the usefulness of the zebrafish model to study addictive behavior. These effects appear mediated by activation of both  $\kappa$ -opioid and cannabinoid CB<sub>1</sub> receptors.

**Keywords** *Salvia divinorum* · Zebrafish · Conditioned place preference · Swimming behavior · SR 141716A · Nor-binalthorphimine

## Introduction

*Salvia divinorum*, a member of the *Lamiaceae* family, has been used for many years by the Mazatec Indians of northeastern Oaxaca, Mexico, primarily for its psychoactive effects (Wasson 1962, 1963; for reviews, see Valdes et al. 1983; Sheffler and Roth 2003). The active ingredient of *S. divinorum*, salvinorin A, is a non-nitrogenous neoclerodane diterpene, which represents the most potent naturally occurring hallucinogen until now known (Valdes et al. 1984; Siebert 1994). Both *S. divinorum* and salvinorin A have been used recreationally for their hallucinogenic properties (Giroud et al. 2000). Salvinorin A induces in humans an intense, short-lived hallucinogenic and positive effects (changes in depth perception, increase in sensual and aesthetic appreciation, creative dream-like experience). All these effects are distinct from those of the classical hallucinogens, such as lysergic acid diethylamide (LSD), psilocybin, and mescaline (Siebert 1994). However, depending on the dose and the route of administration, the

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effects are sometimes extremely negative (overly-intense experiences, fear, terror and panic, increased perspiration and possible difficulty integrating experiences), and people have no desire to repeat the experience (Turner 1996). Users, particularly teenagers, claim the drug is not addictive, but its potential abuse is not known. Salvinorin A is a potent and highly selective  $\kappa$ -agonist, with greater efficacy than that of the synthetic  $\kappa$ -agonist U-50488 and U-69593, as revealed by binding studies (Roth et al. 2002; Sheffler and Roth 2003; Chavkin et al. 2004). In vivo studies revealed that salvinorin A is a relatively low toxic substance after acute or chronic treatment in mice and rats even when given at doses many times greater than those used by humans (Mowry et al. 2003). In a range of doses between 0.5 and 4.0 mg/kg, an antinociceptive activity in the tail-flick and hot-plate test, through the activation of  $\kappa$ -opioid subtype receptor, has been found (McCurdy et al. 2006). Salvinorin A (0.5–2 mg/kg) produces pro-depressant-like effects in the forced swimming test, increasing the occurrence of immobility and decreasing swimming behavior (Carlezon et al. 2006). Until now, the potential abuse of salvinorin A has been poorly investigated. Salvinorin A (0.001–0.032 mg kg<sup>-1</sup> sc<sup>-1</sup>) had discriminative stimulus effects similar to those of  $\kappa$ -agonist U-69593 in rhesus monkey (Butelman et al. 2004). Treatment with salvinorin A (1 and 3 mg/kg i.p.) in C57BL/6J mice produced conditioned place aversion and decreased the dopamine levels in the caudate putamen but not in the nucleus accumbens (Zhang et al. 2005). This effect was completely blocked by the pre-injection with the  $\kappa$ -opioid receptor antagonist nor-binaltorphimine (nor-BNI) at a dose of 10 mg/kg.

Recently, some authors have been developing a rapid method to assess reinforcing properties of cocaine (Darland and Dowling 2001) in zebrafish using a conditioned place preference (CPP) paradigm.

The zebrafish (*Danio rerio*) is a small freshwater teleost rapidly emerging as an important model organism in genetics and developmental neurobiology. Although zebrafish enjoy widespread use in biomedicine (e.g. Shin and Fishman 2002), their usefulness, in studying neurological disorders, remains unexplored. During the recent years, zebrafish has been also used in neurobehavioral toxicology (Dlugos and Rabin 2003; Anichtchik et al. 2004; Bretaud et al. 2004) as a model system for the study of human diseases and behavioral disorders for drug potential therapeutic application (Dooley and Zon 2000; Levin and Chen 2004; Zon and Peterson 2005; Giacomini et al. 2006; Levin et al. 2006).

