

The hallucinogenic herb *Salvia divinorum* and its active ingredient salvinorin A reduce inflammation-induced hypermotility in mice

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Abstract The hallucinogenic plant *Salvia divinorum* has been used for medical treatments of gastrointestinal disorders. Here, we evaluated the effect of a standardized extract from the leaves of *Salvia divinorum* (SDE) and of its active ingredient salvinorin A on motility in vivo, both in physiological states and during croton oil-induced intestinal inflammation. SDE (1–100 mg kg⁻¹) significantly inhibited motility only in inflamed, but not in control, mice. In control mice, salvinorin A (0.01–10 mg kg⁻¹) significantly inhibited motility only at the highest doses tested (3 and 10 mg kg⁻¹) and this effect was not counteracted by naloxone or by the κ -opioid receptor (KOR) antagonist nor-binaltorphimine. Inflammation significantly increased the potency of salvinorin A (but not of the KOR agonist U-50488) in reducing motility. The inhibitory effects of both salvinorin A and U-50488 in inflamed mice were counteracted by naloxone or by nor-binaltorphimine. We conclude that salvinorin A may reduce motility through activation of different targets. In physiological states, salvinorin A, at high doses, inhibited motility through a non-KOR mediated mechanism. Gut inflammation increased the potency of salvinorin A; this effect was mediated by KOR, but it was not shared by U-50488, thus suggesting that salvinorin A may have target(s) other than KOR in the inflamed gut.

Keywords inflammation, intestinal motility, κ -opioid receptors, myenteric plexus, salvinorin A.

INTRODUCTION

The hallucinogenic plant *Salvia divinorum* is a member of the sage family that has been used for divination and shamanism by the Mazatecs.^{1,2} Traditionally, *Salvia divinorum* has been ingested by chewing fresh leaves, drinking the juice or infusion or smoked dry leaves for its hallucinogenic properties and also used in healing practices for a number of ailments including those affecting the digestive tract (i.e. abdominal swelling, diarrhoea and intestinal spasms).³ Currently, *Salvia divinorum* is mainly used for recreational purposes, often as a marijuana substitute, particularly in the USA and Europe; its use as hallucinogenic substance has been facilitated by its availability through Internet suppliers.⁴

The active component of *Salvia divinorum* is salvinorin A, a neoclerodane diterpenoid. Salvinorin A has been reported to be the most potent naturally occurring hallucinogen, with an effective dose, when smoked, of 0.2–1 mg in humans.^{5,6} Thus it rivals the synthetic hallucinogens lysergic acid diethylamide (LSD) and 2,5-dimethoxy-4-bromoamphetamine (DOB) in potency.^{4,5} Salvinorin A represents the first non-nitrogenous natural hallucinogen acting on κ -opioid receptor (KOR).^{7,8} It is a highly potent and selective KOR agonist.⁷ It has negligible affinity for a large number of ion channels, transporters and receptors, including various serotonin receptors, the principal targets of classical hallucinogens.^{7,9}

KOR receptors have been located on the myenteric plexus¹⁰ and their activation results in suppression of

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peristaltic propulsion evoked by elevation of intraluminal pressure in the isolated guinea-pig ileum^{11,12} and suppression of fast excitatory postsynaptic potential in guinea-pig myenteric nerves.¹³ When compared with μ -opioid receptor (MOR) agonists or non-selective opioid agonists, KOR agonists have limited side effects in the respiratory and gastrointestinal tract. Therefore, there is considerable interest in developing therapeutically useful agonists at other opioid receptors.¹⁴ We have recently shown that a *Salvia divinorum* extract and salvinorin A depressed enteric cholinergic transmission in the isolated guinea-pig ileum through activation of KORs,¹⁵ however, the effect of this herb and of its main active ingredient has been never explored on intestinal motility *in vivo* to date.

Given the traditional use of *Salvia divinorum* in treating gastrointestinal disorders and the fact that this herb inhibits enteric transmission *in vitro* through KOR activation, we now evaluate the effect of a standardized extract from *Salvia divinorum* leaves (SDE), and its active ingredient salvinorin A on intestinal motility *in vivo*, both in physiological and in pathophysiological (inflammatory) states.

