Salvinorin A: the 'magic mint' hallucinogen finds a molecular target in the kappa opioid receptor

Douglas J. Sheffler[1] and Bryan L. Roth[2]

[1]Department of Biochemistry, Case Western Reserve University Medical School, 10900 Euclid Avenue, Cleveland, OH 44106, USA
[2]National Institute of Mental Health Psychoactive Drug Screening Program and Departments of Biochemistry, Psychiatry, and Neurosciences, Case Western Reserve University Medical School, 10900 Euclid Avenue, Cleveland, OH 44106, USA

Salvinorin A, a neoclerodane diterpene, is the most potent naturally occurring hallucinogen known and rivals the synthetic hallucinogen lysergic acid diethylamide in potency. Recently, the molecular target of salvinorin A was identified as the kappa opioid receptor (KOR). Salvinorin A represents the only known non-nitrogenous KOR selective agonist. Based on the selectivity of salvinorin A for the KOR, this receptor represents a potential molecular target for the development of drugs to treat disorders characterized by alterations in perception, including schizophrenia, Alzheimer's disease and bipolar disorder.

Salvia divinorum (Diviner's sage) (Fig. 1a) is a rare member of the mint family that has been used for many centuries by the Mazatec people of Oaxaca, Mexico in traditional spiritual practices (Box 1). More recently, *S. divinorum* (also known as 'magic mint') has been used as a marijuana substitute by Mexican youths [1]. Furthermore, a large number of *S. divinorum* plants were discovered recently in Swiss horticulturists' greenhouses and a few more were seized at a large-scale plantation in Switzerland, implicating their increasing use as a recreational drug in Europe [2]. For several years, *S. divinorum* has been cultivated in California for use as a legal hallucinogen. At present, neither Swiss nor US laws for controlled substances ban the use of *S. divinorum* or its active compounds. In traditional spiritual practices, *S. divinorum* is typically ingested by one of three routes: (1) mastication and swallowing of the leaves; (2) crushing the leaves to extract the juices and then swallowing the extract; or (3) smoking the leaves [3]. The hallucinatory effect that results has been reported to be potent and intense, lasting for up to an hour [1,3].

The presumed main active ingredient of *S. divinorum* salvinorin A (Fig. 1b) was first identified by Alfredo Ortega in 1982 and independently isolated by Leander Valdes soon thereafter [1,4]. Salvinorin A is a neoclerodane diterpine of known absolute stereochemistry whose structure has been determined using ^{1}H nuclear magnetic resonance (NMR) and by two independent single-crystal X-ray studies (Fig. 1c) [4–6]. Salvinorin A represents the only known psychoactive terpenoid [7] and is chemically unique. Additionally, salvinorin A has been reported to be the most potent naturally occurring hallucinogen, with an effective dose, when smoked, of 200–1000 μg in humans. Thus, it is similar in potency to the synthetic hallucinogens lysergic acid diethylamide [LSD (the typical human dose used in abuse is 50–250 μg)] and 4-bromo-2,5-dimethoxyphenylisopropylamine [DOB (the typical human dose used in abuse is 500–1000 μg)] [1,3].

The molecular target of salvinorin A

Previous attempts to determine the proximal molecular target(s) of salvinorin A have been unsuccessful. Shortly after the recognition of the psychoactive properties of salvinorin A (by Daniel Siebert in 1994), salvinorin A was submitted for screening to NovaScreen™ to discover its molecular target [3]. Unfortunately, salvinorin A showed no significant inhibition of radioligand binding at any of the receptors, including various biogenic amine receptors, cannabinoid receptors and sigma receptors, screened by the NovaScreen™ process [3]. Recently, the pharmacological profile of salvinorin A at a variety of human G-protein-coupled receptors (GPCRs), ligand-gated

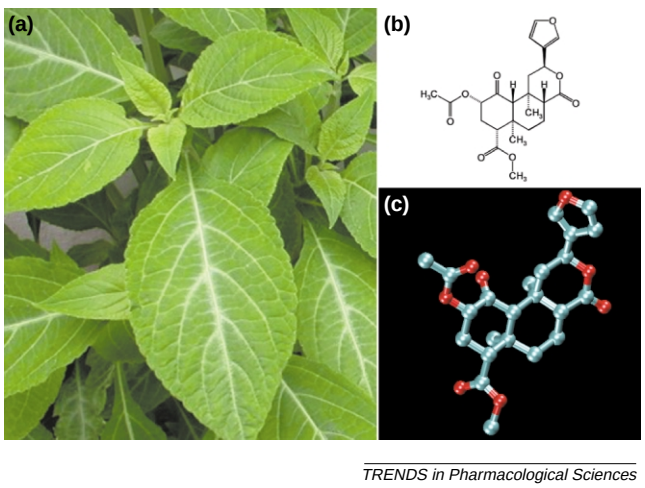

Fig. 1. (a) *Salvia divinorum* is a rare member of the Lamiaceae (mint) family. It has been used in traditional spiritual practices and as a recreational drug. (b) The two-dimensional structure of salvinorin A, the presumed main active ingredient of *S. divinorum*. (c) The three-dimensional structure of salvinorin A.

