Autonomic, Neuroendocrine, and Immunological Effects of Ayahuasca

A Comparative Study With D-Amphetamine

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Abstract: Ayahuasca is an Amazonian psychotropic plant tea combining the 5-HT_{2A} agonist *N*,*N*-dimethyltryptamine (DMT) and monoamine oxidase–inhibiting β-carboline alkaloids that render DMT orally active. The tea, obtained from *Banisteriopsis caapi* and *Psychotria viridis*, has traditionally been used for religious, ritual, and medicinal purposes by the indigenous peoples of the region. More recently, the syncretistic religious use of ayahuasca has expanded to the United States and Europe. Here we conducted a double-blind randomized crossover clinical trial to investigate the physiological impact of ayahuasca in terms of autonomic, neuroendocrine, and immunomodulatory effects. An oral dose of encapsulated freeze-dried ayahuasca (1.0 mg DMT/kg body weight) was compared versus a placebo and versus a positive control (20 mg D-amphetamine) in a group of 10 healthy volunteers. Ayahuasca led to measurable DMT plasma levels and distinct subjective and neurophysiological effects that were absent after amphetamine. Both drugs increased pupillary diameter, with ayahuasca showing milder effects. Prolactin levels were significantly increased by ayahuasca but not by amphetamine, and cortisol was increased by both, with ayahuasca leading to the higher peak values. Ayahuasca and amphetamine induced similar time-dependent modifications in lymphocyte subpopulations. Percent CD4 and CD3 were decreased, whereas natural killer cells were increased. Maximum changes occurred around 2 hours, returning to baseline levels at 24 hours. In conclusion, ayahuasca displayed moderate sympathomimetic effects, significant neuroendocrine stimulation, and a time-dependent modulatory effect on cell-mediated immunity. Future studies on the health impact of long-term ayahuasca consumption should consider the assessment of immunological status in regular users.

Key Words: ayahuasca, autonomic, neuroendocrine, immunity

(*J Clin Psychopharmacol* 2011;31: 00–00)

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Received December 17, 2010; accepted after revision June 13, 2011.

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This work was supported by grant SAF 2002-02746 from the Spanish Ministry of Education and Science and a private donation by Richard Wolfe.

ISSN: 0271-0749
DOI: 10.1097/JCP.0b013e31823607f6

Ayahuasca is a psychoactive beverage consumed throughout the Amazon Basin as the aqueous infusion of *Banisteriopsis caapi* and *Psychotria viridis*, 2 plants endemic to the region.[1,2] The tea is a central element of Amazonian shamanism, being used for magico-religious, ceremonial, and medicinal purposes.[2] Chemical analyses have shown that *P. viridis* contains the orally labile serotonergic agonist and monoamine oxidase (MAO) substrate *N*,*N*-dimethyltryptamine (DMT), whereas *B. caapi* contains several alkaloids with β-carboline structure (harmine, harmaline, and tetrahydroharmine) showing MAO inhibiting properties.[3] The β-carbolines present in ayahuasca reversibly block visceral MAO-A,[4,5] allowing the access of DMT to systemic circulation and the central nervous system (CNS). At the molecular level, DMT binds at 5-HT_{2A}, 5-HT_{1A}, and 5-HT_{2C} receptor sites,[6–8] eliciting psychedelic effects in humans.[9,10]

In recent years, ayahuasca use has spread from the indigenous to the general population not only in the Amazon but also to countries around the world. A relevant factor in this expansion is the beverage's use in syncretic religions. Several religious groups originating from Brazil and using ayahuasca as a sacrament in a ceremonial context have expanded their activities to Europe and North America. These groups typically ingest ayahuasca over extended periods on a bimonthly basis.[11,12] Based on the traditional uses of ayahuasca and both anecdotal and empirical data on potential health benefits derived from ayahuasca use, some authors have proposed its therapeutic use, especially in the field of drug addiction.[13] Two studies among Brazilian regular users of ayahuasca have found a decrease in illicit drug consumption after initiation of regular ayahuasca use.[14,15] However, despite its potential health benefits, data on the impact of ayahuasca on human physiology are still limited and warrant further investigation.

Previous clinical research has shown that ayahuasca displays distinct physiological and psychotropic effects. At doses equivalent to 0.6 to 0.8 mg DMT/kg body weight, ayahuasca leads to moderate increases in cardiovascular measures, with only diastolic blood pressure reaching statistical significance.[10] The effects of the drug on the CNS have been demonstrated by subjective effects measures and neurophysiological recordings. The analysis of self-report questionnaires has shown significant increases in scales measuring psychostimulant-like subjective activation and modifications of perception and thought processes.[10,16] Electroencephalographic (EEG) effects include an increase in relative power in the faster β band.[17] Central nervous system effects after ayahuasca administration have also been demonstrated by means of neuroimaging techniques (single-photon emission computed tomography)[18] and sleep recordings.[19] The time course of subjective effects and EEG measures runs parallel to DMT concentrations in blood.[3] Increases in diastolic blood pressure and normetanephrine excretion[10] suggest that ayahuasca exerts general sympathomimetic effects, together with

more specific serotonergic effects such as the aforementioned increases in EEG relative β[17] and rapid eye movement sleep suppression.[19]

To date, no controlled study has assessed the impact of acute ayahuasca on neuroendocrine measures and the immune function. In a noncontrolled study, researchers found increases in cortisol levels above preadministration values following a single ayahuasca dose.[20] Cortisol release is known to have an impact on cell immunity, leading to lymphocyte redistribution.[21] Lymphocytes are the main cellular components of the immune system. Lymphocyte deficiencies may predispose to infectious diseases. Some viral infections, such as that caused by the human immunodeficiency virus, may cause sustained and marked decreases in some T-cell subpopulations (CD4 lymphopenia). Substance use, such as alcohol intake and cigarette smoking, has shown detrimental effects on lymphocyte subpopulations.[22,23] Regarding psychedelics, a recent study found decreases in CD8 lymphocytes following the administration of 4-iodo-2, 5-dimethoxyphenyl-isopropylamine (DOI, a 5-HT_{2A} receptor agonist) to mice. This effect was antagonized by the 5-HT_{2A} antagonist ketanserin.[24]