MATERIALS AND METHODS

Animals

Male ICR mice (Harlan Italy, S. Pietro al Natisone, UD) (24 – 26 g) were used after 1 week of acclimation. Food was withheld 6 h before transit measurement and 18 h before the induction of intestinal inflammation. All animal experiments complied with the Italian D.L. n 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Intestinal inflammation

Inflammation was induced as previously described.^{16,17} Mice received orally two doses of croton oil (20 μ L per mouse) in two consecutive days. Motility was measured 4 days after the first administration of croton oil. This time was selected on the basis of a previous work,¹⁶ in which maximal inflammatory response occurred 4 days after the first treatment.

Transit

Transit was measured by evaluating the intestinal location of rhodamine-B-labelled dextran.¹⁸ Animals were given fluorescent-labelled dextran (100 μ L of 25 mg mL⁻¹ stock solution) via a gastric tube into the

stomach. Twenty minutes after administration, the animals were killed by asphyxiation with CO₂ and the entire small intestine with its content was divided into 10 equal parts. The intestinal contents of each bowel segment were vigorously mixed with 2 mL of saline solution to obtain a supernatant containing the rhodamine. The supernatant was centrifuged at 40 g to force the intestinal chyme to a pellet. The fluorescence in duplicate aliquots of the cleared supernatant was read in a multi-well fluorescence plate reader (LS55 Luminescence spectrometer; Perkin Elmer Instruments, Waltham, MA, USA; excitation 530 \pm 5 nm and emission 590 \pm 10 nm) for quantification of the fluorescent signal in each intestinal segment. From the distribution of the fluorescent marker along the intestine, we calculated the geometric centre (GC) of small intestinal transit as follows:

$$GC = \sum(\text{fraction of fluorescence per segment} \times \text{segment number}),$$

where GC ranged from 1 (minimal motility) to 10 (maximal motility). This procedure yielded an accurate, non-radioactive measurement of intestinal transit.

Drug administration

SDE (1–100 mg kg⁻¹), SDE without salvinorin A (1–100 mg kg⁻¹), salvinorin A (0.01–10 mg kg⁻¹), and U-50488 (1–30 mg kg⁻¹) or vehicle [dimethyl sulphoxide (DMSO) 4 μ L per mouse or saline 0.1 mL per mouse] were given intraperitoneally (i.p.) 20 min before rhodamine administration, either to control mice or mice with inflammation. In some experiments, naloxone (10 mg kg⁻¹) (to block opioid receptors), nor-binaltorphimine (20 mg kg⁻¹) (to block KORs) were given (i.p.) 30 min before salvinorin A (3 mg kg⁻¹ in control mice or 1 mg kg⁻¹ in croton oil-treated mice) or U-50488 (10 mg kg⁻¹, either to control mice or to croton oil-treated mice). In preliminary experiments, SDE (100 mg kg⁻¹) or Salvinorin A (10 mg kg⁻¹) were given orally 60 min before the administration of rhodamine, either to control mice or mice with inflammation. In these experiments, SDE and Salvinorin A were suspended in 1% carboxymethylcellulose (0.1 mL per mouse).

Plant extracts preparation and salvinorin A isolation/purification

An ethanolic extract (320 g) from dried *Salvia divinorum* leaves (2 kg) was prepared and a portion of this extract (20 g) was standardized to contain 1.6% salvinorin A (SDE) or the equivalent to 0.3% salvinorin A

from dried leaves. The extract was separated by reverse phase C18 vacuum liquid chromatography (VLC) eluted in a gradient manner using methanol: water (70 : 30–100 : 0) until no colour was observed in the fractions. The fractions containing salvininorin A were combined and all VLC fractions and washings, with the exception of the salvininorin A fractions, were recombined and evaporated to dryness to give an extract without salvininorin A (263 g). The salvininorin A from the crystallizations and column fractions was purified by solvent/solvent partitions (aqueous methanol: hexanes) and recrystallizations (95% ethanol). A 1.6% standardized extract was made from the purified salvininorin A (333 mg) and the extract without salvininorin A (19.78 g).

