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Antinociceptive profile of salvinorin A, a structurally unique kappa opioid receptor agonist

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Abstract

Salvinorin A, is a structurally unique, non-nitrogenous, kappa opioid receptor (KOP) agonist. Given the role of KOPs in analgesic processes, we set out to determine whether salvinorin A has antinociceptive activity in thermal and chemo-nociceptive assays. The tail-flick assay was employed to investigate 1) salvinorin A's (0.5, 1.0, 2.0, and 4.0 mg/kg) dose–response and time-course (10, 20, and 30 min) effects in a thermal nociceptive assay, and 2) the ability for the KOP antagonist norBNI (10.0 mg/kg) to prevent salvinorin A antinociception. The hotplate assay was utilized as a second thermal nociceptive measure to test salvinorin A's dose–response effects. The acetic acid abdominal constriction assay was used to study salvinorin A's dose–response and time-course (over 30 min) effects in a chemo-nociceptive assay. Together, these studies revealed that salvinorin A produces a dose-dependent antinociception that peaked at 10 min post-injection but rapidly returned to baseline. Additionally, pretreatment with the KOP antagonist norbinaltorphimine (norBNI) reversed salvinorin A-induced antinociception. These findings demonstrate that salvinorin A produces a KOP mediated antinociceptive effect with a short duration of action.

Keywords: Salvinorin A; Kappa opioid receptor agonist; Tail-flick; Hotplate; Acetic acid writhing; Mouse; Antinociception

1. Introduction

Salvinorin A (Fig. 1) is a potent and highly selective kappa opioid receptor (KOP) agonist as demonstrated in in vitro assays (Roth et al., 2002; Chavkin et al., 2004). A compound lacking nitrogen with such high selectivity for KOP and virtually no affinity for many other psychoactive drug targets (Roth et al., 2002) has not previously been reported. *Salvia divinorum*, the plant from which salvinorin A is isolated, has been used in ritualistic and spiritual practices of the Mazateca curanderos (healers) for many years for its ability to produce lucid and inward thought (Ortega et al., 1982). Interestingly, in smaller doses, when administered orally, *Salvia divinorum* has been utilized by the curanderos to treat various ailments such as providing relief from headaches, rheumatism, and gastrointestinal movement disorders (Valdes et al., 1983). These uses are not surprising given the well-documented role KOPs play in spinal-mediated pain processing, the GI tract, as well as the bladder. Throughout its use, salvinorin A or more specifically, extracts from the plant, have not shown any addictive potential (Valdes et al., 1983; Zhang et al., 2005) and therefore, could serve as a template for non-addictive opioid analgesics if one could delineate the analgesic effects from the psychotropic effects. Salvinorin A has also been shown to produce kappa opioid agonist-like discriminative effects in rhesus monkeys (Butelman et al., 2004), slight aversive properties in mice (Zhang et al., 2005), sedative effects in mice (Fantegrossi et al., 2005) and antidepressant effects in one human case report (Hanes, 2001). Collectively, these studies suggest a range of effects quite similar to known kappa opioid agonists. However, reports of analgesia from salvinorin A have been limited. In

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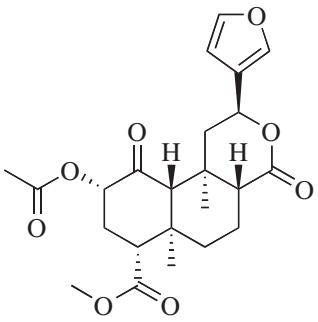

SALVINORIN A

Fig. 1. Structure of salvinorin A.

fact, one report demonstrated that salvinorin A, even at high doses, lacked antinociceptive effects in the abdominal constriction test (Wang et al., 2005). While we were preparing this manuscript, a study by Harding et al. (2005) was published indicating salvinorin A indeed demonstrated analgesic effects in two different nociceptive assays. Although details were not given on time-course or doses used ED_{50} values were presented.

