Changes in aminoacidergic and monoaminergic neurotransmission in the hippocampus and amygdala of rats after ayahuasca ingestion

Eduardo Ferreira de Castro-Neto, Rafael Henrique da Cunha, Dartiu Xavier da Silveira, Mauricio Yonamine, Telma Luciana Furtado Gouveia, Esper Abrão Cavalheiro, Débora Amado, Maria da Graça Naffah-Mazzacoratti

AIM: To evaluate changes in neurotransmission induced by a psychoactive beverage ayahuasca in the hippocampus and amygdala of naïve rats.

METHODS: The level of monoamines, their main metabolites and amino acid neurotransmitters concentrations were quantified using high performance liquid chromatography (HPLC). Four groups of rats were employed: saline-treated and rats receiving 250, 500 and 800 mg/kg of ayahuasca infusion (gavage). Animals were killed 40 min after drug ingestion and the structures were stored at -80 °C until HPLC assay. The data from all groups were compared using Analysis of variance and Scheffé as post test and P < 0.05 was accepted as significant.

RESULTS: The results showed decreased concentrations of glycine (GLY) (0.13 ± 0.03 < 0.29 ± 0.07, P < 0.001) and γ-aminobutyric acid (GABA) (1.07 ± 0.14 vs 1.73 ± 0.25, P < 0.001) in the amygdala of rats that received 500 of ayahuasca. Animals that ingested 800 mg/kg of ayahuasca also showed a reduction of GLY level (0.11 ± 0.01 < 0.29 ± 0.07, P < 0.001) and GABA (0.98 ± 0.06 vs 1.73 ± 0.25, P < 0.001). In the hippocampus, increased GABA levels were found in rats that received all ayahuasca doses: 250 mg/kg (1.29 ± 0.19 < 0.84 ± 0.21, P < 0.05); 500 mg/kg (2.23 ± 0.38 < 0.84 ± 0.21, P < 0.05) and 800 mg/kg (1.98 ± 0.92 vs 0.84 ± 0.21, P < 0.05). In addition, an increased utilization rate of all monoamines was found in the amygdala after ayahuasca administration in doses: 250 mg/kg (noradrenaline: 0.16 ± 0.02 < 0.36 ± 0.06, P < 0.01; dopamine: 0.39 ± 0.012 < 2.39 ± 0.84, P < 0.001; serotonin: 1.02 ± 0.22 < 4.04 ± 0.91, P < 0.001), 500 mg/kg (noradrenaline: 0.08 ± 0.02 < 0.36 ± 0.06, P < 0.01; dopamine: 0.33 ± 0.19 < 2.39 ± 0.84, P < 0.001; serotonin: 0.59 ± 0.08 < 4.04 ± 0.91, P < 0.001) and 800 mg/kg (noradrenaline: 0.16 ± 0.04 vs 0.36 ± 0.06, P < 0.001; dopamine: 0.84 ± 0.65 vs 0.36 ± 0.06, P < 0.001).

Abstract

AIM: To evaluate changes in neurotransmission in...
CONCLUSION: Our data suggest increased release of inhibitory amino acids by the hippocampus and an increased utilization rate of monoamines by the amygdala after different doses of ayahuasca ingestion.

Key words: Ayahuasca; Amino Acids; Monoamines; Hippocampus; Amygdala

INTRODUCTION

Ayahuasca is a Quechua term derived from the juxtaposition of the words: (Aya) “soul”, “dead spirit”, and (Waska) “rope”, “vine”, and thus is loosely translatable as “vine of the souls” or “vine of the dead”. Ayahuasca refers to the vine that is the principal ingredient of a psychoactive beverage used by more than 70 different indigenous groups spread throughout Brazil, Colombia, Peru, Venezuela and Ecuador. In human beings, it is also present in the brain as an endogenous substance and is found in blood, urine and cerebrospinal fluid. After oral administration of ayahuasca at different doses to naïve rats, we found that ayahuasca ingestion could modify neurotransmitter release in limbic brain structures.

MATERIALS AND METHODS

Sample of ayahuasca

The infusion of ayahuasca was supplied by Professor Dr. Dartiu Xavier da Silveira, from the Psychiatry Department of Universidade Federal de São Paulo, and this infusion was prepared by the Núcleo Senhora Santana, Campo Grande, Brazil on May 22nd 2008, for Master José Roberto de Souza under the auspices of Centro Espírita Beneficente União do Vegetal, and in the care of C. Otávio Castelo. The infusion was previously lyophilized and stored at -18 °C under vacuum. After this procedure, each 200 mL of infusion was converted in 40.8 g of powder, which was maintained under proper conditions.