In the study of drugs of abuse, the use of a zebrafish as a model and subsequent genetic studies could provide important insights into the molecular and cellular mechanisms underlying addiction. The nervous system of zebrafish is more comparable to that of humans compared to *Drosophila*. Treatment with ethanol to adult zebrafish lead to dose-

dependent modifications of locomotor activity (Gerlai et al. 2000) and tolerance as has been observed in mammals as well as *Drosophila* (Scholz et al. 2000). Moreover, ethanol-induced hyperlocomotion can be blocked by the addition of a dopamine antagonist, suggesting the involvement of the brain dopamine system (Lockwood et al. 2004). Most validation of the zebrafish model, in studying the process of addiction in vertebrates, comes from the use of CPP test with cocaine (Darland and Dowling 2001) and amphetamine (Ninkovic et al. 2006).

The aim of the present work was to assess the suitability of using the zebrafish as a model system to investigate the behavioral effects of salvinorin A. In particular, the effect of an acute i.m. injection of a wide range of doses on swimming behavior, using a square observation chamber, was tested. Because a conditioned place aversion was obtained in mice with doses (1–3.2 mg/kg) higher than those effective in the smoked *S. divinorum* in humans (200–500  $\mu$ g/human that is about 3–7.5  $\mu$ g/kg; Valdes 1983; Siebert 1994), low doses of salvinorin A were investigated in the CPP paradigm to determine its potential rewarding properties. As a comparison, cocaine and spiradoline, a  $\kappa$ -opioid agonist, were employed. To investigate the mechanism of action, the  $\kappa$ -opioid receptor antagonist, nor-BNI, was given before salvinorin A at a dose (10 mg/kg) that has been reported to reverse salvinorin A-induced aversion and dopamine extracellular levels in the caudate putamen in mice (Zhang et al. 2005). Moreover, because an interaction between both  $\kappa$ -opioid and cannabinoid systems on self-administration has been recently demonstrated (Mendizabal et al. 2005), a possible involvement of the CB<sub>1</sub>-cannabinoid receptor on salvinorin A effect was studied using rimonabant at a dose (1 mg/kg) able to antagonize  $\Delta^9$ -tetrahydrocannabinol-induced CPP and self-administration (Braidia et al. 2004).

## Materials and methods

### Subjects

The experimental protocol was approved by the Italian Governmental Decree No. 13/2004-A. All efforts were made to minimize the number of animals used and their discomfort. Adult zebrafish (*D. rerio*) were kept at approximately 28.5°C on a 14-h light/10-h dark cycle, and behavioral testing took place during the light phase between 900 and 1400 hours. Tank water consisted of deionized H<sub>2</sub>O and sea salts (0.6 g/10 l of water; Instant Ocean, Aquarium Systems, Sarrebourg, France). The home tanks with groups of adult fish were maintained with constant filtration and aeration. Fish were fed daily with brine shrimp and flake fish food. All the fish were drug naive, and each fish was used only once. There were 10–30 fish per group.

## Treatment

Body weight was measured according to Novak et al. (2005). Fish were removed from their tank using a net, momentarily blot dried on gauze, and placed in a container containing tank water, positioned on a torn digital balance. Fish weight was determined as the weight of the container plus the fish minus the weight of the container before the fish was added. The mean of three measurements was recorded. Fish were injected i.m. in the caudal musculature with a volume depending on the weight of the fish (2  $\mu$ l/g) using a Hamilton syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland).

## Swimming behavior

Immediately after treatment, each subject was placed in a square observation chamber (10  $\times$  10 cm) containing home tank water filled at a level of 12 cm. Each fish was observed for 30 s, immediately after injection, every 5 min, for a total of six observation bins, over a 30-min period. Swimming activity was evaluated using a score scale applied to Siamese fighting fish by Abramson and Evans (1954) and adapted by us to zebrafish: 0—"trance-like" effect, horizontal motionless position on bottom tank maintained for 2–3 min at a time at the peak of the narcosis and broken by a very brief change of position by means of a slight stimulus; 2—slowed swimming, normal body position; 4—normality state; 6—accelerated swimming, normal body position; 8—frenetic swimming, with the body suspended in the vertical or some angled from the vertical; 10—frenetic circling behavior.