In view of the expanding use of ayahuasca worldwide, in the present study we aimed to explore (*a*) the physiological impact of acute ayahuasca administration in terms of autonomic and neuroendocrine effects and (*b*) the potential effects of ayahuasca on cell-mediated immunity. As described below, autonomic variables, hormone levels, and distribution of lymphocyte subpopulations were evaluated. Cortisol was assessed for its direct role in lymphocyte regulation,[21] and prolactin and growth hormone (GH) were selected as measures of serotonergic stimulation.[25,26] Cell-mediated immunity changes were analyzed assessing the most relevant lymphocyte subpopulations: T lymphocytes (CD3, CD4, CD8), natural killer (NK) cells, and B lymphocytes (CD19). In addition, to verify alkaloid absorption and CNS effects, we also measured DMT plasma levels, subjective effects, and relative EEG β power. To gain greater insight into the specificity or generality of ayahuasca effects, D-amphetamine, a standard sympathomimetic drug, was used as an active comparator.

MATERIALS AND METHODS

Volunteers

Ten young healthy male volunteers were recruited. Mean age was 29.0 years (range, 20–38 years); mean weight was 67.0 kg (range, 60–85 kg); and mean height was 1.77 m (range, 1.69–1.96 m). Volunteers underwent a structured psychiatric interview (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). Exclusion criteria included presence or history of Axis I disorders and alcohol or other substance dependence. Eligibility criteria included prior use of psychedelics on at least 10 occasions without sequelae derived thereof, that is, psychedelic-related disorders as described in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. A medical examination and laboratory tests were performed before study initiation to rule out any medical condition, allergies, and intolerances.

Participants had used psychedelics from 10 to 100 times. The most commonly used psychedelics were psilocybian mushrooms (10/10) and lysergic acid diethylamide (LSD) (9/10). Less commonly used were ketamine (5/10), peyote (4/10), and mescaline (1/10). None of the participants had used ayahuasca. Besides psychedelics, volunteers had consumed cannabis (10/10), cocaine (10/10), 3,4-methylenedioxymethamphetamine (MDMA) (8/10), and amphetamine (9/10). They reported moderate consumption of alcohol (7 drinks per week), cigarettes (fewer than 10 per day), and caffeinated drinks (<3 per day). Volunteers were in good health, confirmed by medical history, laboratory tests, and electrocardiogram. Prestudy examinations also included drug screening and serological testing (for hepatitis B and C and human immunodeficiency virus). The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of ayahuasca, the general psychological effects of psychedelics, and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drugs

The administered drugs were a placebo (lactose), 20 mg D-amphetamine, and a freeze-dried encapsulated formulation of ayahuasca equivalent to 1 mg DMT/kg body weight. The freeze-dried material was obtained from a Brazilian batch of ayahuasca and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg tetrahydroharmine per gram. The ayahuasca dose administered was chosen based on an earlier work in which it had been proven to elicit full-blown psychotropic effects.[16] The administration of the placebo and the 2 active treatments in capsules allowed for the adequate masking of drug taste.

Study Design

The study was conducted according to a randomized, double-blind, placebo-controlled, crossover design and involved the participation on 3 experimental sessions at least 1 week apart. Volunteers were requested to abstain from any medication or illicit drug use in the 2 weeks before the experimental sessions and until study completion. Volunteers also abstained from alcohol, tobacco, and caffeinated drinks in the 24 hours before each experimental day. Urinalysis for illicit drug use was performed for each experimental session. On each experimental day, volunteers had a light breakfast before 10:00 AM, and at noon, they received capsules containing 1 of the 3 treatments. During measurements, the volunteers remained seated in a comfortable reclining chair in a quiet dimly lit room. All volunteers remained overnight in the laboratory and were discharged at noon of the following day.

Study Methods

Subjective Effects Measures

Subjective effects were measured by means of 2 self-report questionnaires: the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI).

The HRS[9] measures psychedelic-induced subjective effects and includes 6 scales: *somaesthesia*, reflecting somatic effects; *affect*, sensitive to emotional and affective responses; *volition*, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; *cognition*, describing modifications in thought processes or content; *perception*, measuring visual, auditory, gustatory, and olfactory experiences; and finally *intensity*, which reflects the strength of the overall experience. In the present study, a Spanish adaptation of the questionnaire was used.[27] The range of scores for all HRS scales is 0 to 4.

The short version of the ARCI[28] consists of 5 scales or groups: MBG, morphine-benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD scale, measuring

somatic-dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency; and the A scale, an empirically derived scale measuring amphetamine-like effects. The range of scores is 0 to 16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. The questionnaire had been translated into Spanish and validated by Lamas and coworkers.[29] Volunteers answered the ARCI immediately before drug administration and 4 hours after drug intake, whereas the HRS was answered only at 4 hours after administration.

EEG Measures

Spontaneous brain electrical activity was recorded, preprocessed, and quantified following standard procedures as previously described.[17] In brief, recordings were obtained at 19 scalp locations according to the international 10/20 system by means of a Neuroscan SYNAMPS amplifier. Three-minute EEGs with eyes closed were obtained at 0 (baseline) and 30 minutes and at 1, 1.5, 2, and 2.5 hours after administration. The EEG signal was recorded using high-pass and low-pass filters of 0.3 and 30 Hz, respectively, and digitized online with a sampling frequency of 100 Hz. Following ocular artifact rejection and correction steps, spectral analysis was performed using the fast Fourier transform. The target variable: relative power (expressed as percentage) in the β (13–30 Hz) frequency band was calculated from the spectral density curves at each electrode and time point. An average of relative β power in all 19 leads was used in the subsequent statistical analysis.

Autonomic Measures

Body temperature (°C) was measured by means of a mercury thermometer placed in the participant's armpit. Measurements were conducted at −15 (baseline 1), 0 (baseline 2), and 30 minutes and at 1, 1.5, 2, 4.5, 6, 8, and 10 hours after administration.

