Dose-Related Behavioral, Subjective, Endocrine, and Psychophysiological Effects of the κ Opioid Agonist Salvinorin A in Humans

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Background: Salvia divinorum (Salvia) is an increasingly popular recreational drug amongst adolescents and young adults. Its primary active ingredient, Salvinorin A (SA)—a highly selective agonist at the κ opiate receptor—is believed to be one of the most potent naturally occurring hallucinogens. However, there is little experimental data on the effects of SA in humans.

Methods: In a 3-day, double-blind, randomized, crossover, counterbalanced study, the behavioral, subjective, cognitive, psychophysiological, and endocrine effects of 0 mg, 8 mg, and 12 mg of inhaled SA were characterized in 10 healthy individuals who had previously used Salvia.

Results: SA produced psychotomimetic effects and perceptual alterations, including dissociative and somaesthetic effects, increased plasma cortisol and prolactin, and reduced resting electroencephalogram spectral power. The SA administration was associated with a rapid increase of its levels in the blood. SA did not produce euphoria, cognitive deficits, or changes in vital signs. The effects were transient and not dose-related. SA administration was very well-tolerated without acute or delayed adverse effects.

Conclusions: SA produced a wide range of transient effects in healthy subjects. The perceptual altering effects and lack of euphoric effects would explain its intermittent use pattern. Such a profile would also suggest a low addictive potential similar to other hallucinogens and consistent with κ opiate receptor agonism. Further work is warranted to carefully characterize a full spectrum of its effects in humans, to elucidate the underlying mechanisms involved, and to explore the basis for individual variability in its effects.

Key Words: Hallucinogen, κ-opioid, perception, psychosis, Salvia, Salvinorin A

Salvia divinorum (Salvia) is an increasingly popular recreational drug among adolescents and young adults. Salvia, a member of the mint family, has been used for centuries in traditional Mexican religious and medicinal rituals (1,2). Chewing or smoking Salvia leaves produces depersonalization and auditory and visual hallucinations. Salvinorin A (SA), the primary psychoactive component of Salvia is a potent and highly selective agonist at κ opiate receptors (KOR) (3). SA has no activity at other receptor systems—including dopaminergic, serotonergic, or *N*-methyl-D-aspartate (NMDA) receptors—that are involved in the mechanism of other drugs that produce perceptual abnormalities (3).

Several lines of evidence point to the rising popularity of recreational Salvia and SA use in the US (4–8). National Survey on Drug Use and Health (2006) data suggest that the rates of SA use among adolescents (.6%) and young adults (1.7%) are greater than that of other common hallucinogenic drugs such as lysergic acid diethylamide (LSD), ketamine, phencyclidine, and dimethyltryptamine (9). These rates of SA exposure increased from 1.5% in 2006 to 3.7% by 2010. Salvia products are readily available both locally and via the Internet. Salvia and SA are not federally regulated in the US, although the Drug Enforcement Agency has listed them as "drugs of concern," and 13 states have begun to regulate their use.

The human literature on SA effects is limited by a preponderance of anecdotal reports (1,5–7,9–11). Salvia produces a rapid onset of transient mood alterations, dissociative symptoms, and psychotomimetic effects. The anecdotal literature is difficult to interpret because of the use of variable doses and routes of administration; the use of other drugs before, with, or after SA use; variable set and setting; and a lack of characterization of the subject samples.

Experimental data with Salvia/SA in humans include one study that developed a method to detect SA in biological fluids after smoking Salvia (12) and four on the effects of SA (13–16). Seibert (15) described subjective effects of oral, sublingual, and inhaled Salvia and SA administration in an open-label, uncontrolled study in 20 subjects. Mendelson *et al.* (14) reported no effects and undetectable SA blood levels with SA administered sublingually at doses up to 4 mg in eight subjects. The lack of effects in this study was likely due to low bioavailability of sublingual SA. Johnson *et al*. (13) administered 16 doses of inhaled SA in a fixed-order, ascending-dose, placebo-controlled, single-blind study of four subjects. Subjects experienced a rapid onset of transient hallucinogenic effects without any physiological changes. Finally, Addy (16) studied 30 healthy subjects who self-administered 1017 μg of inhaled SA on dried Salvia leaves or placebo (unenhanced dried Salvia leaves) in a partially blinded manner (blinded only to the first dose) (16). The latter two studies, although demonstrating the hallucinatory effects of SA, were also limited in the lack of randomization or objective outcomes, the use of fixed ascending order of doses (13), and the use of Salvia leaves as the vehicle and control (16).

SA has been reported to produce behavioral effects, cognitive impairments, and prolactin elevations in animals. Other KOR ago-

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nists have been reported to increase prolactin and cortisol levels in rodents (17,18) and humans (19) and to reduce resting electroencephalogram (EEG) power in rats (20). Resting EEG is potentially informative, because it is sensitive to drug-induced changes in consciousness (21–23) and is altered in psychosis (24,25). Finally, the pharmacokinetics of SA have not been studied in humans. SA is rapidly metabolized to Salvinorin B (SB), which is a much less potent KOR agonist (26). However, these outcomes have not been studied thus far in humans.

The behavioral, subjective, cognitive, endocrine, and psychophysiological effects of SA and its pharmacokinetic profile in humans were characterized in a controlled study to address the limitations and gaps in the existing literature.