Corresponding author: Bryan L. Roth (roth@biocserver.BIOC.cwru.edu).

Box 1. The ethnopharmacology of *Salvia divinorum*

Salvia divinorum is known by many names including *ska Maria*, *ska Pastora*, *hojas de Maria*, *hojas de la Pastora*, *hierba Maria*, and *la Maria*. These names reflect the Mazatec belief that *S. divinorum* is the incarnation of the Virgin Mary; hence, the plant is highly prized and respected [16–18]. Despite its many names, *S. divinorum* was not identified until 1962 when Wasson and Hofmann collected a member of the Lamiaceae (mint) family that was known by the Mazatec people of Oaxaca, Mexico to have psychoactive properties [19]. This mint only grows in forest ravines and in other moist areas of the Sierra Mazateca between 750 m and 1500 m altitude [16].

Interestingly, *S. divinorum* flowers rarely, blossoming with white corollas and purple chalyces [16]. Seed production is even more rare because *S. divinorum* has never been reported to produce seeds in its native habitat of the Sierra Mazateca. Indeed, only Valdes and Siebert have reported the identification of seeds. *S. divinorum* primarily reproduces by vegetative growth from a shoot that is stuck into the ground, and can be readily grown from cuttings. Some believe that all *S. divinorum* strains present in the USA are one of seven vegetatively propagated clones, originating from the Sierra Mazateca in Oaxaca, Mexico. Most clones in the USA are derived from the original clone identified by Wasson in 1962 [18,20]. Because *S. divinorum* grows easily from cuttings, there is great potential for its increased use as a recreational drug; a yield of even a gram of salvinorin A per kilogram of dried leaves would provide 2000 doses of salvinorin A [21]. For additional information on *S. divinorum* and salvinorin A, see 'The *Salvia divinorum* Research and Information Center' (http://www.sagewisdom.org), an online resource maintained by Daniel Siebert.

ion channels, and transporters was re-examined via the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) (http://pdsp.cwru.edu) [8]. Salvinorin A was discovered to be a potent and selective kappa opioid receptor (KOR) agonist (importantly, KOR binding was not assayed using NovaScreen™ [3]). None of the other 48 tested molecular targets that were screened using the NIMH-PDSP [8], including the mu opioid receptor (MOR) and delta opioid receptor (DOR), showed significant inhibition of radioligand binding by salvinorin A, which demonstrates that salvinorin A is apparently selective for the KOR.

Opioid receptors are heptahelical GPCRs that have an extracellular N-terminus and an intracellular C-terminus. These opioid receptors are subdivided into MORs, DORs and KORs, each of which has receptor subtypes. The KORs, MORs and DORs are coupled to the G-protein subfamily G_i/G_o, and thus opioid receptor activation elicits analgesic (DORs, MORs and KORs) and psychotomimetic (KORs) [9] effects that are expected to involve inhibition of adenylyl cyclases, the activation of inward rectifying K^+ channels, and the inhibition of N-, P-, Q- and R-type voltage-activated Ca^{2+} channels.

Radioligand binding studies [8] disclosed that salvinorin A had high affinity at both cloned ($K_i = 16$ nM) and naturally occurring (guinea-pig brain; $K_i = 4.3$ nM) KORs. Interestingly, salvinorin A did not have detectable affinity for 5-HT_{2A} receptors, nor did it activate 5-HT_{2A} receptors, which represent the primary target for classical hallucinogens [8,10]. In functional studies, salvinorin A was found to be a potent agonist of the human KOR expressed in human embryonic kidney 293 (HEK293) cells with an EC_{50} of 1.05 nM for the inhibition of adenylyl cyclase activity, compared with an EC_{50} of 1.2 nM for the KOR agonist U69593 (see Chemical name) [8]. Recently, the actions of salvinorin A on tail-flick latency (a measure of analgesia) observed in wild-type mice have been shown to be abrogated in KOR knockout animals (J. Pintar, pers. commun).