Drugs

Naloxone hydrochloride and U-50488 hydrochloride were purchased from Sigma (Milan, Italy); nor-binaltorphimine was purchased from Tocris Cookson (Northpoint, UK); SDE (with or without salvininorin A) and salvininorin A were obtained as described above. SDE, SDE without salvininorin A, salvininorin A and nor-binaltorphimine were dissolved in DMSO, U-50488 in saline. The drug vehicles [4 μ L per mouse DMSO (i.p.), 0.1 mL per mouse saline (i.p.) or 0.1 mL per mouse carboxymethylcellulose (orally)] had no effect on intestinal transit.

Statistics

Data are mean values \pm SEM. To determine statistical significance, Student's *t*-test for unpaired data or one-way analysis of variance followed by Tukey–Kramer multiple comparisons test was used. A *P*-value <0.05 was considered significant.

RESULTS

Administration of croton oil produced a significant increase of intestinal transit (GC: 4.98 ± 0.06 ; croton oil 6.24 ± 0.12 $n = 8$, $P < 0.05$). Intraperitoneal administration of SDE (1–100 mg kg^{-1}) did not modify significantly transit under physiological conditions (Fig. 1). However SDE (1–100 mg kg^{-1}) produced a dose-dependent reduction of motility in inflamed mice, with a significant effect at the 100 mg kg^{-1} dose (Fig. 2). SDE extract without salvininorin A (100 mg kg^{-1}) did not affect transit neither in control mice (GC: control: 5.21 ± 0.19 ; SDE without salvininorin A 4.88 ± 0.25) nor in mice with intestinal inflammation (croton oil 6.09 ± 0.13 ; croton oil plus SDE

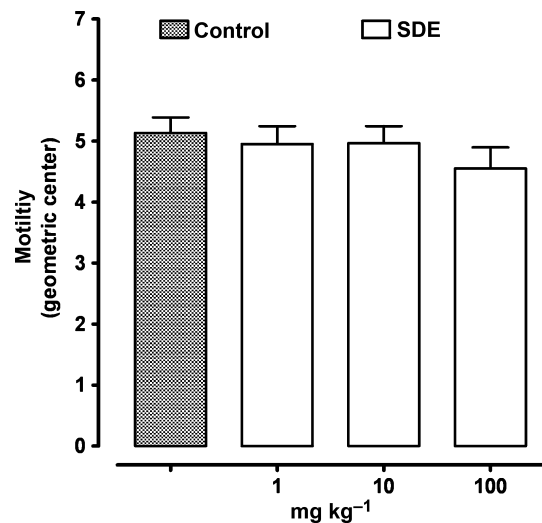


Figure 1 Effect of a standardized extract from the leaves of *Salvia divinorum* (SDE, 1–100 mg kg^{-1} , i.p.) on intestinal transit in control mice. Transit was expressed as the geometric centre of the distribution of a fluorescent marker along the small intestine (see Materials and methods section). Bars represent the mean \pm SEM of 10–12 animals. No significant differences were observed.

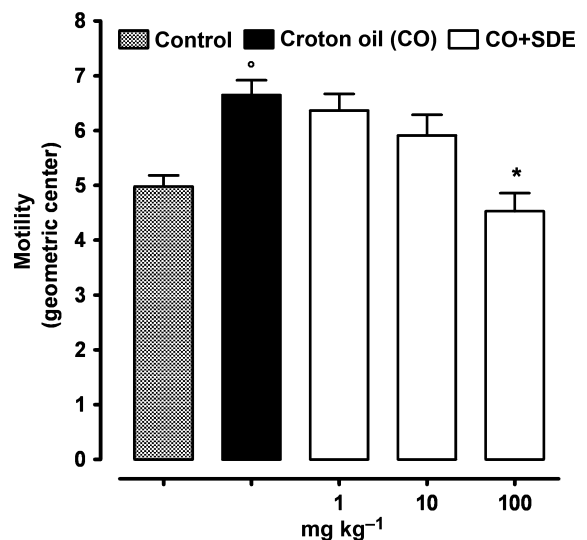


Figure 2 Effect of a standardized extract from the leaves of *Salvia divinorum* (SDE, 1–100 mg kg^{-1} , i.p.) on intestinal transit in croton-oil treated mice. Transit was expressed as the geometric centre of the distribution of a fluorescent marker along the small intestine (see Materials and methods section). Bars represent the mean \pm SEM of 10–12 animals. $^{\circ}P < 0.05$ vs control and $*P < 0.05$ vs croton oil.

without salvininorin A 5.85 ± 0.08 , $n = 10$ for each experimental group).