Methods and Materials

This study was approved by the institutional review boards at Yale University and the Veterans Affairs Connecticut Healthcare System and the US Food and Drug Administration and was carried out in accordance with the Helsinki Declaration of 1975.

Study Design

This double blind, randomized, placebo controlled, counterbalanced, crossover, 3-day study was conducted at the Neurobiological Studies Unit (Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut).

Subjects. As detailed in Supplement 1, a rigorous screening was conducted to include medically and psychiatrically healthy subjects, between 18 and 55 years of age with previous exposure to Salvia. Because Salvia users characteristically use other drugs (27), subjects with exposure to other drugs were included so that the sample would be representative. History provided by subjects was corroborated with an outside informant nominated by the subject. Subjects were instructed to refrain from alcohol, illicit drugs, caffeine, and prescription drugs from a week before the first test day until study completion. Subjects were paid $200/test day for their participation.

General Procedure and Test Days. Subjects presented to the research unit, approximately 1 hour before the scheduled time of administration of drug, during which they underwent a urine toxicology exam and pregnancy test (in women), had an IV line placed, and underwent baseline ratings. In-study safety procedures were in place as described previously (28). Prospective safety assessments were performed the day after the first and last test days and 1 and 3 months after study completion.

Drugs. Subjects on each test day inhaled one of two doses of active SA or placebo (in an aluminum container) administered through a commercially available vaporizer (see Supplement 1 for details). The SA was obtained from the laboratory of Dr. Bruce M. Cohen, McLean Hospital, Belmont, Massachusetts, and stored in the research pharmacy at the Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut. On the morning of each test day, the SA dose was prepared in the designated container by the research pharmacists. Placebo consisted of the container devoid of any SA. Subjects and raters were blinded to the dose administered.

Outcome Measures. See Supplement 1 for greater detail.

Subjective and Behavioral Effects. Subjective feeling states such as "high," "anxious," "drowsy," "irritable," and "anxious" were measured with a self-reported visual analog scale (VAS). Psychotomimetic symptoms were measured with the Positive and Negative Syndrome Scale (PANSS) (29) and the Psychotomimetic States Inventory (PSI) (30). Perceptual alterations were measured with the Clinician Administered Dissociative Symptoms Scale (CADSS) (31) and the Hallucinogen Rating Scale (HRS) (32,33).

Cognitive Effects. Phonological processing, working memory, and attention were assessed with a simple cognitive battery comprising the Digits Forward and Backward and Letter Number Sequence tasks of the Wechsler Adult Intelligence Scale-Revised (34).

Neuroendocrine Effects. Plasma cortisol and prolactin were assayed at various time points before and after SA inhalation. Levels were analyzed in duplicate by the Yale Center for Clinical Investigation, Yale University, New Haven, Connecticut.

SA and SB Levels. Both SA and SB levels were analyzed by Dr. E. Thomas Everhart at the Drug Dependence Research Center (Langley Porter Psychiatric Institute, University of California) with a slightly modified liquid-chromatographic-atmospheric pressure chemical ionization-tandem mass spectrometric method (14) (see Supplement 1 for details). The limits of quantitation were .5 ng/mL for both SA and SB in plasma.

Psychophysiological Effects. Three minutes of resting state EEG was obtained as subjects sat still with their eyes closed immediately after SA inhalation.

Data Analysis

Initially, data were examined descriptively with means, SDs, and graphs. Each outcome was tested for normality with Kolmogorov-Smirnov test statistics and normal probability plots. All PANSS, PSI, HRS and cognitive battery outcomes were approximately normally distributed. These outcomes were analyzed with linear mixed models, which included SA dose (placebo, low [8 mg], and high [12 mg]) and time (pre- vs. postinhalation) as within-subjects explanatory factors and random subject effects. The best-fitting variance-covariance structure was chosen on the basis of information criteria. Significant interactions between dose and time were interpreted by appropriate post hoc tests. Similar models were used to compare physiological measures and serum SA and hormone (log) levels across time. All CADSS and VAS outcomes were highly skewed. Thus, these non-normal outcomes were analyzed with the nonparametric approach for repeated measures data, in which data are ranked and then fitted with a mixed-effects model with an unstructured variance-covariance matrix and *p* values are adjusted for analysis of variance-type statistics (ATS) (35). In these models, SA (placebo, low dose, high dose, and time [pre- vs. posttreatment]) were included as within-subjects explanatory factors. The EEG power frequencies were compared with linear mixed models with dose and electrode (Cz, Pz, Oz) as within-subjects factors. All data were analyzed with SAS (version 9.2; SAS, Cary, North Carolina).

Results

Subjects were young (23.8 ± 3.2 years), predominantly male (90%), with 15.3 (± 1.2) years of education, intelligence quotient scores of 117.2 (± 7.1), and low (2.8 ± 2.8) psychosis proneness scores on the Schizotypal Personality Questionnaire (Table S2 in Supplement 1). Nine subjects completed all 3 test days, and one dropped out after his second test day. All 10 subjects were included in the analyses. None of the subjects met criteria for alcohol or substance dependence. All subjects had previous exposure to SA and other illicit substances (Table S3 in Supplement 1). For parsimony, only positive results are reported in detail here.

Subjective Reports

The following are quotations from subjects describing SA-induced changes:

> Somaesthetic changes: "I felt a cold prickling feeling on my legs," "...tingling in my fingers," "...felt a pattern sweep over me like a wave...I felt as well as saw the waves..."