

# The hallucinogenic herb *Salvia divinorum* and its active ingredient salvinorin A inhibit enteric cholinergic transmission in the guinea-pig ileum

R. CAPASSO,\* F. BORRELLI,\* F. CAPASSO,\* D. J. SIEBERT,† D. J. STEWART,‡ J. K. ZJAWIONY‡ & A. A. IZZO\*

\*Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy

†The *Salvia divinorum* Research and Information Center, Malibu, CA, USA

‡Department of Pharmacognosy and National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS, USA

**Abstract** *Salvia divinorum* is a widespread hallucinogenic herb traditionally employed for divination, as well as a medicament for several disorders including disturbances of gastrointestinal motility. In the present study we evaluated the effect of a standardized extract from the leaves of *S. divinorum* (SDE) on enteric cholinergic transmission in the guinea-pig ileum. SDE reduced electrically evoked contractions without modifying the contractions elicited by exogenous acetylcholine, thus suggesting a prejunctional site of action. The inhibitory effect of SDE on twitch response was abolished by the opioid receptor antagonist naloxone and by the  $\kappa$ -opioid antagonist nor-binaltorphimine, but not by naltrindole (a  $\delta$ -opioid receptor antagonist), CTOP (a  $\mu$ -opioid receptor antagonist), thioperamide (a  $H_3$  receptor antagonist), yohimbine (an  $\alpha_2$ -receptor antagonist), methysergide (a 5-hydroxytryptamine receptor antagonist),  $N^G$ -nitro-L-arginine methyl ester (an inhibitor of NO synthase) or apamin (a blocker of  $Ca^{2+}$ -activated  $K^+$  channels). Salvinorin A, the main active ingredient of *S. divinorum*, inhibited in a nor-binaltorphimine- and naloxone-sensitive manner electrically induced contractions. It is concluded that SDE depressed enteric cholinergic transmission likely through activation of  $\kappa$ -opioid receptors and this may provide the pharmacological basis underlying its traditional antidiarrhoeal use. Salvinorin A might be the chemical ingredient responsible for this activity.

**Keywords** *Salvia divinorum*, salvinorin A, intestine, cholinergic transmission,  $\kappa$ -opioid receptors, gastrointestinal motility.

## INTRODUCTION

*Salvia divinorum* belongs to the family Labiatae (Lamiaceae), commonly referred to as the mint family. Shamanic healers of the Mazatec region in Oaxaca, Mexico, traditionally use the psychoactive herb by chewing fresh leaves or by drinking the juice of the leaves.<sup>1–3</sup> It also has been used traditionally as a medicine for the treatment of a number of diseases including headache, rheumatism, abdominal swelling and diarrhoea.<sup>1,4</sup> More recently, there have been reports of using *S. divinorum* recreationally for its hallucinogenic effects. *Salvia divinorum* leaf preparations or concentrated extracts are indeed widely available in western Europe and the USA, notably on Internet sites,<sup>5</sup> and also used as marijuana substitute.<sup>6,7</sup> The hallucinatory effect that results has been reported to be potent and intense, lasting for up to an hour when smoked.<sup>6,8</sup> Although it is an emerging intoxicant in the USA and Europe<sup>9</sup> very little pharmacological research has been conducted on this plant.

The main active constituent of *S. divinorum* is salvinorin A, a neoclerodane diterpene. Salvinorin A represents the only known hallucinogenic terpenoid and has been reported to be the most potent naturally occurring hallucinogen, with an effective dose, when smoked, of 0.2–1 mg in humans.<sup>7</sup> Salvinorin A induces an intense, short-lived hallucinogenic experience qualitatively distinct from that induced by the classical hallucinogens such as lysergic acid

### Address for correspondence

Raffaele Capasso, Department of Experimental Pharmacology, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy.

Tel: +39 081 678432; fax: +39 081 678403;

e-mail: rafcapas@unina.it

Received: 7 June 2005

Accepted for publication: 24 August 2005

diethylamide (LSD), psilocybin and mescaline.<sup>7</sup> Quite recently, it has been reported that salvinorin A has high affinity and selectivity for the cloned  $\kappa$ -opioid receptor.<sup>10</sup> Indeed, it has been suggested, that salvinorin A is a  $\kappa$ -opioid receptor agonist,<sup>10-13</sup> although there is no definitive evidence that hallucinations are mediated by  $\kappa$ -opioid receptors. However, the effects of salvinorin A on neural functions are largely unexplored.

Because of anecdotal reports that extracts of *S. divinorum* may possess antidiarrhoeal activity<sup>1</sup> and because  $\kappa$ -opioid receptors may modulate intestinal peristalsis,<sup>14,15</sup> we investigated the effect of *S. divinorum*, and its main active ingredient, salvinorin A, on myenteric cholinergic transmission. For this purpose we evaluated the effect of a standardized extract from *S. divinorum* leaves (SDE) and of isolated salvinorin A on the contractions elicited either by electrical stimulation or by exogenous acetylcholine in the guinea-pig ileum.