Salvinorin A (0.01–10 mg kg^{-1}) (Fig. 3) and U-50488 (1–30 mg kg^{-1}) (Fig. 4) produced a dose-related

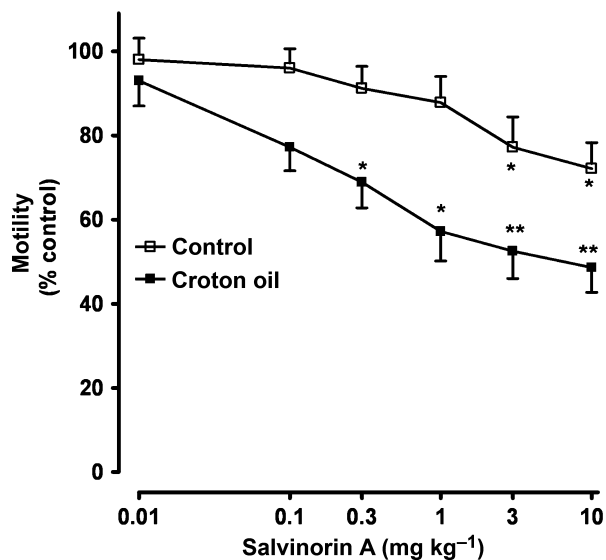


Figure 3 Dose-related inhibition of intestinal motility by salvinorin A (0.01–10 mg kg⁻¹, i.p.) in control or in croton oil-treated mice. Results are expressed as percentage of the corresponding control. * $P < 0.05$; ** $P < 0.01$ vs corresponding control ($n = 10$ –12 animals for each experimental group). Note that the curve representing the inhibitory effect of salvinorin A in control mice was statistically different from the curve representing the inhibitory effect of salvinorin A in croton oil-treated mice.

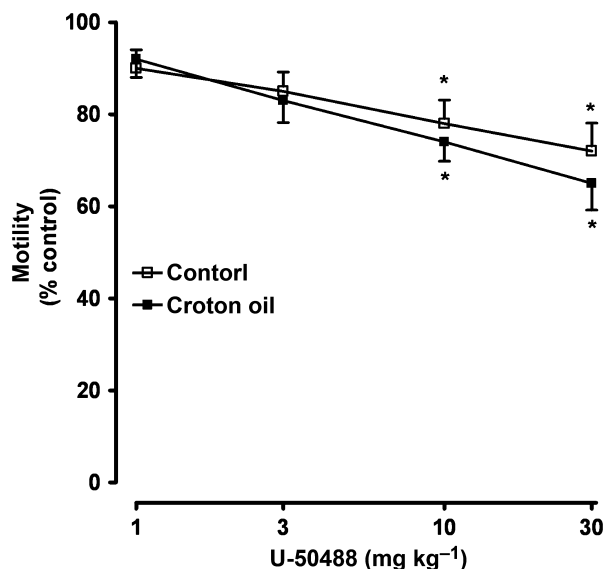


Figure 4 Dose-related inhibition of intestinal motility by U-50488 (1–30 mg kg⁻¹, i.p.) in control or in croton oil-treated mice. Results are expressed as percentage of the corresponding control. * $P < 0.05$ vs corresponding control ($n = 10$ –12 animal for each experimental group). No statistical difference between the two curves was observed.

inhibition of transit in both physiological and pathophysiological states. The activity of salvinorin A, but not U-50488, was significantly higher in mice with inflammation. In fact, the curve representing the inhibitory effect of salvinorin A on transit in control mice was statistically different from the curve representing the inhibitory effect of salvinorin A in croton oil-treated mice (Fig. 3). Salvinorin A significantly inhibited motility starting from 3 mg kg⁻¹ in control mice and from 0.3 mg kg⁻¹ in croton-oil treated mice (Fig. 3).

In control animals, both the opioid antagonist naloxone (10 mg kg⁻¹) and the KOR antagonist nor-binaltorphimine (20 mg kg⁻¹) counteracted the inhibitory effect of U-50488, but not the inhibitory effect of salvinorin A (Fig. 5A,B). However, in croton oil-treated animals, naloxone and nor-binaltorphimine antagonized the effect of both salvinorin A and U-50488 (Fig. 6A,B).

