Acute Physiologic and Chronic Histologic Changes in Rats and Mice Exposed to the Unique Hallucinogen Salvinorin A.

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Abstract

Background. Salvinorin A is a unique hallucinogen that is seeing increased use in humans. It is not currently a controlled substance and is used as a legal alternative to controlled substances. Usually smoked or buccally absorbed by chewing, doses of approximately 200mcg can produce profound hallucinogenic effects of short duration. The mechanism of action of salvinorin A is at the k-opioid recpetor. Little data is available on the medical effects of this substance so animal studies were undertaken to explore the acute toxic effects of this substance in rats and the chronic effects in mice

Methods. Rats were anesthetized and administered salvinorin A at 1600mcg/kg or vehicle. Recordings were made of galvanic skin response, EKG, temperature, and pulse pressure for 100 minutes.

Mice were chronically exposed to vehicle or 400, 800, 1600, 3200, or 6400 mcg/kg of salvinorin A for two weeks. After exposure the animals were sacrificed and brain, heart, kidney, bone marrow, blood and spleen were removed, fixed, sectioned, stained and examined by light microscopy.

Results. No effects were seen on cardiac conduction, temperature, or galvanic skin response. A non-significant rise was seen in pulse pressure.

Histologic studies of spleen, blood, brain, liver, kidney, and bone marrow did not find any significant histologic changes at any of the doses examined

Conclusion. These data suggests that the toxicity of salvinorin A is relatively low, even at doses many times that of what humans are exposed to. However, further

studies should be done on blood pressure effects. The psychological impact of this potent hallucinogen should also be investigated.

Introduction

Throughout history humans have been in search of mind-altering substances (1) and have generated an awe-inspiring list of mood and mind altering substances. Some of these are fairly benign (eg., marijuana) while others have high toxic potential (eg., methamphetamine) and most falling in between depending on dosage and route of administration (eg., alcohol).

The search for psychogenic substances is continuing even today, and while some potential users of recreational substances are turning to synthetic substances (eg: ecstasy) others are searching the ethnobotanical histories of other cultures to uncover previously little known substances used for indigenous magic, divination, or religious ceremonies. One such substance is Salvia divinorum (aka: diviner's mint, Mexican sage). Salvia has been used by native tribes in Mexico to achieve religious mystical states for the divination of health and spiritual ailments. It is now being used in the United States as an alternative to illegal hallucinogens such as marijuana. Currently, there are no specific legal prohibitions to the sale or possession of Salvia in the United States. Salvia can be purchased from companies listed in the Internet. We have contacted one Internet source of Salvia who reported that 30% of their retail sales and 20% of the web site visits are for Salvia divinorum, even though it represents only 1% of

their product line. A report out of Switzerland indicated that Salvia is becoming a new recreational drug (2) and a number of newspaper articles and television programs have recently appeared discussing Salvia divinorum.

We have performed a search of the National Library of Medicine using the PubMed search engine and have found 7 articles that matched the key words Salvia divinorum. Nearly all of these are ethnologic studies and self-reports of the effects of Salvia. Information on the medical and biological aspects of Salvia is very sparse.

A review of the literature points to several clear facts. The chief active ingredient of Salvia divinorum appears to be salvinorin A, although other minor active ingredients may be present (salvinorin B and C). Salvinorin A ($C_{23}H_{28}O_8$, mw 432) is a unique diterpene hallucinogen with an unknown mechanism of action. Salvinorin A forms slender off-white crystals when extracted from the plant. The crystals are insoluble in water but soluble in many organic solvents. Details of salvinorin A chemistry can be found in Koreeda et al. (3), Ortega et al. (4) & Valdes et al. (5). Studies by Siebert (6) using NovaScreen™ receptor site screening did not reveal activity at any of the examined sites, including forskolin, PCP, serotonin, GABA and benzodiazepine receptors, among others. A recent study by Roth et al. (7) has inicated that the mechanism of action for salvinorin A is at the kappa opiate receptor site.

Salvia is typically consumed by smoking a quantity of dried leaves, although buccal absorption by chewing dried leaves or a tincture is also used. It

has been estimated that the dose of salvinorin A is in the 200-500mcg range. The onset of action is relatively rapid, on the order of 30 seconds for smoking, to 5-10 minutes for buccal absorption. The duration of action is relatively short, 20-30 minutes. Pharmacokinetic studies of salvinorin A are not available. The effects of Salvia are varied but include frank visual hallucinations and auditory distortions. Siebert (6) list common themes to a Salvia experience including: becoming objects, visions of two-dimensional surfaces, revisiting the past, loss of body identity, sensations of motion, laughter, and overlapping realities. After-effects of the experience seem to consist of minor somnolence and occasional "hangover". If high dosages are consumed stuporous states have been reported, prompting users of the drug to recommend "sitters" during the experience to prevent accidental injury.