## MATERIAL AND METHODS

Male guinea-pigs weighing between 250 and 350 g were purchased from Harlan Italy (S. Pietro al Natissone, UD, Italy), and were maintained under controlled conditions of temperature ( $24 \pm 2$  °C) and humidity (60%) until used. The guinea-pigs had free access to water and food. All experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Guinea-pigs were killed by asphyxiation with CO<sub>2</sub> and segments (1–1.5 cm) of the distal ileum lying 5–15 cm proximal to the ileocaecal valve were quickly removed and flushed of luminal contents and placed in Krebs solution (mmol L<sup>-1</sup>: NaCl 119, KCl 4.75, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.5 and glucose 11). The segments were set up (in such a way so as to record contractions mainly from the longitudinal axis) in an organ bath containing Krebs (20 mL) equilibrated with 95%O<sub>2</sub>, 5%CO<sub>2</sub> at 37 °C. The tissues were connected to an isotonic transducer (load 0.5 g) connected to PowerLab system (Ugo Basile, Comerio, Italy).

After a minimal 1 h equilibration period, the strips were subjected to electrical field stimulation (EFS, 2.5 Hz for 1 s, 400 mA, 1 ms pulse duration), delivered through electrodes placed around the tissue.

Stable and reproducible contractions for a time period of 4 h were obtained with stimulation every 20 s. Contractions were expressed as % of contractions produced by 10<sup>-5</sup> mol L<sup>-1</sup> carbachol; this concentration of carbachol produced a maximal contractile effect

(100% contraction). After stable control contractions evoked by EFS had been recorded, the contractile responses were observed in the presence of increasing cumulative concentrations of SDE (0.01–300 ng mL<sup>-1</sup>). The contact time for each concentration was 10 min. Preliminary experiments showed that this contact time was sufficient for SDE to achieve maximal effect.

The effect of SDE was also evaluated after the administration in the bath (contact time 30 min) of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME,  $3 \times 10^{-4}$  mol L<sup>-1</sup>) (to block NO synthesis), apamin ( $10^{-7}$  mol L<sup>-1</sup>) (a blocker of Ca<sup>2+</sup>-activated K<sup>+</sup> channels which blocks the enteric inhibitory component mediated by ATP or a related purine), yohimbine ( $10^{-7}$  mol L<sup>-1</sup>) (to block  $\alpha_2$ -adrenergic receptors), thioperamide ( $10^{-6}$  mol L<sup>-1</sup>) (to block H<sub>3</sub> receptors), methysergide ( $10^{-6}$  mol L<sup>-1</sup>) [to block 5-hydroxytryptamine (5-HT) receptors], naloxone ( $10^{-6}$  mol L<sup>-1</sup>) (to block opioid receptors), naltrindole ( $3 \times 10^{-8}$  mol L<sup>-1</sup>) (to block  $\delta$ -opioid receptors), CTOP ( $10^{-6}$  mol L<sup>-1</sup>) (to block  $\mu$ -opioid receptors) and nor-binaltorphimine ( $3 \times 10^{-8}$  mol L<sup>-1</sup>) (to block  $\kappa$ -opioid receptors). In preliminary experiments the effect of tetrodotoxin ( $3 \times 10^{-7}$  mol L<sup>-1</sup>) or atropine ( $10^{-6}$  mol L<sup>-1</sup>) (contact time 10 min) on EFS-induced contractions was evaluated. These concentrations were selected on the basis of previous work.<sup>15-18</sup>

The effect of SDE was also evaluated (contact time 10 min) on the contractions produced by exogenous acetylcholine ( $10^{-6}$  mol L<sup>-1</sup>). This concentration of acetylcholine gave a contractile response that was similar in amplitude to that of electrical stimulation. Acetylcholine was left in contact with the tissue for 60 s and then washed out. The interval between each contraction was 10 min.

In another set of experiments, the effect of salvinorin A ( $10^{-12}$ – $10^{-6}$  mol L<sup>-1</sup>), the  $\kappa$ -opioid antagonist U-50488 ( $10^{-12}$ – $10^{-6}$  mol L<sup>-1</sup>) or SDE without salvinorin A (1–100 000 ng mL<sup>-1</sup>) on EFS- or acetylcholine-induced contractions was also evaluated (contact time: 10 min for each concentration). Salvinorin A was also evaluated in the presence of naloxone ( $10^{-6}$  mol L<sup>-1</sup>), naltrindole ( $3 \times 10^{-8}$  mol L<sup>-1</sup>), CTOP ( $10^{-6}$  mol L<sup>-1</sup>) and nor-binaltorphimine ( $3 \times 10^{-8}$  mol L<sup>-1</sup>).