In absence of any drug, the opioid receptor antagonist naloxone (10 mg kg⁻¹) and nor-binaltorphimine (20 mg kg⁻¹) did not modify significantly motility neither in control ($n = 7$ –8) nor in croton oil-treated mice ($n = 7$ –8) (data not shown).

When given orally, both SDE (100 mg kg⁻¹) and salvinorin A (10 mg kg⁻¹) did not modify significantly intestinal transit, either in control mice (GC: control 5.10 ± 0.41; SDE 5.35 ± 0.44; salvinorin A 4.95 ± 0.61, $n = 10$ for each experimental group) or in croton oil-treated-mice (GC: control 4.85 ± 0.46, croton oil 6.98 ± 0.48; croton oil + SDE 6.88 ± 0.46, croton oil + salvinorin A 6.17 ± 0.65, $n = 10$ for each experimental group).

DISCUSSION

Previous studies have shown that *Salvia divinorum*, and its main active ingredient salvinorin A, inhibited enteric cholinergic transmission *in vitro*.¹⁵ In the present study, we have shown that a standardized extract from *Salvia divinorum* leaves, and salvinorin A normalize intestinal motility in pathophysiological states with either weak or no effects in control animals. The inhibition of motility in pathophysiological states, without appreciable effects in control animals (at least for the lower doses of salvinorin A), is relevant because one of the major side effects associated with oral administration of opiates is their constipating effect. Interestingly, in our experiments, both SDE and salvinorin A were not active after oral administration. This observation is intriguing in the light of the fact that *Salvia divinorum* was consumed by Mazatecs Indians of Oaxaca by chewing fresh leaves which made absorption of salvinorin A possible through the oral mucosa.¹⁹