## Conditioned place preference

The fish were tested in a two-chamber tank (10-cm wide  $\times$  20-cm long  $\times$  15-cm high) according to Darland and Dowling (2001), with slight modifications. The tank was divided into two halves (10  $\times$  10 cm) containing distinct visual cues (two black pois) with a perforated wall that allowed complete, albeit somewhat impeded, movement. On the first day, after an initial introduction to the apparatus, the fish were tested for baseline preference by calculating the percent time spent on a given side during a 15-min trial (preconditioning phase). The fish that displayed abnormal behavior in the apparatus, such as deficient or excessive swimming or a baseline preference greater than 70%, were not tested further (rarely more than 3 or 4 of 20 fish tested from a given family). The fish were then treated i.m. with the drug and then restricted to the least preferred side for 30 min. The next day, fish receiving vehicle were confined in the opposite compartment for 30 min. Drug-texture pairings were always counterbal-

anced. On the third day, the fish were free to access to two sides for 15-min and the time spent in each compartment was recorded. The change in preference, obtained by subtracting baseline percentage from the final value, reflected reinforcing or aversive properties.

## Drugs

Salvinorin A (Daniel Siebert, The *Salvia divinorum* Research and Information Center, Malibu, CA) was dissolved in a vehicle containing cremophor, ethanol, and saline (1:1:8) and injected i.m. in a range of doses between 0.1 and 80  $\mu$ g/kg. *N*-piperidino-5-(4-chlorophenyl) 1-(2,4-dichlorophenyl)-4-methyl pyrazole 3-carboxamide (rimonabant), kindly supplied by Synthelabo–Sanofi Recherche, Montpellier, France (1 mg/kg), was dissolved in cremophor, ethanol, and saline (1:1:18). As  $\kappa$ -opioid agonist, ( $\pm$ )-(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro [4.5]dec-8-yl] benzeneacetamide mesylate (spiradoline) was dissolved in saline and given i.m. at a dose of 1 mg/kg. Nor-BNI 2HCl (10 mg/kg) and cocaine HCl (Sigma, Chemical, St. Louis, MO, USA; 20 mg/kg) were dissolved in physiological saline.  $\kappa$ -Opioid and cannabinoid CB<sub>1</sub> receptor antagonists were given 2 h and 15 min before salvinorin A, respectively. Cocaine HCl (Sigma), used as drug reference, was given at a dose of 20 mg/kg. This dose of cocaine was within the range of doses shown to be effective within the place preference design in rats (Busse et al. 2004). The dose of spiradoline was chosen on the basis of a previous study in rats (Smith et al. 2003) and our pilot experiments in zebrafish. The doses of the drugs were calculated as salt. All drugs were prepared fresh daily.

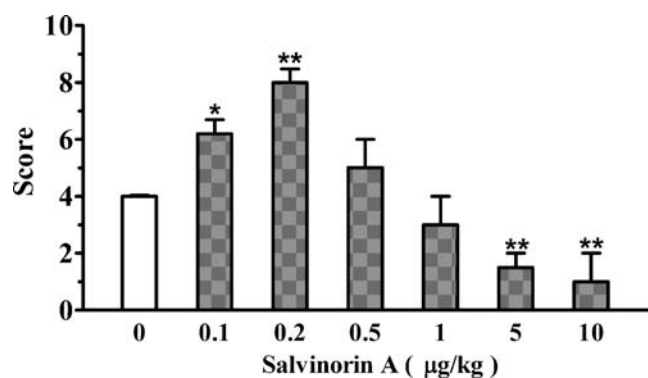
## Statistics

The data were expressed as mean  $\pm$  SEM. Swimming activity, evaluated as mean score of six observation bins, was analyzed by Kruskal–Wallis test followed by Dunn's post-hoc test. Data from CPP test were assessed by one-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey's post-hoc test. All statistical analyses were done using software Prism, version 4 (GraphPad, San Diego, CA, USA). The accepted level of significance was  $P < 0.05$ .