## Drugs

An ethanolic extract (320 g) from dried *S. 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. Briefly, 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 salvinorin A were combined and all VLC fractions and washings, with the exception of the salvinorin A fractions, were recombined and evaporated to dryness to give an extract without salvinorin A (263 g). The salvinorin 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 salvinorin A (333 mg) and the extract without salvinorin A (19.78 g). Acetylcholine hydrochloride, carbachol chloride, apamin, yohimbine hydrochloride, methysergide, L-NAME hydrochloride, thioperamide, U-50488, naloxone hydrochloride, tetrodotoxin, were purchased from Sigma (Milan, Italy). Naltrindole, CTOP and nor-binaltorphimine were purchased from Tocris Cookson (Avonmouth, UK). Salvinorin A, naltrindole, CTOP, nor-binaltorphimine, tetrodotoxin, thioperamide were dissolved in dimethyl sulphoxide (DMSO). SDE and SDE without salvinorin A were dissolved in DMSO (stock solution: 1 mg mL<sup>-1</sup> and 100 mg mL<sup>-1</sup>, respectively) and then diluted in distilled water for the lower concentrations. The other drugs were dissolved in distilled water. DMSO (<0.01%) did not modify EFS-induced contractions.

### Statistic

Results are expressed as mean ± SEM. Nonlinear regression analysis for all concentration response curves were performed (Graph Pad InStat program version 4.01; GraphPad Software, Inc., San Diego, CA, USA). Data were analysed by two way ANOVA. A value of  $P < 0.05$  was considered significant. The concentrations of SDE, SDE without salvinorin A, salvinorin A or U-50488 that produced 50% inhibition of EFS-induced contractions (IC<sub>50</sub>) or maximal effect ( $E_{max}$ ) were used to characterize their potency and efficacy respectively. The IC<sub>50</sub> and  $E_{max}$  values [geometric mean ± 95% confidence limits (CL)] were calculated using the Graph Pad InStat program version 4.01.

## RESULTS

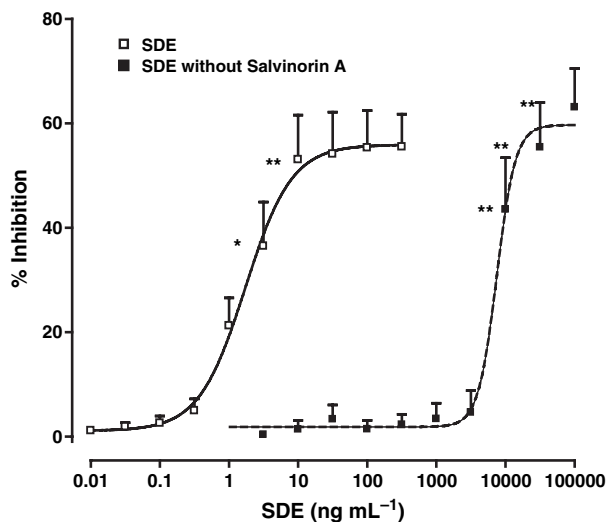
### EFS-induced contractions: effect of various antagonists or inhibitors

The EFS (2.5 Hz for 1 s, 400 mA, 0.25 ms pulse duration) of the guinea-pig ileum evoked a contractile response that was 49 ± 5% of the contraction produced by 10<sup>-4</sup> carbachol ( $n = 7$ ). These EFS-induced contrac-

tions were abolished by tetrodotoxin (3 × 10<sup>-7</sup> mol L<sup>-1</sup>) or atropine (10<sup>-6</sup> mol L<sup>-1</sup>), thus indicating that these contractions were because of release of acetylcholine from enteric nerves. EFS-induced contractions were not significantly modified by naloxone (10<sup>-6</sup> mol L<sup>-1</sup>) (5 ± 3% increase), yohimbine (10<sup>-7</sup> mol L<sup>-1</sup>) (6 ± 4% increase), L-NAME (3 × 10<sup>-4</sup> mol L<sup>-1</sup>) (22 ± 5% increase), thioperamide (10<sup>-6</sup> mol L<sup>-1</sup>) (4 ± 5% increase), naltrindole (3 × 10<sup>-8</sup> mol L<sup>-1</sup>) (12 ± 4% increase), CTOP (10<sup>-6</sup> mol L<sup>-1</sup>) (10 ± 5% increase), ( $n = 7-8$  experiments for each antagonist used). By contrast, nor-binaltorphimine (3 × 10<sup>-8</sup> mol L<sup>-1</sup>) and apamin (10<sup>-7</sup> mol L<sup>-1</sup>) significantly increased EFS-induced contractions (36 ± 5% and 46 ± 4% increase, respectively,  $P < 0.05$ ,  $n = 7-8$  experiment for both compounds). Methysergide (10<sup>-6</sup> mol L<sup>-1</sup>) produced a transient contractile effect, but did not modify EFS-induced contractions after 30-min contact (data not shown).