Pupillary diameter and pupillary light reflex (PLR) were determined using a Compact Integrated Pupillometer (AMTech GmbH, Weinheim, Germany), tested in darkness after a 5-minute dark adaptation period. Participants were instructed to fix their gaze on a target point located on the wall of the examination room at a distance of about 3 m to prevent pupillary near response or accommodation adjustments. Pupillary light reflexes were elicited by standardized light stimuli (whole-field stimulation) from a light-emitting diode with a duration of 200 milliseconds and using 3 increasing intensities (2.35×10^3, 4.7×10^3, and 9.4×10^3 cd/m^2), stimulus intensity being measured at the source. Changes in pupillary diameter were recorded for 2 seconds with a sampling rate of 250 Hz and stored on a personal computer.[30]

Three consecutive measures were made for each level of intensity, and the mean value was obtained. The different intensities were administered at 2-minute intervals. The target variables were as follows: initial pupillary diameter (in millimeters) and latency (in milliseconds) and amplitude (in millimeters) of the miotic light reflex response. The initial pupillary diameter is the diameter obtained just before light stimulation. The latency period is the interval between the light stimulation and the onset of the pupil contraction. The light reflex amplitude was determined as the difference between the initial and the minimum pupillary diameter after light stimulation.[31] The initial pupillary diameter reflects the sympathetic/parasympathetic balance, whereas latency and light reflex amplitude are parameters reflecting parasympathetic pupillary modulation.[31,32] Measurements were conducted at 0 (baseline) and 30 minutes and at 1, 2, 4, 6, 8, 10, and 24 hours after administration.

Respiration rate (in breaths per minute) was measured by means of a respiratory band placed around the participant's chest. The respiratory signal was digitized and recorded on a computer and later analyzed offline. The number of respiratory events in 1 minute was counted at each recording time point. Measurements were conducted at −15 (baseline 1), 0 (baseline 2), and 30 minutes and at 1, 1.5, 2, and 2.5 hours after administration.

Neuroendocrine Measures

Blood samples (3 mL, plain tubes without clot activator) were drawn at −40 (baseline 1), −10 (baseline 2), and 30 minutes and at 1, 1.5, 2, 4.5, and 6 hours after administration and were allowed to stand at room temperature. Serum was separated by centrifugation and aliquots stored for the analysis of GH, prolactin, and cortisol.

Serum GH and prolactin concentrations were determined by a chemiluminescence immunoassay system (Immulite 2000; Diagnostic Products Corp, EURO/Diagnostic Products Corporation, Llanberis, UK). The GH immunoassay, with a sensitivity of 0.06 mIU/L, uses the WHO first IRP 80/505 and shows intra-assay and interassay coefficients of variation (CVs) of 5.3% to 6.1% and 5.7% to 6.5%, respectively. The prolactin immunoassay uses the third IS 84/500, with an analytical sensitivity of 3.4 mIU/L and intra-assay and total CV between 2.2% to 2.3% and 6.9% to 7.9%, respectively. Serum cortisol concentrations were measured by electrochemiluminescent immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics GmbH Manheim, Germany) with functional sensitivity of less than 8 nmol/L and intra-assay and total CVs of 1.7% and 2.8%, respectively, for mean human serum concentrations between 129 and 717 nmol/L.

Obtained values were transformed to nanograms per milliliter (prolactin and GH) and micrograms per deciliter (cortisol).

Lymphocyte Subpopulations

Blood samples (3 mL, heparin tubes) were drawn at baseline and at 1.5, 2, 4.5, and 24 hours after administration and were subjected to lymphocyte immunophenotyping. The following lymphocyte subpopulations were quantified: CD8 T, CD4 T, CD3 T, CD19 B, and NK cells.

For lymphocyte immunophenotyping, blood samples were stained with the Lymphogram (Cytognos, Salamanca, Spain) reagent kit; each tube contains 5 different murine MoAbs with 3 fluorochromes: CD8 and CD19 with fluorescein isothiocyanate, CD3 and CD56 with phycoerythrin. CD4 were labeled in tandem with PE and cyanate 5. The procedure has been detailed elsewhere.[33] Lymphocyte subpopulations were expressed as percentage of all blood cells.

DMT Plasma Levels

Blood samples (10 mL, EDTA tubes) were drawn at −10 (baseline) and 30 minutes and at 1, 1.5, 2, 2.5, 4.5, 6, and 10 hours after administration for analysis of DMT. Samples were centrifuged at 2000 revolutions per minute for 10 minutes at 4°C, and plasma was immediately frozen at −20°C. Frozen plasma samples were stored at −80°C until analysis. *N,N*-dimethyltryptamine was quantified by the method described by McIlhenny and coworkers,[34] which uses high-pressure liquid chromatography with electrospray ionization and tandem mass spectrometry. The method was adapted to quantify DMT in a plasma matrix using a protein precipitation/dilution protocol.

Protein precipitation 96-well plates (Thermo Scientific, Waltham, Mass) were used to prepare the samples. Analyses were conducted using a Thermo Open Autosampler and a Thermo Accela pumping system interfaced to a Thermo Velos linear ion trap-ion trap system with a heated electrospray ionization probe and operated in the positive ion mode as described.[35] The observed maximum concentration in plasma (C_{max}) and the time to reach this concentration (t_{max}) were determined for each individual.

Statistical Analyses

Subjective Effect measures

Before statistical analysis, ARCI scores were transformed to differences from preadministration values. The transformed ARCI scores and the raw HRS scores were analyzed using a 1-way repeated-measures analysis of variance (ANOVA) with drug (placebo, ayahuasca, amphetamine) as factor. When a significant effect was observed, pairwise comparisons were performed by means of Student *t* test.