## Results

### Swimming behavior

Salvinorin A modified normal swimming activity in zebrafish (Fig. 1). Kruskal–Wallis test revealed a significant difference between groups ( $\chi^2 = 36.05$ ,  $P < 0.001$ ). Post-hoc



**Fig. 1** Effect of salvinorin A given i.m. on swimming behavior ( $n=10$  per group) in the zebrafish. Data are expressed as mean ( $\pm$ SEM) of six observation bins evaluated every 5 min within 30 min. Salvinorin A produced an increase of mean score (frenetic swimming with the body suspended in the vertical plane) at low doses, while it induced a score decrease (slowed swimming) at highest ones. \* $P<0.05$ ; \*\* $P<0.01$  compared with 0 (vehicle; Dunn's test)

test showed that the low doses of Salvinorin A (0.1 and 0.2  $\mu\text{g}/\text{kg}$ ) induced accelerated swimming behavior, whereas the higher ones (5 and 10  $\mu\text{g}/\text{kg}$ ) slowed down in comparison with vehicle. Doses of 0.5 and 1  $\mu\text{g}/\text{kg}$  were ineffective.

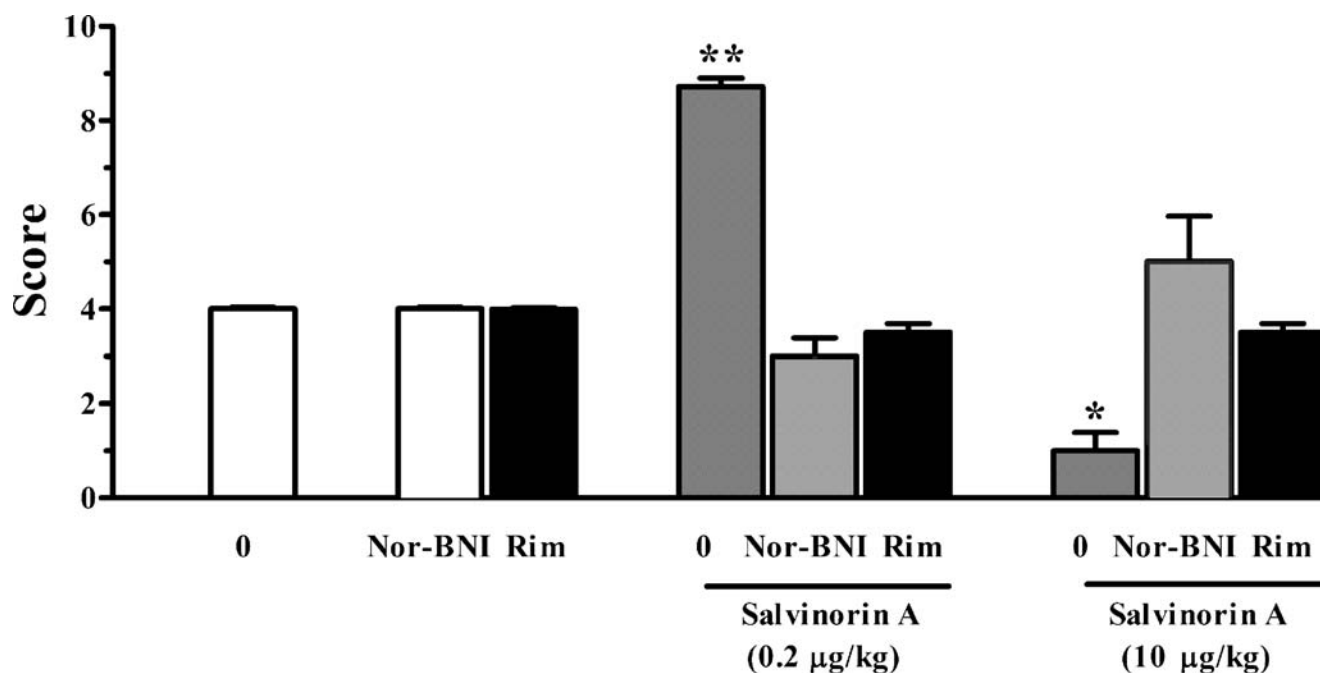
The effect of the combination of nor-BNI and rimonabant with salvinorin A (0.2 and 10  $\mu\text{g}/\text{kg}$ ) on swimming activity was shown in Fig. 2. Kruskal–Wallis test showed a significant difference between groups ( $\chi^2=43.18$ ,  $P<0.001$ ). Further analyses evidenced a significant block of salvinorin A-induced both stimulating and depressive