Animal studies are alluded to in the literature. Valdes et al (5) reported that a single injected dose of 1g/kg did not have any apparent toxic effects in mice, although histology or other objective evaluation was not provided. Behavioral effects on animals included sedation and reduced open-field activity, although data were not presented.

A search of the Internet revealed in excess of 100 relevant hits for the term "Salvia divinorum" using the "Yahoo" search engine. Those, combined with anecdotal reports and the recent report from Switzerland, newspaper and television reports, have prompted us to investigate Salvia's acute physiologic effects and chronic toxicity. Our intent was to provide a description of the drug

effects when first administered and to determine if long term administration might be problematic. We hope that these data might be useful to the medical community to aid in determining what adverse effects can and cannot be assigned to Salvia use. Animals were used as the subjects because Institutional Review Board approval for human subjects is unlikely for substances where no published toxicology data is available.

Methods

The use of animals was approved by the IACUC of the University of Nebraska at Kearney.

Extraction and preparation of salvinorin A

Dried Salvia divinorum leaves were purchased from Pureland Ethnobotanicals (Racine, WI; ethnobotanicals.com), a source considered to be a reliable supplier of the herb. A sample of crushed Salvia divinorum leaves (~75.0 g) was added to a 2L flask containing 1.5 L chloroform. The resulting greenish-brown slurry was refluxed on a hot plate for 30 minutes with stirring. The resulting mass was hot filtered through a paper filter and washed with hot chloroform. The greenish-brown filtrate was then evaporated on a rotary evaporator at ambient temperature. The dark green residue (~8 g) was then subjected to flash chromatography on silica gel (8:2 hexane : ethyl acetate) to provide the desired product as a yellowish orange oil. Co-elution of a minor orange-colored product necessitated further purification. Trituration of the yellow

oil with methanol provided the crude product as an off-white solid. Redissolution in 8:2 hexane : ethyl acetate followed by addition of methanol provided the purified product as off-white needles (~0.2 g). Verification of the structure of the product was determined by two-dimensional NMR techniques (NOESY, COSY, long-range COSY): mp 237-239 (uncorrected), lit. mp 238-240, H-NMR ($CDCl_3$, in ∂): 1.10 (s, 3H); 1.43 (s, 3H); 1.60 (m, 2H); 1.77 (m, 2H); 2.05 (dd, J = 2.4, 11.7Hz, 1H); 2.19 (s, 3H); 2.28 (m, 3H); 2.49 (dd, J = 5.2, 13.5, 2H); 2.73 (dd, J = 6, 10.6, 1H); 3.74 (s, 3H); 5.14 (t, J = 10 Hz, 1H); 5.51 (dd, J = 6, 12 Hz, 1H); 6.38 (m, 1H); 7.41 (m, 2H). C-NMR ($CDCl_3$, in ∂): 15.3, 16.0, 18.2, 20.5, 30.9, 35.5, 38.1, 42.1, 43.3, 51.3, 51.9, 53.5, 63.9, 72.0, 75.0, 108.4, 125.3, 139.5, 143.7, 169.9, 171.2, 171.6, 202.0. The physical data for this compound agreed with experimental data given elsewhere (4, 5).

Electrophysiologic responses

Seventeen male and female Long-Evans rats from 2 to 6 months of age were weighed and anesthetized with I. P. chloral hydrate (450mg/kg for females; 600 mg/kg for males). The thorax was shaved and electrodes placed for EKG recording (lead II). An oral temperature probe was placed. An optical pulse plethysmograph was placed on the right hindpaw and one galvanic skin response electrode was placed on each forepaw. Data was equipped with the use of an MP30 bio-amplifier and recorded on a Macintosh computer. A baseline was recorded and the animals were injected I.P. with either salvinorin A 1600mcg/kg

or an equal volume of vehicle. Salvinorin A was prepared for injection by dissolving 1mg of the substance in 10ul of DMSO and adding 10ml of water to form an extremely fine suspension suitable for injection. Recordings were made every 10 minutes for 100 minutes. After recording the animals were placed in their home cage to recover.