### EFS-induced contractions: effect of SDE or salvinorin A

The SDE (0.01–300 ng mL<sup>-1</sup>) as well as SDE without salvinorin A (3–100 000 ng mL<sup>-1</sup>) decreased, in a concentration-dependent manner, the amplitude of EFS-evoked contractions (Fig. 1). Statistical significance was achieved starting from the 3 ng mL<sup>-1</sup> concentration (SDE) and 10 000 ng mL<sup>-1</sup> concentrations. The IC<sub>50</sub> (95% CL) values were 1.72 ng mL<sup>-1</sup> (0.93–3.1) for



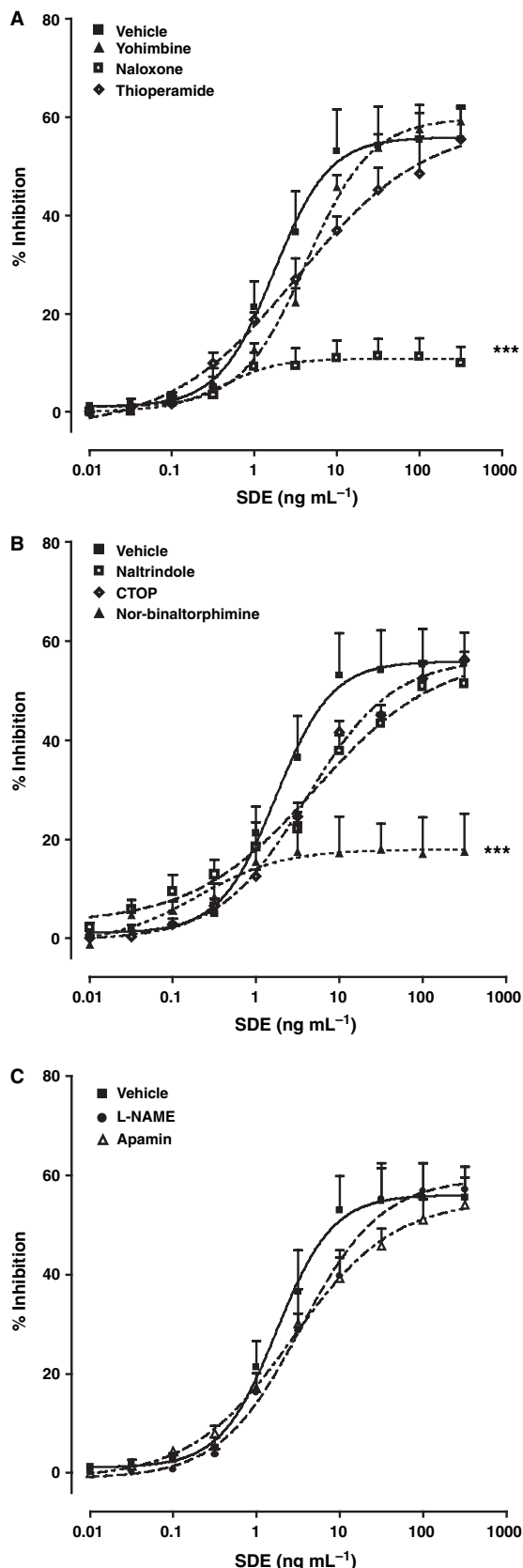
**Figure 1** Effect of *Salvia divinorum* extract (SDE) with or without salvinorin A on the contractile response produced by electrical field stimulation (EFS) in the isolated guinea-pig ileum. Each point represents the mean of 7–8 experiments. Vertical lines show SEM. \* $P < 0.05$  and \*\* $P < 0.01$  vs corresponding controls.

SDE and  $7504 \text{ ng mL}^{-1}$  (5406–1042) for SDE without salvinorin A. The  $E_{\text{max}}$  (95% CL) values were 55.89% (48.89–62.88) for SDE and 59.67% (52.19–67.16)% for SDE without salvinorin A.

Figure 2 shows the effect of SDE on EFS-evoked contractions in the presence of drugs that block main prejunctional receptors (i.e. opioid,  $\alpha_2$ -adrenergic and histamine  $H_3$  receptors) (Fig. 2A), or in the presence of selective opioid receptor antagonists (Fig. 2B), or in the presence of drugs that block the enteric inhibitory transmission (i.e. L-NAME and apamin) (Fig. 2C). Naloxone and nor-binaltorphimine, but not the other drugs, counteracted the inhibitory effect of SDE on EFS-induced contractions. Moreover, the inhibitory effect of SDE on EFS-induced contractions was not modified by methysergide [(% variation, SDE):  $0.01 \text{ ng mL}^{-1}$ :  $1.1 \pm 1$ ;  $0.03 \text{ ng mL}^{-1}$ :  $1.90 \pm 1$ ;  $0.1 \text{ ng mL}^{-1}$ :  $2.61 \pm 2$ ;  $0.3 \text{ ng mL}^{-1}$ :  $4.98 \pm 3$ ;  $1 \text{ ng mL}^{-1}$ :  $21.24 \pm 4$ ;  $3 \text{ ng mL}^{-1}$ :  $36.49 \pm 5$ ;  $10 \text{ ng mL}^{-1}$ :  $53.07 \pm 4$ ;  $30 \text{ ng mL}^{-1}$ :  $54.13 \pm 5$ ;  $100 \text{ ng mL}^{-1}$ :  $55.13 \pm 4$ ;  $300 \text{ ng mL}^{-1}$ :  $55.52 \pm 5$ ; (% variation, SDE + methysergide)  $0.01 \text{ ng mL}^{-1}$ :  $1.5 \pm 4$ ;  $0.03 \text{ ng mL}^{-1}$ :  $2.0 \pm 4$ ;  $0.1 \text{ ng mL}^{-1}$ :  $3.0 \pm 4$ ;  $0.3 \text{ ng mL}^{-1}$ :  $5.10 \pm 5$ ;  $1 \text{ ng mL}^{-1}$ :  $20.04 \pm 4$ ;  $3 \text{ ng mL}^{-1}$ :  $31.63 \pm 6$ ;  $10 \text{ ng mL}^{-1}$ :  $49.50 \pm 5$ ;  $30 \text{ ng mL}^{-1}$ :  $50.82 \pm 5$ ;  $100 \text{ ng mL}^{-1}$ :  $52.64 \pm 4$ ;  $300 \text{ ng mL}^{-1}$ :  $55.91 \pm 5$ ;  $n = 7-8$ ].