effects by both the antagonists, which per se did not modify swimming behavior.

#### Conditioned place preference

The development of a CPP or aversion by salvinorin A, cocaine, and spiradoline injection is shown in Fig. 3. ANOVA revealed that there was a significant difference between groups ( $F_{6,63}=15.01$ ,  $P<0.0001$ ). Tukey's test showed that salvinorin A produced a progressive increase in the time spent in the drug-paired side during the postconditioned phase at the doses of 0.2 and 0.5  $\mu\text{g}/\text{kg}$  in comparison with vehicle group. The reinforcing effect induced by the lowest dose of salvinorin A was similar to that exhibited by cocaine (20 mg/kg), whereas a dose of 0.5  $\mu\text{g}/\text{kg}$  elicited a greater reinforcing effect, as compared with cocaine. The dose of 1  $\mu\text{g}/\text{kg}$  was ineffective, whereas the highest (80  $\mu\text{g}/\text{kg}$ ) produced a significant aversion similar to that obtained by spiradoline.

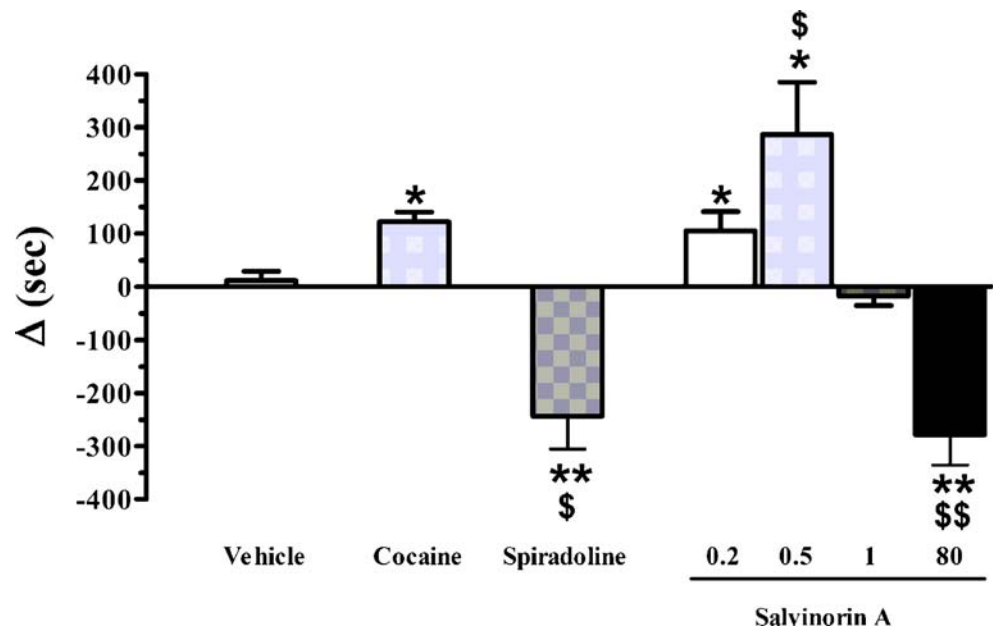
The involvement of  $\kappa$ -opioid and  $\text{CB}_1$ -cannabinoid receptors in the salvinorin A-induced CPP is shown in Fig. 4. ANOVA showed that there was a difference among the groups ( $F_{5,50}=18.63$ ,  $P<0.00015$ ). Tukey's test revealed that pretreatment with nor-BNI or rimonabant per se did not modify the time spent in the two compartments, whereas the combination with salvinorin A, at the maximally reinforcing dose (0.5  $\mu\text{g}/\text{kg}$ ), fully reduced the time spent in the drug-paired compartment in comparison with salvinorin A alone.