EEG, Autonomic, Neuroendocrine, and Lymphocyte Measures

Preadministration values were subtracted from postadministration measures. Subsequently, we calculated peak variations (the maximum absolute change from baseline values). The obtained values were analyzed using a 1-way repeated-measures ANOVA with drug (placebo, ayahuasca, amphetamine) as factor. When a significant effect was observed, pairwise comparisons were performed by means of Student *t* test. In addition, a 2-way repeated-measures ANOVA was conducted, with drug (placebo, ayahuasca, amphetamine) and time point as factors to study the time course of effects. When this ANOVA yielded a significant drug or drug-by-time interaction, individual repeated-measures ANOVAs with drug as factor were performed at each postadministration time point followed by pairwise comparisons using Student *t* test.

DMT Plasma Levels

Descriptive statistics were used to report the characteristics of the time course of plasma DMT concentration. Maximum concentration (C_{max}) and time taken to reach the maximum concentration (t_{max}) were calculated and are reported as mean (SD) and as median and range, respectively.

In all tests performed, differences were considered statistically significant for $P < 0.05$. However, given the exploratory nature of the present study with regard to autonomic, neuroendocrine, and lymphocyte measures, pairwise comparisons between treatments are also reported in those cases where the main ANOVA yielded a $P < 0.1$.

RESULTS

Subjective Effects

Subjective effects results are shown in Table 1.

Compared with placebo, the administration of ayahuasca led to significant increases in all scales of the HRS and in the A, MBG, and LSD of the ARCI. On the other hand, differences from placebo were found for amphetamine in the affect, cognition, and intensity scales of the HRS and in the amphetamine scale of the ARCI. When the 2 active treatments were compared, ayahuasca led to significantly higher scores in the perception, cognition, volition, and intensity scales of the HRS. Regarding the ARCI, the comparison between active treatments found statistically significant differences in the BG scale, with amphetamine leading to increases and ayahuasca to decreases; in the PCAG scale where amphetamine showed decreases and ayahuasca increases; and finally in the LSD scale. Here both treatments led to increases that were much larger after ayahuasca.

EEG Effects

Treatment effects on relative β power are shown in Table 2 and Figure 1. As shown therein, ayahuasca induced significant increases in this variable, which was not modified by

TABLE 1. Subjective Effects Induced by Placebo, D-Amphetamine 20 mg, and Ayahuasca 1 mg DMT/kg

					Pairwise Comparisons		
	Placebo	D-Amphetamine	Ayahuasca	GLM	PLA:AMP	PLA:AYA	AYA:AMP
HRS							
Somaesthesia	0.07 (0.22)	0.5 (0.60)	1.23 (0.70)	<0.01	NS	*	NS
Affect	0.28 (0.09)	0.79 (0.52)	1.36 (0.69)	<0.01	†	*	NS
Perception	0.03 (0.09)	0.24 (0.45)	1.46 (0.97)	<0.001	NS	*	*
Cognition	0.01 (0.03)	0.49 (0.062)	1.58 (1.16)	<0.01	†	*	†
Volition	0.87 (0.74)	0.86 (0.43)	1.84 (0.75)	<0.01	NS	†	*
Intensity	0.00 (0.00)	1.10 (0.85)	2.23 (1.10)	<0.001	*	‡	†
ARCI							
A	0.30 (0.67)	2.20 (2.25)	3.30 (2.67)	<0.01	†	*	NS
BG	0.70 (0.82)	2.90 (2.85)	−0.70 (3.34)	<0.01	NS	NS	*
MBG	−0.10 (0.99)	2.20 (3.26)	3.10 (4.48)	0.069	NS	†	NS
PCAG	−1.60 (3.50)	−3.9 (3.38)	0.30 (5.38)	0.039	NS	NS	†
LSD scale	0.40 (1.65)	1.10 (1.52)	4.20 (2.25)	<0.01	NS	*	*

Values are mean (SD) of the scores obtained for the HRS and ARCI questionnaires subscales (n = 10) and results of the statistical analysis performed.

A indicates Amphetamine; AMP, D-amphetamine; AYA, ayahuasca; BG, benzedrine group; GLM, general linear model; MBG, morphine-benzedrine group; NS, not statistically significant; PCAG, pentobarbital-chlorpromazine-alcohol group; PLA, placebo.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.001$.

amphetamine or placebo. The analysis of the time course of effects showed that ayahuasca-induced increases were significant, relative to placebo at 1.5 and 2 hours after dosing. In addition, ayahuasca was different from amphetamine at 1.5 hours.

Autonomic Measures

Autonomic effects results are shown in Table 2 and Figure 1.

Body Temperature

After placebo administration, body temperature showed a steady increase throughout the day. After amphetamine and ayahuasca administration, however, a biphasic pattern was observed; an initial decrease between 0 and 1 hour was followed by a gradual increase thereafter. This increase was larger for amphetamine. The overall analysis did not find any significant modification in peak values after either of the active treatments. However, the analysis of the time course of effects showed a significant decrease for ayahuasca as compared with placebo at 30 minutes after dosing. A significant increase relative to placebo was observed for amphetamine at 2 hours after administration.

Pupillometry

As shown in Figure 1, mean pupillary diameter values before light stimulation were larger for amphetamine and ayahuasca than for placebo. The overall statistical analysis showed a trend increase in peak values. Pairwise comparisons showed significant increases for amphetamine and no overall effect for ayahuasca. The analysis of the time course of effects showed significant elevations relative to placebo for amphetamine from 1 hour onward. Values remained significantly elevated at 24 hours at which time point they also differed significantly from those obtained after ayahuasca. This latter treatment significantly increased pupillary diameter relative to placebo between 0.5 and 2 hours after dosing.

Mean amplitude of the PLR was reduced to varying degrees by the 2 active treatments. The overall statistical analysis showed a trend effect in the general ANOVA for peak values. Pairwise comparisons showed a significant decrease after ayahuasca relative to placebo and no significant effect after amphetamine. The analysis of the time course of effects showed a significant decrease relative to placebo for ayahuasca at 30 minutes and a significant decrease relative to amphetamine at 2 hours.

Opposed patterns were seen between ayahuasca and amphetamine for pupillary reflex latency. Whereas placebo-like variations were observed for amphetamine, ayahuasca increased the mean values. Again, the overall ANOVA only showed a trend to significance. Pairwise comparisons showed trend increases relative to placebo for ayahuasca and amphetamine. Analysis of the time course of effects showed significant increases for ayahuasca relative to placebo and amphetamine at 2 hours.