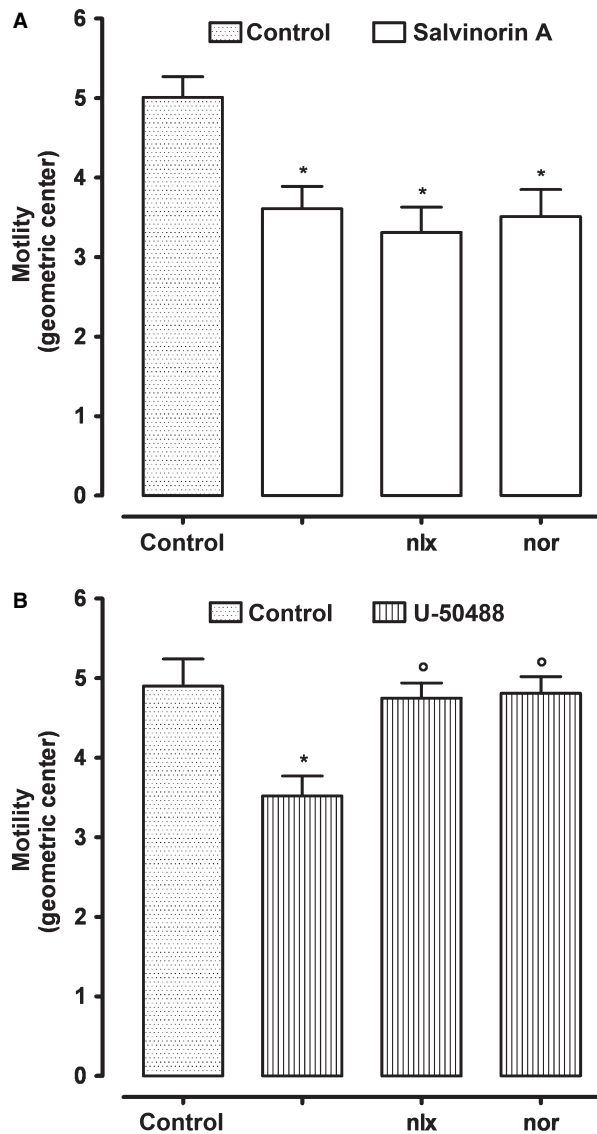


Figure 5 Effect of IP-injected salvinorin A (3 mg kg^{-1}) (A) or U-50488 (10 mg kg^{-1}) (B) in control mice alone or in the presence of the opioid receptor antagonist naloxone (nlx, 10 mg kg^{-1} , i.p.) or of the κ -opioid receptor (KOR) antagonist nor-binaltorphimine (nor, 20 mg kg^{-1} , i.p.). Transit was expressed as the geometric centre of the distribution of a fluorescent marker along the small intestine (see Materials and methods section). Bars represent the mean \pm SEM of 10–12 animals. * $P < 0.05$ vs corresponding control; ° $P < 0.05$ vs U-50488.

We have previously shown that *Salvia divinorum* and its active ingredient salvinorin A inhibited cholinergic twitch contractions in the isolated guinea-pig ileum through KOR activation.¹⁵ This is consistent with previous reports showing a KOR-mediated inhibition of cholinergic transmission²⁰ and peristalsis in the isolated guinea-pig ileum.¹¹ However, there are

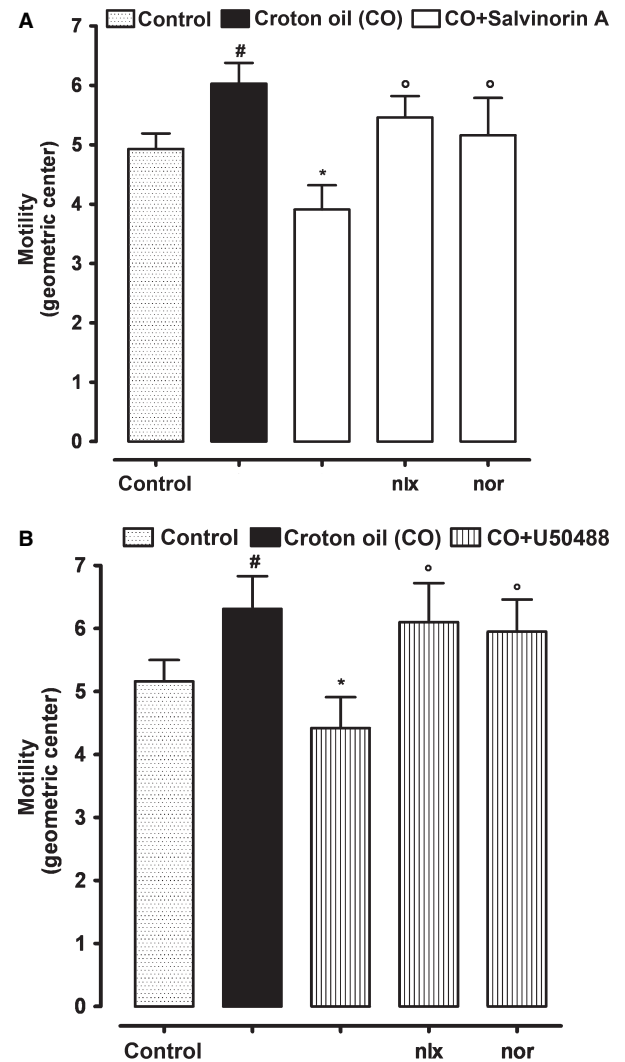


Figure 6 Effect of IP-injected salvinorin A (1 mg kg^{-1}) (A) or U-50488 (10 mg kg^{-1}) (B) in croton oil-treated mice alone or in the presence of the opioid receptor antagonist naloxone (nlx, 10 mg kg^{-1} , i.p.) or of the KOR antagonist nor-binaltorphimine (nor, 20 mg kg^{-1} , i.p.). Transit was expressed as the geometric centre of the distribution of a fluorescent marker along the small intestine (see Materials and methods section). Bars represent the mean \pm SEM of 10–12 animals. # $P < 0.05$ vs control; * $P < 0.05$ vs croton oil; ° $P < 0.05$ vs salvinorin A or U50488.

conflicting results about the effect of KOR agonists on intestinal transit in mice. Indeed, some authors have shown that KOR agonists inhibited charcoal meal transit in mice,^{21,22} but these findings have not been confirmed by others.^{16,23} These studies were performed using a charcoal 'meal' method. This method is not quantitative in contrast to radioisotopic ones; it is merely an indicator of the leading edge of visible marker. Therefore, such method gives no information

about the amount of marker at each point along the intestine.