The effect of salvinorin A, the main psychoactive compound in *S. divinorum*, on EFS-induced contractions is shown in Fig. 3. Salvinorin A ( $10^{-12}$ – $10^{-6} \text{ mol L}^{-1}$ ) decreased, in a concentration-dependent manner, the amplitude of EFS-evoked contractions [ $\text{IC}_{50}$  (95% CL):  $3.9 \times 10^{-9} \text{ mol L}^{-1}$  ( $8.40 \times 10^{-10}$ – $1.48 \times 10^{-8}$ );  $E_{\text{max}}$  (95% CL): 68.12 (50.62–87.28)%]. The inhibitory effect of salvinorin A was counteracted by naloxone and nor-binaltorphimine, but not by CTOP or naltrindole. The selective  $\kappa$ -opioid receptor agonist U-50488, used as a reference compound, also inhibited EFS-induced contractions [( $\text{IC}_{50}$  (95% CL):  $1.86 \times 10^{-9} \text{ mol L}^{-1}$  ( $1.48 \times 10^{-9}$ – $2.23 \times 10^{-9}$ );  $E_{\text{max}}$  (95% CL): 51.32% (49.47–53.18)].

**Figure 2** Electrical field stimulation (EFS)-induced contractions in the isolated guinea-pig ileum: Effect of *Salvia divinorum* extract (SDE;  $0.01$ – $300 \text{ ng mL}^{-1}$ ) alone (vehicle) or in presence of yohimbine ( $10^{-6} \text{ mol L}^{-1}$ ), naloxone ( $10^{-6} \text{ mol L}^{-1}$ ), thioperamide ( $10^{-6} \text{ mol L}^{-1}$ ) (A), naltrindole ( $3 \times 10^{-8} \text{ mol L}^{-1}$ ), CTOP ( $10^{-6} \text{ mol L}^{-1}$ ), nor-binaltorphimine ( $3 \times 10^{-8} \text{ mol L}^{-1}$ ) (B),  $N^G$ -nitro-L-arginine methyl ester (L-NAME,  $3 \times 10^{-4} \text{ mol L}^{-1}$ ) or apamin ( $10^{-7} \text{ mol L}^{-1}$ ) (C). Each point represents the mean of 7–8 experiments. Vertical lines show SEM. \*\*\* $P < 0.001$  vs vehicle (significance between curves).



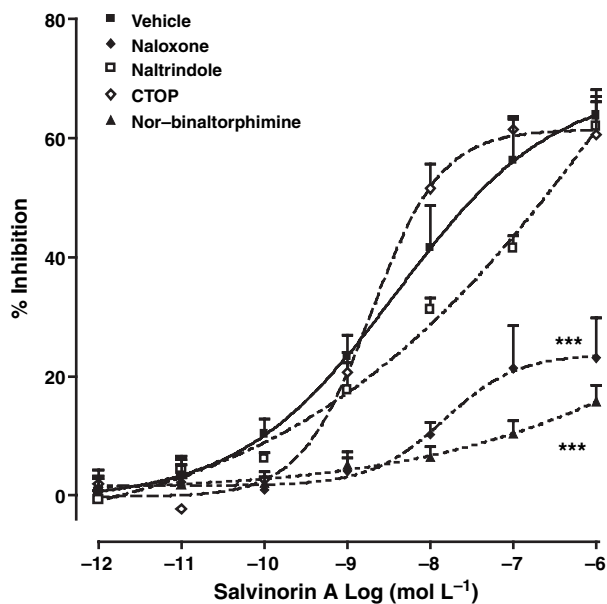
### Acetylcholine-induced contractions: effect of SDE or salvinorin A