TABLE 2. Effects Induced by Placebo, D-Amphetamine 20 mg, and Ayahuasca 1 mg DMT/kg on Peak Values for EEG and Autonomic Measures, Neuroendocrine Parameters, and Lymphocyte Subpopulations

					Pairwise Comparisons		
	Placebo	D-Amphetamine	Ayahuasca	GLM	PLA:AMP	PLA:AYA	AYA:AMP
EEG measures							
EEG relative β power	−1.41 (4.96)	−2.05 (4.46)	8.89 (10.56)	<0.01	NS	*	†
Autonomic measures							
Body temperature	0.33 (0.16)	0.49 (0.28)	0.43 (0.28)	>0.1	—	—	—
Pupillary diameter	0.58 (1.11)	1.66 (1.13)	1.50 (1.02)	0.053	†	NS	NS
PLR amplitude	0 (0.31)	−0.07 (0.26)	−0.33 (0.23)	0.065	NS	†	0.074
PLR latency	−3.5 (15.64)	−4.3 (13.67)	11.8 (17.63)	0.068	NS	0.093	0.051
Respiration rate	0.1 (2.86)	−0.45 (4.75)	0.05 (3.20)	>0.1	—	—	—
Hormones							
Prolactin	3.86 (5.07)	1.72 (5.06)	15.53 (12.03)	<0.01	NS	*	†
Cortisol	−5.28 (4.68)	5.31 (6.42)	11.64 (7.39)	<0.001	*	‡	0.070
GH	5.41 (4.74)	6.30 (9.34)	14.06 (15.25)	>0.1	—	—	—
Lymphocyte subpopulations							
Total lymphocytes	−2.40 (7.66)	−2.80 (7.10)	−4.40 (14.39)	>0.1	—	—	—
CD3	0.30 (6.22)	−7.30 (2.67)	−9.10 (6.61)	0.009	†	†	NS
CD4	−2.60 (6.87)	−8.90 (3.45)	−10.50 (6.52)	0.008	†	†	NS
CD8	−0.10 (3.84)	−1.00 (4.57)	−2.70 (4.08)	>0.1	—	—	—
CD19	−0.89 (4.01)	1.89 (6.51)	−1.44 (6.27)	>0.1	—	—	—
NK cells	1.70 (5.25)	7.70 (3.37)	11.60 (10.06)	0.014	†	†	NS

Mean (SD) of the scores obtained (n = 10), and results of the statistical analysis performed. Peak relative β power expressed as percentage; peak body temperature in °C; peak pupillary diameter in mm; peak PLR amplitude in mm; peak PLR latency in milliseconds; peak respiration rate in breaths/min; peak prolactin and peak GH in ng/mL; peak cortisol in μg/dL; peak lymphocyte subpopulations expressed as percentage.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.001$.

AMP indicates D-amphetamine; AYA, ayahuasca; NS, not statistically significant; PLA, placebo.

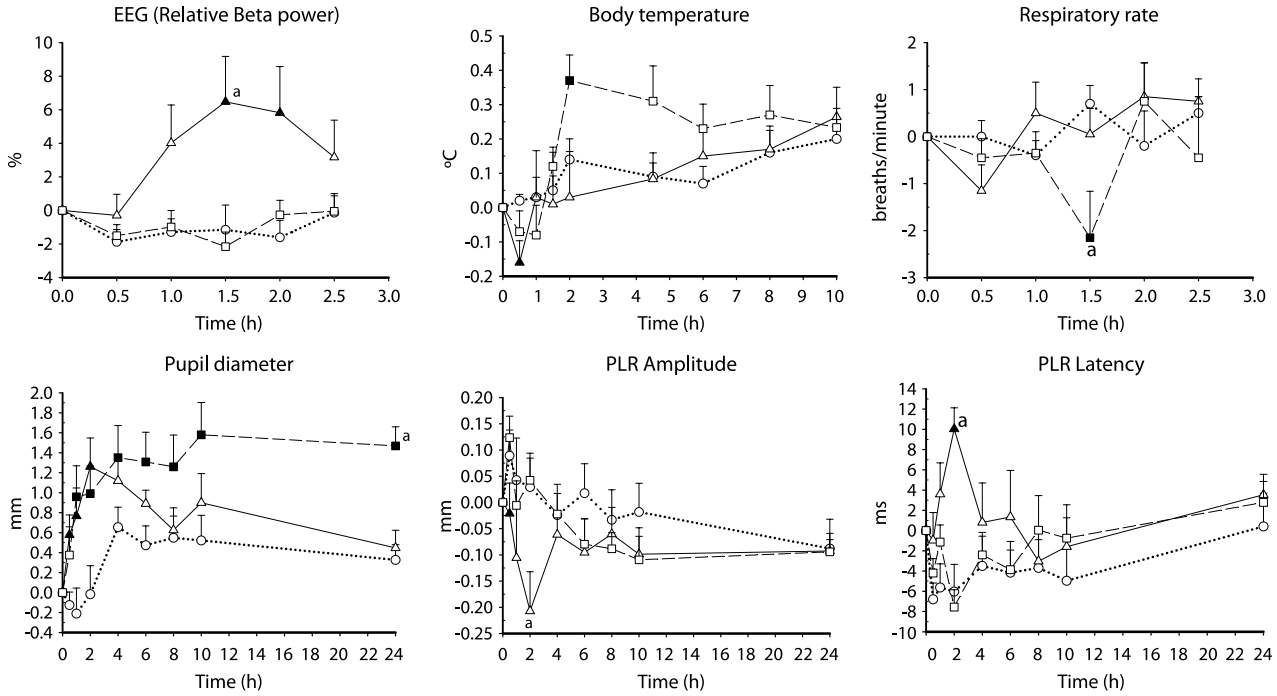

FIGURE 1. Time course of EEG and autonomic measures (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. Error bars denote 1 SEM.