In the present study, using more sensitive method, we noticed that SDE had no effect on intestinal motility under physiological conditions, whereas salvinorin A, a potent KOR opioid agonist, inhibited transit only at high doses. In addition, the selective KOR agonist U-50488 inhibited motility in a dose-dependent fashion. Both the non-selective opioid receptor antagonist naloxone and the selective KOR antagonist nor-binaltrophimine counteracted the effect of U-50488, but not the effect of salvinorin A on intestinal motility. These results suggest that in physiological states, salvinorin A may reduce intestinal motility by a KOR-independent mechanism. The ability of KOR antagonist to counteract the effect of U-50488 but not the effect of salvinorin A could be explained by hypothesizing that this plant compound, in addition to its potent action on KOR,^{7,8} could exert inhibitory actions in the gut which mask the KOR-mediated inhibition in motility. However, a direct antispasmodic effect on smooth muscle seems unlikely, as salvinorin A did not affect the contractions induced by exogenous acetylcholine in the isolated guinea-pig ileum.¹⁵

Croton oil is an irritant that produces experimental chronic inflammation in the mouse small intestine. Inflammation is characterized by a clear disruption of the mucosa and infiltration of lymphocyte in the submucosa.¹⁶ Previous investigators have shown that inflammation induced by two consecutive doses of croton oil given 24 h apart (as in this study), produces maximal inflammatory response and maximal increase in transit 4 days after the first dose of croton oil.¹⁶ Therefore, the influence of SDE and salvinorin A on intestinal motility was assessed at this time point. We have shown that SDE, which exerts no effect on motility under physiological states (see above), dose-dependently reduced motility in croton-oil treated animals. In addition, we provide strong evidence that the pharmacological activity of SDE on intestinal transit was mainly the result of salvinorin A content. Indeed, (i) SDE without salvinorin A did not affect motility in the present study; and (ii) salvinorin A itself reduced motility, an effect mimicked by the synthetic KOR agonist U-50488H. The effect of salvinorin A (and the effect of U-50488H) on motility was counteracted by the non-selective opioid receptor antagonist naloxone and by the selective KOR antagonist nor-binaltrophimine, thus, suggesting the involvement of the KOR in the inhibitory effect of salvinorin A in the inflamed gut.

An interesting finding of our present study lies in the observation that salvinorin A preferentially inhibited motility in mice with inflammation

compared to control mice. It is very unlikely that the increased potency of salvinorin A could involve KOR. This is because the selective KOR agonist U-50488H inhibited motility both in control and in croton-oil treated mice with similar potency. On the other hand, others revealed increased transcription of MOR and DOR (but not KOR) genes in the murine myenteric plexus of croton oil-treated animals,²⁴ which argues against a KOR-mediated mechanism. The ability of salvinorin A to have target(s) other than the KOR, which allows this plant compound to exert motility changes preferentially in inflamed mice, is interesting due to possible use of salvinorin A as a lead compound for the development of drugs being able to reduce motility in inflammatory bowel diseases. Interestingly, others have shown that KOR agonists modulate visceral nociception at a novel, peripheral site of action in the rat colon.¹⁴ However, it is not known whether or not salvinorin A modulates visceral pain.

In conclusion, we have shown that the KOR agonist salvinorin A may have different effects and targets on intestinal motility, depending upon whether this plant compound is evaluated under physiological or pathophysiological states. Under physiological states, salvinorin A exerts a weak effect on motility, by its inhibition only at the high doses tested; this effect is independent from activation of KOR receptors. In an inflammatory model of intestinal dysfunction, salvinorin A inhibited motility at doses that were inactive in control animals through a KOR-dependent mechanism. From an ethnopharmacological point of view, the data presented here could validate the traditional use of *Salvia divinorum* in the treatment of diarrhoea and other gut diseases. Moreover, the ability of salvinorin A (in contrast to the selective KOR agonist U-50488H) to inhibit motility, preferentially during inflammation, makes this compound a good candidate for the development of new drugs retaining not only KOR activation, but also the ability to reduce motility in inflamed gut without appreciable effects under physiological states.

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