Chronic exposure to salvinorin A

Male and female Swiss-Webster mice, aged 4-6 months, housed under laboratory conditions were separated into groups of 6 by gender. The groups then received salvinorin A by IP injection everyday for 14 days. Doses were 400, 800, 1600, 3200, or 6400mcg/kg or a volume of vehicle equivalent to that of the 1600mcg/kg group to serve as control. This produced a 2x4 design based on gender (2) and dose (4). A total of 114 animals were used for this portion of the study. Salvinorin A was prepared for injection by dissolving 1mg of salvinorin A in 10ul of DMSO and then adding 10ml of water to produce a fine suspension suitable for injection.

After 14 days of exposure blood smears were prepared by nicking the tail vein of each animal. The animals were then killed with an overdose of chloroform. The heads were quickly opened by incising through the skull and the chest and abdomen opened before being placed in 3% phosphate buffered formalin where they fixed for at least 1 week before histology was performed.

Formalin fixed tissue samples of liver, spleen, kidney, bone marrow and brain were obtained. The samples were paraffin embedded, sectioned at 5 um, processed routinely and stained with hemotoxalin and eosin. Light microscopic examination was then carried out.

Results

Electrophysiologic Findings.

Electrocardiogram. Injections of salvinorin A at the dose of 1600mcg/Kg had no statistically significant effect on rat heart rate when compared to control animals. In addition, there appeared to be no discernable effect on cardiac conduction. There was no difference in the PR interval or the QT interval between control and Salvia treated animals.

Temperature, Galvanic Skin Response and Pulse Pressure. Treatment with salvinorin A did not produce a change in body temperature or galvanic skin response (GSR) that was different from that seen in control animals. Pulse pressure did appear to increase with salvinorin A exposure twenty and fourty minutes after exposure, however, this increase was not statistically significant (Figure 1).

Histologic Findings

No histologic differences were noted between the controls and animals treated at any of the salvinorin A doses for either sex in the liver, spleen, kidney bone marrow or brain tissue.

Discussion

A number of hallucinogens in use today have significant and potentially harmful effects. The synthetic amphetamine derivatives (DOM, MDA, DMA, MDE, TMA, MDMA) in particular can produce a variety of cardiovascular effects, potentially leading to ischemic or arrhythmic events. Additionally, many are suspected of being neurotoxic leading to Parkinsonian like disorders. On the other hand, some hallucinogens, in particular lysergic acid diethylamide (LSD) have fairly minor physiologic activity, despite their profound psychological effects.

Based on the evidence produced by these experiments salvinorin A appears to have little physiologic effects. Temperature, sympathetic nervous system activity (as measured by GSR), and cardiac effects appear to be minimal. The non-significant increase in pulse pressure is intriguing, suggesting that in some situations salvinorin might increase blood pressure. However, this is only suggestive data and the studies should be conducted to investigate this effect specifically. These general findings seem to confirm the experiences reported in the literature. There are no reports of cardiovascular events, nor of any symptoms that would suggest autonomic nervous system effect. At times some users report slight changes in body temperature, however, that may be related to

the hallucinogenic effects. One of the investigators in this study (WB) has followed the discussions of an informal e-mail based group that investigates the halluncinogenic effects of Salvia, and remarkably few adverse effects are reported.

Long term administration to mice also seemed to confirm the fairly benign effects of salvinorin A. Our study did not find any histologic changes at doses well in excess of those that humans could be expected to be exposed to for a length of time that would seem unlikely in humans.

Our report here should not be interpreted as suggesting that salvinorin A is a completely benign substance. Our measures were limited and were made on species with nervous systems not especially prone to the effects of hallucinogens. Nor were our studies sensitive to the effects of psychological and physiologic interaction. For example, chest pain may not be directly produced by salvinorin, yet the hallucinogenic effects may lead to panic with the production of chest pain as a secondary event. We would also like to emphasize that individuals predisposed to psychopathology may experience “psychotic breaks” if they use any hallucinogen. Nor have there been adequate studies done to determine if salvinorin A is a teratogen, certainly pregnant women should avoid this substance.

In conclusion, the acute administration of salvinorin A had little discernable effect on the cardiovascular function of rats, with the exception of potential increases in pulse pressure. Its long term administration in mice failed to

produce detectable histologic changes. This suggests that salvinorin A, while a potent hallucinogen, has relatively low toxicity.

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Figures

Figure 1

Effect of salvinorin A (1600mcg/kg) exposure on rats. Temperature is in degrees centigrade, GSR and PPW are in millivolts.