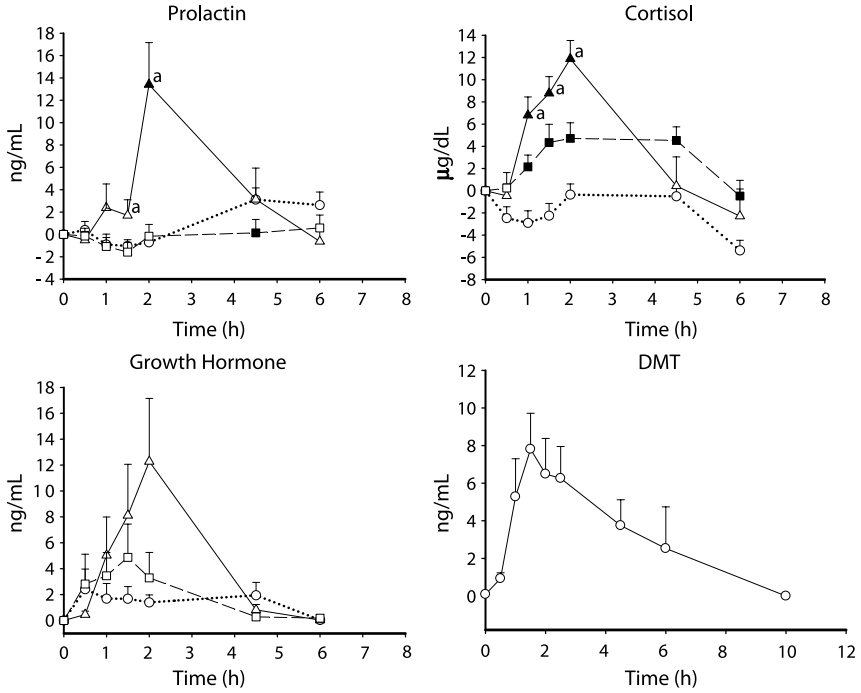

FIGURE 2. The upper panels and the lower left panel show the time course of neuroendocrine measures (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. The lower right panel shows the time course of DMT plasma concentrations after 1.0 mg DMT/kg body weight ayahuasca. Circles indicate means from 10 volunteers. Error bars denote 1 SEM.

Respiration Rate

No significant effect was observed for either active treatment in the overall ANOVA. The analysis of the time course of effects showed significant decreases for amphetamine relative to placebo and ayahuasca at 1.5 hours after dosing.

Neuroendocrine Measures

Neuroendocrine effects results are shown in Table 2 and Figure 2.

Prolactin peak levels after ayahuasca were significantly higher than those after placebo and amphetamine. The analysis of the time course of effects showed that ayahuasca significantly increased prolactin levels relative to placebo at 2 hours. Ayahuasca differed from amphetamine at 1.5 and 2 hours. Interestingly, although amphetamine did not modify prolactin in the peak value analysis, the time course analysis showed significantly reduced levels at 4.5 hours as compared with placebo.

Both active treatments significantly increased cortisol levels relative to placebo. Peak increases after ayahuasca showed a trend to be larger than those after amphetamine. Analyses at the different time points showed significant differences versus placebo in the time interval between 1 and 2 hours for ayahuasca and between 1 and 6 hours for amphetamine. Ayahuasca induced significantly higher cortisol levels than amphetamine at 1, 1.5, and 2 hours.

Regarding GH, although mean values after ayahuasca were higher than after placebo and amphetamine, no significant results were found in the ANOVA or at the individual time points.

Lymphocyte Subpopulations

Treatment effects on lymphocyte subpopulations are shown in Table 2 and Figure 3.

Total lymphocyte percentages in the 24-hour period did not show any significant changes after either of the 2 active treatments. However, the time course analysis showed an increase after ayahuasca relative to placebo at 1.5 hours and a decrease at 4.5 hours. The decrease was significant versus placebo and versus amphetamine. Interestingly, amphetamine nonsignificantly decreased total lymphocyte percentage at this time point. No differences were observed between treatments at 24 hours.

CD3 lymphocyte levels were found to be significantly decreased after ayahuasca and amphetamine. Time course analysis showed significant decreases at 1.5 and 2 hours after ayahuasca and at 1.5, 2, and 4.5 hours after amphetamine. No differences were found between treatments at 24 hours, although mean values were still lower than those after placebo.

Peak CD4 levels were significantly decreased after both active treatments. Time course analysis showed significant decreases at 1.5 and 2 hours for both ayahuasca and amphetamine. Again, no differences were found between treatments at 24 hours.

No significant changes were found for CD8 lymphocytes in the global and time course analyses. CD19 levels were also not found to be modified by any treatment in the ANOVA. However, the time course analysis found a significant decrease after ayahuasca at 2 hours.

Natural killer cells were significantly increased after ayahuasca and amphetamine. The time course analysis showed significant increases versus placebo at 1.5 and 2 hours after ayahuasca and at 1.5, 2, and 4.5 after amphetamine.

DMT Plasma Levels

The time course of DMT plasma concentrations is shown in Figure 2. The mean (SD) of the maximum concentration values