**Fig. 2** Effect of i.m. pretreatment of the  $\kappa$ -opioid receptor antagonist, nor-BNI (10 mg/kg), or  $\text{CB}_1$ -cannabinoid receptor antagonist, rimonabant (1 mg/kg; Rim) given 2 h or 15 min before salvinorin A (0.2 and 10  $\mu\text{g}/\text{kg}$  i.m.), respectively, on swimming behavior ( $n=10$  per group; Veh = vehicle). Data are expressed as mean ( $\pm$ SEM) of six

observation bins evaluated every 5 min within 30 min. Pretreatment of both antagonists by themselves failed to change swimming behavior compared with vehicle group, but they did antagonize salvinorin A-induced stimulating and depressant effect. \* $P<0.05$ ; \*\* $P<0.01$  compared with all the remaining groups (Dunn's test)

**Fig. 3** CPP produced by cocaine (20 mg/kg i.m.) or increasing doses of salvinorin A ( $n=10$  per group). Low doses (0.2 and 0.5  $\mu\text{g}/\text{kg}$  i.m.) and cocaine induced an increase in the mean difference time spent in the drug-paired compartment, whereas the highest dose of salvinorin A and spiradoline produced place aversion. \* $P<0.05$ ; \*\* $P<0.01$  compared with vehicle (Veh); \$ $P<0.05$ ; \$\$ $P<0.01$  compared with vehicle, cocaine, and salvinorin A (0.2 and 0.5  $\mu\text{g}/\text{kg}$ ; Tukey's test)



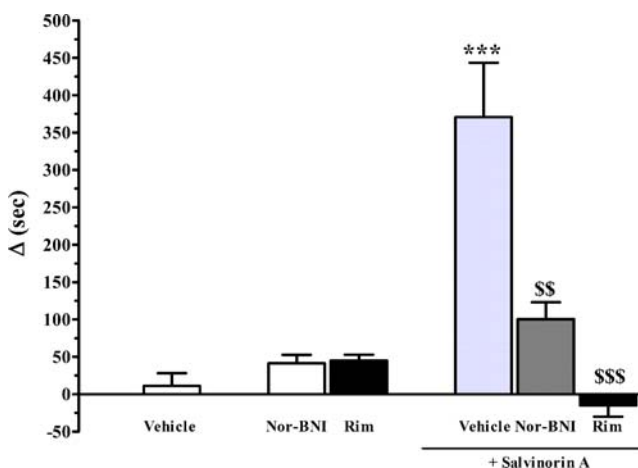
## Discussion

This study shows for the first time that naturally occurring hallucinogen salvinorin A has specific effects on zebrafish behavior as observed in changes of swimming behavior and CPP test. About swimming behavior, a biphasic effect was observed, being a stimulant at low doses and depressant at highest ones. We observed that the general pattern of swimming behavior of vehicle-injected fish was different

from that of salvinorin A-treated fish. The control fish tended to swim in a circular pattern along the walls of the observation tank infrequently changing direction. As reported by Anichtchik et al. (2004), in the same species, and by Clements et al. (2002), in goldfish and salmon, this indicates a normal pattern of swimming with a tendency to follow the wall. In contrast, salvinorin A, depending on the dose, produced alterations in the swimming behavior of the zebrafish. An accelerated/frenetic pattern of swimming in a vertical plane with a frequent change of direction at low doses whereas a slowing of swimming at high doses were obtained. The body usually suspended in the vertical plane or some angle from the vertical has been previously reported (Abramson and Evans 1954) in Siamese fighting fish under LSD treatment, suggesting that salvinorin A has a hallucinatory behavior. The depressive motor activity obtained at high doses agrees with the findings of Zhang et al. (2005) who reported that high doses of salvinorin A decreased locomotor activity in mice.