When the ileum was stimulated with exogenous acetylcholine ( $10^{-6}$  mol L $^{-1}$ ), both SDE and salvinorin A were without effect [(% inhibition): SDE 0.01 ng mL $^{-1}$ :  $1.8 \pm 4$ ; SDE 0.03 ng mL $^{-1}$ :  $2.3 \pm 5$ ; SDE 0.1 ng mL $^{-1}$ :  $4.0 \pm 4$ ; SDE 0.3 ng mL $^{-1}$ :  $8.10 \pm 5$ ; SDE 1 ng mL $^{-1}$ :  $6.04 \pm 4$ ; SDE 3 ng mL $^{-1}$ :  $8.53 \pm 5$ ; SDE 10 ng mL $^{-1}$ :  $8.53 \pm 4$ ; SDE 30 ng mL $^{-1}$ :  $9.02 \pm 5$ ; SDE 100 ng mL $^{-1}$ :  $7.74 \pm 4$ ; SDE 300 ng mL $^{-1}$ :  $8.71 \pm 5$ ,  $P > 0.05$ ,  $n = 7-8$ ; salvinorin A  $10^{-12}$  mol L $^{-1}$ :  $0.8 \pm 4$ ; salvinorin A  $10^{-11}$  mol L $^{-1}$ :  $1.3 \pm 4$ ; salvinorin A  $10^{-10}$  mol L $^{-1}$ :  $5.0 \pm 5$ ; salvinorin A  $10^{-9}$  mol L $^{-1}$ :  $6.50 \pm 5$ ; salvinorin A  $10^{-8}$  mol L $^{-1}$ :  $8.04 \pm 5$ ; salvinorin A  $10^{-7}$  mol L $^{-1}$ :  $7.53 \pm 5$ ; salvinorin A  $10^{-6}$  mol L $^{-1}$ :  $8.0 \pm 4$ ,  $P > 0.05$ ,  $n = 7-8$ ]. At the higher concentrations tested neither SDE (300 n mol L $^{-1}$ ) nor salvinorin A ( $10^{-6}$  mol L $^{-1}$ ) significantly modified the concentration–response curve to acetylcholine ( $10^{-8}$ – $10^{-4}$  mol L $^{-1}$ ) (data not shown).

### DISCUSSION

*Salvia divinorum* is a hallucinogenic sage that has been gaining recreational popularity. Although traditionally the herb is mainly used for its psychoactive effects, it is also reportedly used to treat a variety of disorders, including those affecting the digestive tract (e.g. abdominal swelling and diarrhoea).<sup>1,19</sup> However, the potential pharmacological activities of this plant related to the central and peripheral nervous system are largely under-researched. In the present study we have shown that SDE, as well as its main active ingredient salvinorin A, inhibited enteric cholinergic transmission in the guinea-pig ileum. These results could provide the pharmacological basis underlying its traditional antidiarrhoeal use.

We have shown that SDE inhibited the contractions induced by EFS in the guinea-pig ileum, which are mediated by the release of acetylcholine from myenteric nerves. Moreover, when the ileum was exposed to SDE at concentrations that markedly inhibited the twitch response, the contractile response to exogenous acetylcholine (which is mediated by activation of postjunctional muscarinic receptors) remained unchanged. These observations strongly support the hypothesis that SDE inhibits the twitch response by acting prejunctionally rather than through a direct action on intestinal smooth muscle. Consequently, we tried to investigate the mechanism(s) underlying the inhibition of neural function by SDE.



**Figure 3** Electrical field stimulation (EFS)-induced contractions in the isolated guinea-pig ileum: effect of salvinorin A ( $10^{-12}$ – $10^{-6}$  mol L $^{-1}$ ) (vehicle) alone or in presence of naloxone ( $10^{-6}$  mol L $^{-1}$ ), of naltrindole ( $3 \times 10^{-8}$  mol L $^{-1}$ ), of CTOP ( $10^{-6}$  mol L $^{-1}$ ) and of nor-binaltorphimine ( $3 \times 10^{-8}$  mol L $^{-1}$ ). Each point represents the mean of 7–8 experiments. Vertical lines show SEM. \*\*\* $P < 0.001$  vs vehicle (i.e. the naloxone and nor-binaltorphimine concentration–response curves were significantly different from the vehicle curve).

It is well known that opioid peptides exert inhibitory effects on gastrointestinal motor function.<sup>20</sup> Opioid agonists inhibit acetylcholine release from myenteric neurones and attenuate twitch contraction of the longitudinal muscle in response to transmural electrical stimulation.<sup>21</sup> Immunohistochemical studies have revealed that the opioid receptor subtypes  $\mu$ ,  $\delta$  and  $\kappa$  are present in neural tissues of the guinea-pig enteric nervous system, but not in smooth cells,<sup>22</sup> and their activation results in inhibition of enteric cholinergic transmission.<sup>22</sup> In the present study we provide strong evidence that the inhibitory effect of SDE is mediated by  $\kappa$ -opioid receptors. Indeed the inhibitory effect of SDE on electrically evoked contractions was abolished by the non-selective opioid antagonist naloxone and the selective  $\kappa$ -opioid antagonist nor-binaltorphimine, but not by the  $\mu$ -opioid antagonist CTOP or by the  $\delta$ -opioid antagonist naltrindole.