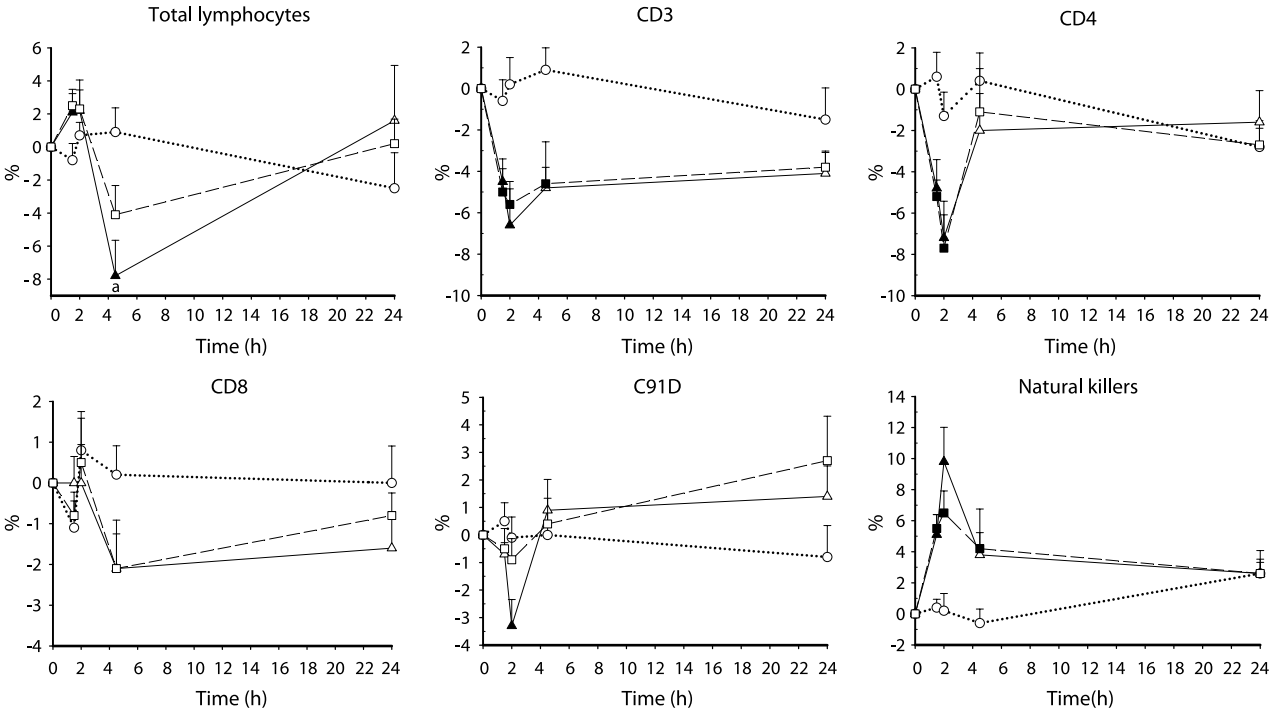

FIGURE 3. Time course of effects on lymphocyte subpopulations (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. Error bars denote 1 SEM.

(C_{max}) was 11.8 (SD, 6.4) ng/mL. The median (range) time at which the C_{max} was attained was 1.8 hours (range, 1–4.5 hours) after dosing.

DISCUSSION

The present investigation was undertaken to explore the autonomic, neuroendocrine, and immunomodulatory profile of ayahuasca. Considering the current expansion of ayahuasca use worldwide, we wished to further investigate the impact of this plant tea on human physiology. Results showed that ayahuasca induces relevant modifications in autonomic, neuroendocrine, and immune parameters as discussed below.

Systemic and CNS access of its main active principle was confirmed, respectively, by measurable DMT plasma levels and significant subjective effects. Maximum DMT concentrations were attained at 1.8 hours, in line with previously reported data.[10] Subjective effects included stimulant-like activation (ARCI-A), positive mood (ARCI-MBG), and somatic effects (ARCI-LSD, HRS-somaesthesia), in addition to perceptual modifications (HRS-perception), changes in thought processes and content (HRS-cognition), increased impairment (HRS-volition), and increased emotional lability (HRS-affect). These results replicate previous results after acute ayahuasca administration.[10,16]

The inclusion of amphetamine as an active control helped further characterize the psychotropic effect profile of ayahuasca. Thus, ayahuasca led to significantly higher scores than amphetamine in 4 of the 6 HRS scales (except HRS-somaesthesia and HRS-affect) and in the ARCI-LSD scale. On the other hand, as compared with ayahuasca, amphetamine significantly increased subjective feelings of intellectual energy and efficiency (ARCI-BG) and decreased scores on the sedative-sensitive ARCI-PCAG scale. Increases in the BG and decreases in the PCAG scale are known features of psychostimulants.[28] Although ayahuasca and amphetamine share some sympathomimetic properties, such as mydriasis and increases in blood pressure,[10] the divergences in subjective effects point to differential central mechanisms. Whereas amphetamine was perceived subjectively to increase intellectual energy, ayahuasca effects were rather felt as impairing. Differential effects on the CNS were further evidenced by the statistically significant increases observed in EEG relative β power. This effect was absent after amphetamine and replicates previous findings.[17] Although $5HT_{2A}$ receptor activation has repeatedly been found to cause physical signs of sympathetic activation,[36,37] the serotonergic mechanism leads to a pattern of CNS effects, which clearly differs from that by dopamine- and noradrenaline-enhancing drugs.

Ayahuasca effects on autonomic measures were not particularly robust. Body temperature showed a biphasic time course after ayahuasca and amphetamine. Mean temperature decreased below placebo levels between drug administration and the first hour and gradually rose thereafter. The initial decrease was significant for ayahuasca, but the subsequent increase was not. Previous studies involving the parenteral administration of DMT found inconsistent results for this measure, with 1 study reporting increases and 3 others reporting no change or ambiguous results.[36–39] Amphetamine, on the other hand, caused a nonsignificant initial decrease in body temperature followed by an increase that was larger than after ayahuasca, attaining statistical significance at 2 hours after dosing. Interestingly, a similar biphasic pattern had been previously described for body temperature after amphetamine and after the amphetamine derivative and serotonin releaser MDMA, although changes were statistically different from placebo only for the latter drug.[40,41] Weak changes were also found in the present study for respiration, which was reduced after amphetamine but only at 1 time point. Ayahuasca did not modify this variable. Early controlled studies with parenteral DMT did not find significant changes for this variable,[38] and it has not been measured in more recent studies.