Identification and quantification of ayahuasca components

The concentration of the main alkaloid ayahuasca components was determined in this work using a gas chromatography procedure, as previously reported. Briefly, analyses were performed using an Agilent gas chromatography
graph equipped with a nitrogen-phosphorous detector (GC-NPD). Chromatographic separation was achieved on an HP ultra-2-fused-silica capillary column (25 m × 0.2 mm × 0.33 µm) film thickness using ultra-pure nitrogen as the carrier gas at a constant flow rate of 0.6 mL/min. Injections of 1 µL were made in split mode (ratio 1:20). The injector port and detector temperature were maintained at 200 and 250 °C respectively. The oven temperature was maintained at 150 °C for 1 min and programmed to rise at 10 °C/min to 250 °C before being held at this latter temperature for 7 min.

A sample solution containing ayahuasca (0.5 mL), borate buffer (pH 9.0, 2.0 mL) and the internal standard diphenhydramine (100 µL, of a solution 1.0 mg/mL) was loaded onto a C18 cartridge mounted on a vacuum manifold and conditioned with methanol (2 mL), deionized water (1.0 mL) and borate buffer (pH 9.0; 2.0 mL). The loaded cartridge was further washed with deionized water and with a solution of acetonitrile-water (1:9). After drying the cartridges under full vacuum for 7 min, the sample was eluted with methanol (2 mL). This solution (1 µL) was injected in the GC-NPD system and the retention time and concentration were obtained after comparison with stock standard solution.[13]

Animal groups
At least 1 wk before the experiments, adult male Wistar rats, weighing 220-280 g, were randomly selected from the same pool and allocated to groups of five, housed under conditions of controlled temperature and humidity on a standard light/dark cycle of 12 h (lights off at 7:00 pm). Rat chow pellets and water were provided ad libitum. The experiments were performed with the approval of the Institutional Ethics Committee (DHEW Publication, NIH, 80-23), (number 1050/09) and all efforts were made to minimize animal suffering. Four groups of rats were employed: saline-treated (n = 5) and rats receiving 250 mg/kg (n = 8), 500 mg/kg (n = 8) and 800 mg/kg (n = 8) of lyophilized ayahuasca orally. The animals’ behavior after drug administration was analyzed by three different observers and the rats were killed 40 min after drug ingestion. The brain structures were separated and stored at −80 °C until assay. Another group of rats (n = 8) was employed to study their behavior after drug administration (500 mg/kg). Changes in the behavior were analyzed by three different observers during 60 min.

Sample preparation and HPLC assay
Monoamines and amino acids were identified and quantified using a HPLC system with electrochemical and fluorescence detectors, respectively.

Brain structures were removed, placed on an ice-chilled plate, weighed and stored at −80 °C until assay. Tissues were ultrasonically homogenized in a 0.1 mol/L solution of HClO4 containing 0.02% Na2S2O3 (15 µL of solution for each milligram of tissue), dihydroxybenzylamine (DHBA, 146.5 ng/mL), as the internal standard for monoamines, and homoserine (HSER, 10 µg/mL), as the internal standard for amino acids. The samples were centrifuged at 11000 g at 4 °C for 40 min, and then the supernatant was filtered and injected into an HPLC system. The monoamines NA, DA and 5-HT, as well as their metabolites, were quantified as previously described by our group.[14] In summary, a Shimatsuzu LC-10AD isocratic system was employed, with a 20 µL injection loop and a Spheri-5 RP-18 5 µm column (220 × 4.6 mm), using electrochemical detection at 0.75 V and a mobile phase composed of phosphate/citrate pH 2.64, 0.02 mol/L, 0.12 mmol/L ethylene diamine tetraacetic acid and 0.006% heptane sulphonic acid, in 10% methanol, at a flow rate of 1 mL/min. Concentrations of monoamines and metabolites were expressed as mean ± SD ng/mg wet tissue. Turnover rates of monoamines were calculated by the ratio between metabolites and monoamine concentrations.