Traditional folk literature reports suggest the use of leaf material from *Salvia divinorum* for analgesic purposes (Valdes et al., 1983). It is reasonable to predict salvinorin A would be the analgesic component of the leaf material, given the well-documented role kappa opioid receptors play in spinal-mediated pain processing (Pasternak, 1993). The purpose of the present research is to characterize the antinociceptive properties of purified salvinorin A in thermal and chemo-nociceptive assays, establishment of a peak effect time for salvinorin A for antinociceptive activity and a reversal study with norBNI demonstrating KOP selectivity in vivo.

2. Materials and methods

2.1. Subjects

For all experiments, male Swiss mice (23–30 g, Harlan, Indianapolis, IN, USA) were group housed at a population density of $n=2–3$ in polycarbonate cages ($20\times35\times12$ cm). Food (Purina 5001 Laboratory Rodent Chow, St. Louis, MO, USA) and water were available ad libitum. Room temperature was maintained at 22 ± 1 °C and overhead fluorescent illumination was maintained on a 12-h light–dark cycle.

2.2. Drugs

Salvinorin A was obtained by reported extraction and purification methods (Munro and Rizzacasa, 2003) from *Salvia divinorum* leaves harvested from plants (Theatrum Botanicum, Laytonville, CA, USA) propagated at the University of Mississippi. Purified, crystalline salvinorin A agreed with published characterization data (Ortega et al., 1982). Salvinorin A was dissolved in a vehicle consisting of 10% DMSO and 90% propylene glycol. This vehicle was utilized based on previous work with other lactones in our laboratory that were freely soluble in propylene glycol. Salvinorin A was not soluble in 100% propylene glycol to our surprise. However, solubility increased in propylene glycol by adding increasing concentrations of DMSO. A 9:1 mixture ended up being suitable for our studies and did not interfere with analgesia on its own. For the kappa antagonist challenge study, norBNI (Tocris, Ellisville, MO) was dissolved in 0.9% physiological saline. All drugs were delivered IP.

2.3. Tail-flick studies

The tail-flick test was used to characterize 1) the dose–response and time-course effects of salvinorin A on thermal nociception and 2) the KOR antagonist effects of norBNI on salvinorin A antinociception. Thermal nociception was quantified using a tail-flick apparatus that integrated both a thermal nociceptive stimulus and an automated response timer (Columbus Instruments, Model #0104-300M; intensity setting 4). For tests, mice were lightly restrained in a soft cloth with their tail positioned in a grove above an aperture that presented the onset of the thermal stimulus with the start of the timer. The average of two trials, taken 20 to 30 s apart, served as the dependent measure for each subject. A cut-off score of 10 s was utilized to minimize the risk of tissue damage. Baseline measures were taken 10 min prior to administration of drug probes; no statistical differences were detected in this measure across assigned groups and these data are not presented herein.

In the dose–response/time-course experiment, mice received vehicle or 0.5, 1.0, 2.0, or 4.0 mg/kg salvinorin A and tested 10, 20 and 30 min post-injection. In the norBNI challenge experiment, mice were given 10 mg/kg norBNI 1 h before administration of 2.0 mg/kg salvinorin A; tail-flick tests were conducted 10 min after the salvinorin A injections. These dose selections and injection-to-test intervals were selected from pilot studies and from previously published reports (Endoh et al., 1992). Sample sizes were $n=8–10$.

2.4. Hotplate study

The hotplate was used to characterize the dose–response effects of salvinorin A on thermal nociception. Although this assay is generally considered to be less sensitive to KOP analgesics, it does assess involvement of both spinal and supraspinal nociceptive processing. Prior to testing, mice received one apparatus habituation trial (2 min) in which they were placed inside the acrylic enclosure with the hotplate (Harvard, Model #52-8570) surface temperature maintained at 40 °C. Nociceptive tests (hotplate temperature = 52 °C) were conducted 10 min after injections of vehicle or 0.5, 1.0 or 2.0 mg/kg salvinorin A (higher doses were found to produce motor side effects that confounded the hotplate response measure). A timer was manually started when all four paws made contact with the apparatus floor. Latency to flutter or lick a hindpaw or perform an escape response (i.e., jumping or scurrying) served as the nociceptive measure (45 s cut-off score). Sample sizes were $n=8–10$ for all groups.