Our experiments enable us to exclude a number of factors as potential contributory mechanisms involved in the inhibition of cholinergic transmission by SDE. In particular we can exclude the involvement of (a) other prejunctional systems (i.e. histamine H $_3$  and  $\alpha_2$ -adrenergic receptors) negatively coupled to cholinergic

transmission as the inhibitory effect of SDE was not affected by  $\alpha_2$ -adrenergic receptor antagonist yohimbine or by the histamine  $H_3$  receptor antagonist thioperamide; (b) enteric inhibitory nerves as apamin [a blocker of  $Ca^{2+}$  activated  $K^+$  channels which blocks the enteric inhibitory component mediated by ATP or related purine<sup>23</sup>] or L-NAME (an inhibitor of NO synthase) did not modify the inhibitory effect of SDE on twitch response; (c) 5-HT receptors as the depressant effect of SDE was insensitive to the 5-HT receptor antagonist methysergide. The lack of involvement of 5-HT receptors is relevant in the light of the observation that 5-HT<sub>2A</sub> receptors represents the primary molecular target responsible for the actions of classic hallucinogens such as LSD, psilocybin and mescaline.<sup>24,25</sup> *Salvia divinorum* contains numerous biologically active constituents, including salvinorins, divinatorins and hardwickiic acid.<sup>2</sup> Salvinorin A is believed to be the compound responsible for the hallucinogen properties of *S. divinorum*. Salvinorin A has been reported to be the most potent naturally occurring hallucinogen, with an effective dose, when smoked, of 200–1000 g in humans.<sup>7,8</sup> In the present study we provide strong evidence that the pharmacological activity of SDE on myenteric nerves observed here is mainly the result of salvinorin A content. Indeed, extracts of SDE without salvinorin A were about 4500-fold less active than the same extract with salvinorin A, and salvinorin A itself inhibited myenteric cholinergic transmission. Moreover, we confirmed with salvinorin A the main findings reported for SDE. In particular, we found that salvinorin A: (a) inhibited electrically evoked contractions without affecting the contractions induced by exogenous acetylcholine (which is consistent with a prejunctional site of action); (b) exerted this inhibitory effect likely through activation of  $\kappa$ -opioid receptors, as the inhibitory effect of this compound (as well as of the  $\kappa$ -opioid agonist U-50488, used as a reference compound) on electrically induced contractions was abolished by the non-selective opioid antagonist naloxone as well as by the selective  $\kappa$ -opioid antagonist nor-binaltrophimine. Consistent with our results, it has been shown that salvinorin A has been found to be a potent agonist of the human  $\kappa$ -opioid receptor expressed in human embryonic kidney 293 (HEK293) cells with an EC<sub>50</sub> of 1.05 nmol L<sup>-1</sup>.<sup>10</sup> Moreover, our finding that salvinorin A has similar potency and efficacy as U50488 in inhibiting cholinergic transmission is in accord with other experiments in isolated cells (i.e. oocytes from *Xenopus laevis*, Chinese hamster ovary, HEK293) expressing  $\kappa$ -opioid receptors.<sup>10–12</sup> It is interesting to note that both salvinorin A and U50488 did not

produce a total inhibition of the contractions evoked by EFS ( $E_{max}$  values in the 50–70% range), which is in line with the ability of U50488 to produce a partial inhibition (maximal inhibition: 55%) of electrically evoked acetylcholine release from guinea-pig myenteric neurones.<sup>26</sup> In preparing our manuscript, others have found that salvinorin A produced  $\kappa$ -opioid agonist-like discriminative effects in rhesus monkeys<sup>27</sup> and decreased dopamine levels in the mouse caudate putamen through activation of  $\kappa$ -opioid receptors.<sup>28</sup>

In conclusion, we have reported here that the hallucinogenic herb *S. divinorum* exerted inhibitory effects on enteric cholinergic transmission in the guinea-pig ileum through activation of prejunctional  $\kappa$ -opioid receptors. Salvinorin A may be the chemical constituent responsible for this activity. These data might justify the traditional use of the herb in the treatment of diarrhoea.

## ACKNOWLEDGMENTS

This work was supported by Enrico and Enrica Sovena Foundation, SESIRCA (Regione Campania), and Cofinanziamento Murst.

## REFERENCES

- Valdes LJ III, Diaz JL, Paul AG. Ethnopharmacology of ska Maria Pastora (*Salvia divinorum*, Epling and Jativa-M.). *J Ethnopharmacol* 1983; **7**: 287–312.
- Siebert DJ. Localization of salvinorin A and related compounds in glandular trichomes of the psychoactive sage, *Salvia divinorum*. *Ann Bot* 2004; **93**: 763–71.
- Halpern JH. Hallucinogens and dissociative agents naturally growing in the United States. *Pharmacol Ther* 2004; **102**: 131–8.
- Valdes LJ, III, Hatfield GM, Koreeda M, Paul AG. Studies of *Salvia divinorum* (Lamiaceae), an hallucinogenic mint from the Sierra Mazateca in Oaxaca, Central Mexico. *Econ Bot* 1987; **41**: 283–91.
- Halpern JH, Pope HG Jr. Hallucinogens on the Internet: a vast new source of underground drug information. *Am J Psychiatry* 2001; **158**: 481–3.
- Valdes LJ, III. *Salvia divinorum* and the unique diterpene hallucinogen, Salvinorin (divinorin) A. *J Psychoactive Drugs* 1994; **26**: 277–83.
- Siebert DJ. *Salvia divinorum* and salvinorin A: new pharmacologic findings. *J Ethnopharmacol* 1994; **43**: 53–56.
- Sheffler DJ, Roth BL. Salvinorin A: the “magic mint” hallucinogen finds a molecular target in the kappa opioid receptor. *Trends Pharmacol Sci* 2003; **24**: 107–9.
- Halpern JH. Hallucinogens: an update. *Curr Psychiatry Rep* 2003; **5**: 347–54.
- Roth BL, Baner K, Westkaemper R *et al.* Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc Natl Acad Sci USA* 2002; **99**: 11934–9.