The uniqueness of salvinorin A is underscored by the fact that it is: (1) the first non-nitrogenous, naturally occurring, KOR selective agonist; (2) the first non-nitrogenous, KOR selective ligand; and (3) the only known non-alkaloidal hallucinogen. Thus, from a medicinal chemistry standpoint, salvinorin A represents a structurally novel class of KOR selective compounds. Molecular modeling performed in the original study [8] resulted in a docked structure of salvinorin A to the KOR (Fig. 2a) and implicated residues that might be involved in the binding of salvinorin A (Fig. 2b) [8]. Mutagenesis studies of these residues to determine their potential contribution to the binding of salvinorin A to the KOR will provide a wealth of insight for selective KOR drug development.

Chemical name

U69593: (+)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide

KOR antagonists as therapeutic agents?

KOR agonists have been shown previously to be psychotomimetic [9] and the psychoactive properties of salvinorin A in humans are probably mediated by KORs. Indeed, there is one anecdotal report that the hallucinogenic effects of salvinorin A are blocked by pretreatment with naloxone, a nonspecific opioid receptor antagonist with modest affinity for KORs (D. Siebert, pers. commun). Thus, overdoses of salvinorin A are likely to respond to naloxone (Narcan®) or naltrexone administration. There are numerous diseases characterized by hallucinations, including schizophrenia, depression with psychotic features, and the hallucinosis associated with certain dementias such as Alzheimer's, Huntington's and Pick diseases. Diseases characterized by hallucinosis could, conceivably, reflect alterations in KOR number or KOR signal transduction; thus, KOR selective antagonists might represent novel therapeutic targets for diseases in which hallucinations are prominent [11]. However, there are few examples in the literature to corroborate this hypothesis. For example, administration of naloxone and naltrexone to schizophrenics showed only modest results [12]. Clearly, further studies using KOR selective antagonists are warranted, although it is

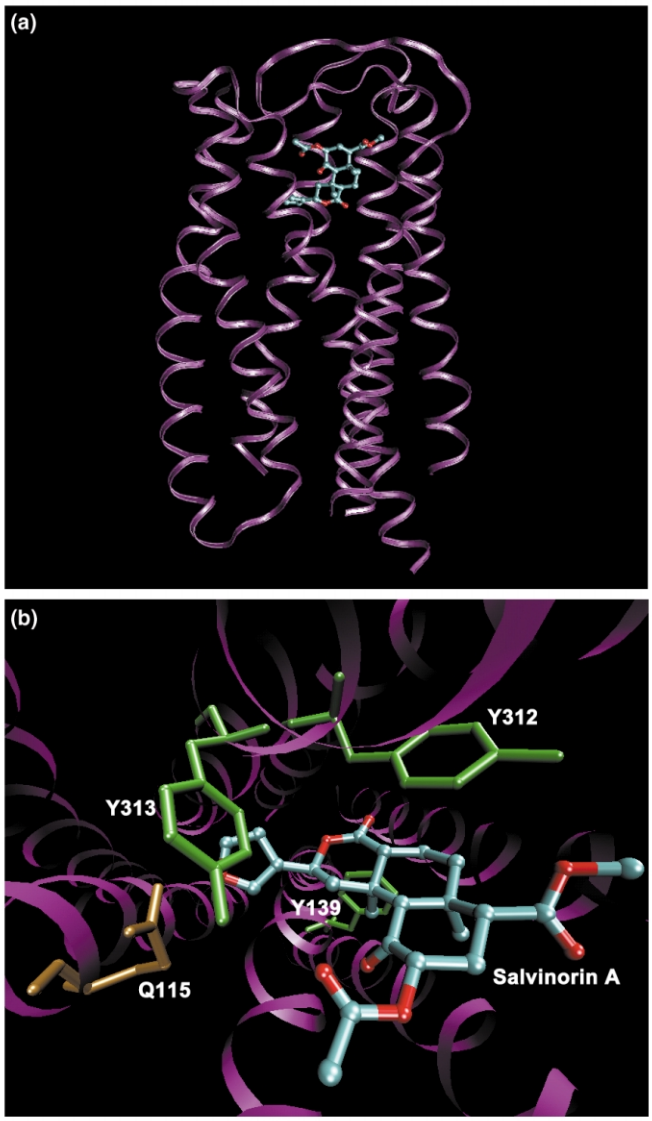

Fig. 2. (a) A model of the interactions of salvinorin A (blue) with the kappa opioid receptor (KOR; purple). (b) A close-up view of residues on the KOR, identified by molecular modeling, that might interact with salvinorin A.

possible that KOR selective antagonists will have no efficacy in diseases manifested by psychosis. Intriguingly, two independent post-mortem studies of Alzheimer's disease have reported an upregulation of KOR expression in the amygdala, putamen and cerebellar cortex [13,14]. In these same studies, the expression of MORs and DORs remained unchanged or was downregulated in these regions [13,14].