Effects on pupillary diameter were more intense than on other autonomic variables, and a mydriatic effect was observed for both ayahuasca and amphetamine. However, whereas the effect of amphetamine was long lasting (still significant at 24 hours after administration), the effect after ayahuasca was significant only until 2 hours after dosing; mean values had fallen back to placebo levels at 8 hours. These findings are consistent with many previous studies. Mydriasis has been demonstrated in several controlled studies for parenteral DMT[36,38,39] and also for oral amphetamine and other psychostimulants.[28,40] As pupillary diameter is controlled by a balance between sympathetic and parasympathetic tone, based on our present findings ayahuasca seems to display sympathomimetic properties like those of amphetamine. However, ayahuasca was also found to decrease the amplitude and increase the latency of the PLR, effects typically ascribed to anticholinergic drugs. However, none of the components of ayahuasca seem to display affinity at muscarinic receptor sites.[3] A potential explanation is that the observed effects could be due to the noradrenergic inhibition of parasympathetic neurotransmission in the Edinger-Westphal nucleus, the CNS nucleus that controls constriction of the iris. The mixed serotonin/noradrenaline reuptake inhibitor venlafaxine has also been found to increase PLR latency and decrease PLR amplitude in the absence of any affinity of the compound for muscarinic receptors.[42,43] Results in those studies were also interpreted in terms of parasympatholytic effects mediated by noradrenergic inhibition of the Edinger-Westphal nucleus.

This is the first study in which the aforementioned autonomic variables have been measured after ayahuasca in a controlled clinical trial. In the only previous study known to us, the authors reported increases in respiration rate, pupillary diameter, and oral temperature.[20] However, as they did not include a nondrug (placebo) condition, the reported effects cannot be directly compared with ours. The autonomic effects of ayahuasca suggest sympathetic activation of lower intensity than that elicited by parenteral DMT in a dose range of 0.05 to 0.4 mg/kg.[36]

Ayahuasca and amphetamine produced significant time-dependent modifications in neuroendocrine variables and lymphocyte subpopulations. Ayahuasca, which contains the direct serotonergic agonist DMT, increased prolactin levels, whereas amphetamine, which increases noradrenergic and dopaminergic neurotransmission, did not. These results are in line with published data showing prolactin increases after DMT and serotonergic drugs such as MDMA, fenfluramine, and citalopram but not after amphetamine.[40,44] It is well known that dopamine is a potent inhibitor of prolactin secretion.[45] On the other hand, stimulation of the serotonin $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, and $5\text{-}HT_{2C}$ receptors, which are all targets of DMT, increases prolactin release.[26] Neither of the active treatments produced significant changes in GH levels, but mean values were increased after ayahuasca. Growth hormone secretion is stimulated by selective $5\text{-}HT_{1A}$ agonists.[25] The lack of a significant result could be due to the lower affinity of DMT for the $5\text{-}HT_{1A}$ receptor.[7,8] Both ayahuasca and amphetamine produced significant increases in cortisol and variations in lymphocyte subpopulations. Activation of the sympathetic nervous system (SNS) and cortisol release have a well-known modulatory effect on lymphocytes.[21] Thus, besides a potentially direct receptor-mediated action, either

mechanism could be responsible for the changes observed in the present study, that is, reductions in CD3 and CD4 and enhanced NK cell levels. These changes appeared to be transient, with baseline values recovered after 24 hours.

A recent study found DOI reduces CD8 lymphocytes in mice via activation of the 5-HT_{2A} receptor.[24] Although no significant changes in CD8 percentages were found in the present study, data from the study in mice indicate that serotonergic psychedelics can modulate the immune function using a direct mechanism. Nevertheless, the remarkable similarity between the effects of ayahuasca and amphetamine on lymphocyte distribution in the present study suggests that changes may rather have been caused by an indirect mechanism common to both treatments rather than by specific drug-target interactions with immune cells. As mentioned above, this indirect mechanism could involve the hypothalamic-pituitary-adrenal axis and the SNS. Experimental evidence shows that despite their different molecular mechanisms of action; both DMT and amphetamine stimulate the hypothalamic-pituitary-adrenal axis, as reflected by increases in adrenocorticotropic hormone release.[36,46] Regarding the SNS, there is abundant literature on the procatecholaminergic effects of amphetamine,[28] and there is also evidence for increased urine excretion of noradrenaline metabolites after ayahuasca, suggesting an increased activity of the SNS after both drugs.[10] Further evidence on the nonspecific nature of ayahuasca effects is the similarity with the profile of changes induced by MDMA. Effects by this drug have been consistently replicated in many studies and basically involve decreases in CD3 and CD4 T lymphocytes and increases in NK cells.[47,48] Other psychoactive substances, such as cocaine, cannabis, alcohol, nicotine, and opiates, have also been found to modify the status of the immune system, both after acute intake and following chronic exposure.[23,48–50]

The health impact of ayahuasca ingestion in terms of susceptibility to disease is difficult to ascertain with the present data. Reductions in CD3 and CD4 are usually interpreted as detrimental. CD4 cells regulate cytotoxic T cells such as CD8 lymphocytes, which in turn destroy cells infected with intracellular microbes. CD4 cells also regulate B lymphocytes (CD19), which are responsible for antibody secretion.[51] On the other hand, increases in NK cells could be beneficial, these cells being involved in fighting virally infected and cancerous cells.[52,53] However, the overall time-dependent neuroendocrine and immunological profile observed in the present study mimics that observed in humans under stress.[54,55] Increased glucocorticoid levels and lymphocyte redistribution in acute stress have traditionally been regarded as immunosuppressant.[21] However, more recent views emphasize that, contrary to chronic stress, acute stress may have modulatory rather than inhibitory effects on immunity.[56] Considering the increasing popularity of ayahuasca and that ingestion of the tea on a regular basis is a central feature of the ayahuasca religions, the long-term impact of regular use on immunity warrants further investigation.

To conclude, the present findings indicate that acute ayahuasca has a moderate impact on the autonomous nervous system and a more robust activation of the hypothalamic-pituitary-adrenal axis. In addition, acute ayahuasca administration shows modulatory capacity on cell-mediated immunity, inducing a time-dependent redistribution of lymphocyte subtypes. A limitation of this study is the use of single doses only of the administered active drugs, thus precluding the assessment of dose-response relationships for the studied variables. Future studies should evaluate both the acute impact of different ayahuasca doses on immune function and the effects of chronic exposure in frequent users.

AUTHOR DISCLOSURE INFORMATION

The authors declare no conflicts of interest.

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