- 11 Chavkin C, Sud S, Jin W *et al.* Salvinorin A, an active component of the hallucinogenic sage *Salvia divinorum* is a highly efficacious kappa-opioid receptor agonist: structural and functional considerations. *J Pharmacol Exp Ther* 2004; **308**: 1197–203.
- 12 Wang Y, Tang K, Inan S *et al.* Comparison of pharmacological activities of three distinct kappa ligands (Salvinorin A, TRK-820 and 3FLB) on kappa opioid receptors in vitro and their antipruritic and antinociceptive activities in vivo. *J Pharmacol Exp Ther* 2005; **312**: 220–30.
- 13 Munro TA, Rizzacasa MA, Roth BL, Toth BA, Yan F. Studies toward the pharmacophore of salvinorin A, a potent kappa opioid receptor agonist. *J Med Chem* 2005; **48**: 345–8.
- 14 Waterman SA, Costa M, Tonini M. Modulation of peristalsis in the guinea-pig isolated small intestine by exogenous and endogenous opioids. *Br J Pharmacol* 1992; **106**: 1004–10.
- 15 Shahbazian A, Heinemann A, Schmidhammer H, Beubler E, Holzer-Petsche U, Holzer P. Involvement of mu- and kappa-, but not delta-, opioid receptors in the peristaltic motor depression caused by endogenous and exogenous opioids in the guinea-pig intestine. *Br J Pharmacol* 2002; **135**: 741–50.
- 16 Izzo AA, Costa M, Mascolo N, Capasso F. The role of histamine H1, H2 and H3 receptors on enteric ascending synaptic transmission in the guinea pig ileum. *J Pharmacol Exp Ther* 1998; **287**: 952–7.
- 17 Storr M, Hahn A, Gaffal E, Saur D, Allescher HD. Effects of endomorphin-1 and -2 on mu-opioid receptors in myenteric neurons and in the peristaltic reflex in rat small intestine. *Clin Exp Pharmacol Physiol* 2002; **29**: 428–34.
- 18 Nocerino E, Izzo AA, Borrelli F *et al.* Relaxant effect of capsazepine in the isolated rat ileum. *Naunyn Schmiedebergs Arch Pharmacol* 2002; **365**: 187–92.
- 19 Wasson RG. A new Mexican psychotropic drug from the mint family. *Bot Mus Leaflet* 1962; **20**: 77–84.
- 20 Wood JD, Galligan JJ. Function of opioids in the enteric nervous system. *Neurogastroenterol Motil* 2004; **16** (Suppl. 2): 17–28.
- 21 Burks TF. Opioid peptides in gastrointestinal function. In: Tseng LF, ed. *The Pharmacology of Opioid Peptides*. Australia: Harwood Academy Publishers, 1995: 397–409.
- 22 Sternini C, Patierno S, Selmer IS, Kirchgessner A. The opioid system in the gastrointestinal tract. *Neurogastroenterol Motil* 2004; **16** (Suppl. 2): 3–16.
- 23 Crist JR, He XD, Goyal RK. Both ATP and the peptide VIP are inhibitory neurotransmitters in guinea-pig ileum circular muscle. *J Physiol* 1992; **447**: 119–31.
- 24 Glennon RA, Titeler M, McKenney JD. Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci* 1984; **35**: 2505–11.
- 25 Roth BL, Willins DL, Kristiansen K, Kroeze WK. 5-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine2C): where structure meets function. *Pharmacol Ther* 1998; **79**: 231–57.
- 26 Kojima Y, Takahashi T, Fujina M, Owyang C. Inhibition of cholinergic transmission by opiates in ileal myenteric plexus is mediated by kappa receptor. Involvement of regulatory inhibitory G protein and calcium N-channels. *J Pharmacol Exp Ther* 1994; **268**: 965–70.
- 27 Butelman ER, Harris TJ, Kreek MJ. The plant-derived hallucinogen, salvinorin A, produces kappa-opioid agonist-like discriminative effects in rhesus monkeys. *Psychopharmacology (Berl)* 2004; **172**: 220–4.
- 28 Zhang Y, Butelman ER, Schlussman SD, Ho A, Kreek MJ. Effects of the plant-derived hallucinogen salvinorin A on basal dopamine levels in the caudate putamen and in a conditioned place aversion assay in mice: agonist actions at kappa opioid receptors. *Psychopharmacology (Berl)* 2005; **179**: 551–8.