More interestingly, we have demonstrated for the first time the potential abuse of salvinorin A when given at the lowest doses. The fact that cocaine also induced reward confirms the validity of the CPP model in the zebrafish. Furthermore, the i.m. injection in place of dissolving drug in the water, as previously reported (Darland and Dowling 2001), made the test more precise and accurate. In fact, when dissolving drugs in the water, the amount of drug reaching the brain has been reported to be undetectable (Ninkovic et al. 2006).

The aversive effect obtained at the highest dose was not unexpected. Zhang et al. (2005) found that salvinorin A led to a place aversion in mice when administered i.p. at high doses (1 and 3.2 mg/kg). Differences in the used species and in the route of administration may account for the non-



**Fig. 4** Effect of i.m. pretreatment of the  $\kappa$ -opioid receptor antagonist, nor-BNI (10 mg/kg), or  $\text{CB}_1$  cannabinoid receptor antagonist, rimonabant (1 mg/kg; *Rim*) given 120 or 15 min before salvinorin A (0.5  $\mu\text{g}/\text{kg}$  i.m.), respectively, on CPP test ( $n=10$  per group). Data are expressed as mean ( $\pm$ SEM). Pretreatment of both antagonists by themselves did not lead to a significant change in time spent in the drug-paired compartment compared with that for vehicle group, but they blocked the salvinorin A-induced reinforcing effect. \*\*\* $P<0.001$  compared to vehicle and the antagonists alone; \$\$\$ $P<0.01$  compared with salvinorin A alone (Tukey's test)

complete overlapping of this dosage. The rewarding properties of low doses of salvinorin A obtained in zebrafish agree with the potential abuse of *S. divinorum* in humans. The amount of salvinorin A contained in the leaves, to give psychotropic effects, is about 200–500 µg (Valdes et al. 1983; Siebert 1994), which corresponds to 3–7.5 µg/kg. This dosage is in a range similar to that employed in our experiments to induce CPP. Furthermore, the reinforcing effect was obtained at either a stimulating or ineffective dose on swimming behavior suggesting that the obtained reward was independent from motor activity.

Behavioral effects of salvinorin A were significantly blocked by pretreatment with nor-BNI and rimonabant, suggesting that both swimming behavior and CPP are specifically mediated by both  $\kappa$ -opioid and cannabinoid CB<sub>1</sub> receptors. The zebrafish central nervous system, although less complex, is essentially organized like the mammalian, where both opioid and cannabinoid CB<sub>1</sub> receptors are distributed in different regions (Gonzalez-Nuñez et al. 2006; Lam et al. 2006). Furthermore, anatomical studies have demonstrated that the tyrosine hydroxylase-positive neurons of the posterior tuberal nucleus projects to the basal forebrain in a manner reminiscent of the ventral tegmental area-nucleus accumbens connection in mammals (Rink and Wullmann 2001). Although, it is not yet certain that this circuit governs reward in fish; however, *c-Fos*-like immunoreactivity in the forebrain of cocaine treated fish was detected (Darland and Dowling 2001). Moreover, the reported colocalization of cannabinoid CB<sub>1</sub> receptors and tyrosine hydroxylase-positive neurons in the periventricular hypothalamus of zebrafish (Lam et al. 2006) suggest that this is a peculiar region of the CNS in which the activity of dopaminergic neurons, i.e. the release of DA, is directly modulated by CB<sub>1</sub> receptors.