Concluding remarks

Salvinorin A represents a new class of non-nitrogenous KOR selective agonists, and KORs and/or KOR signaling probably play a role in the modulation of human cognition and perception. Because salvinorin A, a KOR selective agonist, has psychoactive properties, KOR selective antagonists could conceivably prove useful in the treatment of certain psychiatric disorders (e.g. Alzheimer's disease, schizophrenia, bipolar disorder and dementia). Previous development of antipsychotic drugs has primarily focused on the 5-HT_{2A} and the D2 dopamine receptor [15]. Knowledge that the KOR mediates the hallucinatory effects of salvinorin A provides a novel molecular candidate for the development of antipsychotic drugs.

References

1 Valdes, L.J. III (1994) *Salvia divinorum* and the unique diterpene hallucinogen, salvinorin (divinorin) A. *J. Psychoactive Drugs* 26, 277–283
2 Giroud, C. *et al.* (2000) *Salvia divinorum*: an hallucinogenic mint which might become a new recreational drug in Switzerland. *Forensic Sci. Int.* 112, 143–150
3 Siebert, D.J. (1994) *Salvia divinorum* and salvinorin A: new pharmacologic findings. *J. Ethnopharmacol.* 43, 53–56
4 Ortega, A. *et al.* (1982) Salvinorin, a new *trans*-neoclerodane diterpene from *Salvia divinorum* (Labiatae). *J. Chem. Soc. Perkins Trans.* 1, 2505–2508
5 Valdes, L.J. *et al.* (1984) Divinorin A, a psychotropic terpenoid, and divinorin B from the hallucinogenic Mexican mint *Salvia divinorum*. *J. Org. Chem.* 49, 4716–4720
6 Koreeda, M. *et al.* (1990) The absolute stereochemistry of salvinorins. *Chem. Lett.*, 2015–2018
7 Abramov, M.M. and Yaparova, S.A. (1963) *J. Appl. Chem (USSR)* 36, 2471
8 Roth, B.L. *et al.* (2002) Salvinorin A: a potent naturally occurring nonnitrogenous κ opioid selective agonist. *Proc. Nat. Acad. Sci. U. S. A.* 99, 11934–11939
9 Roth, B.L. *et al.* (1998) 5-Hydroxytryptamine$_2$-family receptors (5-hydroxytryptamine$_{2A}$, 5-hydroxytryptamine$_{2B}$, 5-hydroxytryptamine$_{2C}$): where structure meets function. *Pharmacol. Ther.* 79, 231–257
10 Pfeiffer, A. *et al.* (1986) Psychotomimesis mediated by κ opiate receptors. *Science* 233, 774–776
11 Ni, Q. *et al.* (1995) Opioid peptide receptor studies. 3. Interaction of opioid peptides and other drugs with four subtypes of the κ2 receptor in guinea pig brain. *Peptides* 16, 1083–1095
12 Marchesi, G.F. *et al.* (1995) The therapeutic role of naltrexone in negative symptom schizophrenia. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* 19, 1239–1249
13 Mathieu-Kia, A. *et al.* (2001) μ-, δ-, and κ-opioid receptor populations are differentially altered in distinct areas of postmortem brains of Alzheimer's disease patients. *Brain Res.* 893, 121–134
14 Bard, J. *et al.* (1993) Opioid receptor density in Alzheimer amygdala and putamen. *Brain Res.* 632, 209–215
15 Roth, B.L. *et al.* (1999) Activation is hallucinogenic and antagonism is therapeutic: role of 5-HT_{2A} receptors in atypical antipsychotic drug actions. *The Neuroscientist* 5, 254–262
16 Valdes, L.J. *et al.* (1982) Ethnopharmacology of *Ska Maria Pastora* (*Salvia divinorum*, Epling and Javita-M.). *J. Ethnopharmacology* 7, 287–312
17 Valdes, L.J. (2001) The early history of *Salvia divinorum*. *The Entheogen Review* X, pp. 73–75
18 Valdes, L.J. *et al.* (1987) Studies of *Salvia divinorum* (Lamiaceae), an hallucinogenic mint from the Sierra Mazateca in Oaxaca, Central Mexico. *Economic Botany* 41, 283–291
19 Wasson, W.G. (1962) A new Mexican psychotropic drug from the mint family. In *Botanical Museum Leaflets, Harvard University Press* 20, pp. 77–84
20 Siebert, D.J. (1994) *Salvia divinorum* and salvinorin A: new pharmacologic findings. *J. Ethnopharmacol.* 43, 53–56
21 Valdes, L.J. (1994) *Salvia divinorum* and the unique diterpine hallucinogen Salvinorin (Divinorin) A. *J. Psychoactive Drugs* 26, 277–283

0165-6147/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0165-6147(03)00027-0