The possibility that nonspecific effects were responsible for the observed antagonism in both tasks may be ruled out, as the antagonists per se had no effect. At least, for the CPP paradigm, the obtained antagonism agrees with the findings of Zhang et al. (2005), where pre-injection of nor-BNI blocked salvinorin A-induced place aversion and hypomotility and a decrease in dopamine levels in nucleus accumbens.

It is surprising that a  $\kappa$ -opioid agonist as salvinorin A produced CPP. In fact, in our experiments, another  $\kappa$ -opioid agonist, spiradoline, produced place aversion in accordance with other authors who reported that  $\kappa$ -opioid agonists (U-50488, U-69593, and TRK-820) induced conditioned place aversion in rats (Shippenberg and Herz 1987; Bals-Kubik et al. 1989; Suzuki et al. 1992; Mori et al. 2002). Only in one report dynorphin A (1–17) was found to show significant CPP when given i.c.v. (Iwamoto 1989). On the other hand, salvinorin A has been recently reported to be an unusual  $\kappa$ -opioid receptor agonist. In fact, it was

similarly potent as U-50488 in stimulating [<sup>35</sup>S]GTP $\gamma$ S binding, 40-fold less potent in promoting internalization of the human  $\kappa$ -opioid receptors, causing less down-regulation of surface receptors than U-50488 and showing little antiscratching and no abdominal constriction activities in mice (Wang et al. 2005).

The fact that nor-BNI antagonized the rewarding effects of salvinorin A indicates the involvement of  $\kappa$ -opioid receptors. A possible explanation could be that nor-BNI is a nonselective compound when used in vivo as previously reported (Cui et al. 2000; Spanagel et al. 1994; Carroll et al. 2005) where an exacerbation of withdrawal signs in morphine-dependent rats treated with nor-BNI but not with a more selective  $\kappa$ -opioid antagonist, JDTic, was found.

More surprisingly, the CB<sub>1</sub>-cannabinoid antagonist, rimonabant, reversed salvinorin A-induced reinforcing effect. Previous experiments (Hampson et al. 2000) reported that, in primary cultures of rat hippocampal neurons, CB<sub>1</sub> and  $\kappa$ -opioid receptor activation is addictive with respect to potassium K current ( $I_K$ ) amplitude, suggesting that CB<sub>1</sub> and  $\kappa$ -opioid receptors share a common Gs protein signaling pathway involving protein kinase C. Furthermore, the  $\kappa$ -opioid receptor antagonist, nor-BNI, per se actually increased ( $I_K$ ) amplitude and significantly reduced the effect of coadministered WIN 55212-2. Even if speculative, this similar pathway can explain how CB<sub>1</sub>-cannabinoid and  $\kappa$ -opioid receptor systems could act in synergy to modulate responses (reward?) produced by the activation of either receptor alone. Of course, this tentative explanation should be verified by further experiments. The final consideration concerns the similarity of chemical structure between salvinorin A and the CB<sub>1</sub>-cannabinoid agonist,  $\Delta^9$ -tetrahydrocannabinol. These drugs are both terpenoids (Erowid: <http://www.erowid.org/ask/ask.cgi?ID=2936>) and share a common pharmacological profile (analgesia, sedation, disphoria, and perceptual distortion; Ameri 1999; Bucheler et al. 2005). In addition, nor-BNI blocked tetrahydrocannabinol-induced antinociception after both central and systemic administration, suggesting an interaction between cannabinoid and  $\kappa$ -opioid systems (Smith et al. 1994).

Taken together, the present findings demonstrate for the first time the validity of zebrafish model to stress hallucinatory and rewarding effects of salvinorin A, when injected i.m. at extraordinarily low doses, according to the recreational use of *S. divinorum* and salvinorin A in humans (Giroud et al. 2000). Thus, the zebrafish employing behavioral testing model is a promising way to uncover the potential abuse of new drugs, as previously reported for cocaine (Darland and Dowling 2001) and amphetamine (Ninkovic et al. 2006). Using this method we also evidenced, for the first time, a possible interaction of salvinorin A with the CB<sub>1</sub>-cannabinoid receptors.

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