To assay amino acids, the supernatant was filtered and submitted to an o-phthalaldehyde (OPA) derivatization and then injected into the HPLC system. Amino acid derivatization was done by dissolving 27 mg of OPA in 1 mL of methanol, adding 5 µL of 2-mercaptoethanol and 9 mL of 0.1 mol/L sodium tetraborate (pH 9.3) solution. Before sample analysis, a solution was prepared with 1 mL of stock solution and 2 mL of sodium tetraborate 0.1 mol/L. The pre-column derivatization was completed by reacting 100 µL of this solution with 50 µL of sample or amino acid standard solution for 2 min before the injection.[13] An isocratic HPLC system was used with a fluorescence detector, a 20 µL sample injector and an RP-18 column (50 × 4.6 mm). The mobile phase consisted of sodium phosphate 0.05 mol/L (pH 5.95) with methanol 11.5%. The flow rate of this HPLC system was 3.5 mL/min and the detector was employed with an excitation of 348 nm and emission of 460 nm.

Standard concentrations of amino acids and monoamines were tested and the retention time was verified for each substance to certify that there were no peaks overlapping on sample delivery. The amino acids were expressed as mean ± SD (nmol/L per milligram) wet tissue.

Statistical analysis
The data from all groups were compared using Analysis of variance and Scheffé as post test and P < 0.05 was accepted as significant.

RESULTS
Animal behavior
After ayahuasca administration, the behavior of the animals was evaluated qualitatively and compared with that of saline-treated rats. Ten min after infusion administration, all rats that received ayahuasca showed increased exploratory behavior, with increased sniffing and chewing. After this period, they exhibited hyperkinesia, oral and masticatory movements and blinking. The hyperkinesia evolved to loss of foot strength, enlarged base and semi-closed eyes, 30 min after ayahuasca administration. The number of fecal residues was similar in control and...
A

Mean amino acid hippocampus

B

Mean amino acid amygdala

Table 1 Concentration of each active principle measured by gas-chromatography

| Ayahuasca lyophilized | 250 mg/kg | 500 mg/kg | 800 mg/kg |
|-----------------------|-----------|-----------|-----------|
| DMT                   | 6.02      | 12.04     | 19.26     |
| THH                   | 1.94      | 3.88      | 6.20      |
| HRL                   | 51.92     | 103.89    | 166.19    |
| HRM                   | 1.94      | 3.88      | 6.20      |

The concentrations of ayahuasca components present in the sample were measured using Gas-Chromatography. DMT: N,N-dimethyltryptamine; THH: Tetrahydroharmine; HRL: Harmalina; HRM: Harmine.

treated animals. This altered behavior was progressively normalized and 60 min after ayahuasca administration, all rats showed normal activity.

The original infusion of ayahuasca employed contained DMT = 0.59 mg/mL, THH = 0.99 mg/mL, HRL = 0.19 mg/mL and HRM = 5.09 mg/mL. Table 1 summarizes the concentration of each active principle administered to each group of rats.

Changes in amino acid content in the amygdala and hippocampus

When the concentrations of amino acids were analyzed in the hippocampus, we found an increased level of GABA in the groups of rats that received 250, 500 and 800 mg/kg of ayahuasca, when compared with saline-treated animals (Figure 1A). In contrast, the data illustrated in Figure 1B show that the amygdala presented a decreased concentration of GLY in all drug-treated groups and decreased GABA only in those rats that received the highest ayahuasca concentrations (500 and 800 mg/kg).

Changes in concentrations of monoamines and their metabolites

The hippocampus of ayahuasca-treated rats showed greater variation when 500 and 800 mg/kg of drug was administered. Animals that received these doses showed increased concentrations of 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid and homovanillic acid (data not shown). In addition, the level of 5-HT was also increased in those rats that received 500 or 800 mg/kg of ayahuasca (Figure 2A). However, the comparison between drug-treated and saline-treated groups showed only a decreased utilization rate for 5-HT in the hippocampus of rats that received 800 mg/kg (Figure 3A).

The amygdala presented the biggest change with regard to monoamine levels. NA, DA and 5-HT all showed increased concentrations in all the studied groups. Analyzing the utilization rates of monoamines in the amygdala, we found that NA, DA and 5-HT were less utilized or less degraded, as shown in Figure 3B.

DISCUSSION

Ayahuasca infusions prepared in different South American countries contain different concentrations of psychotropic agents. According to McKenna et al.[5], each milliliter of ayahuasca from Peru contains DMT (0.6 mg), HRM (4.67 mg), HRL (0.41 mg) and THH (1.6 mg). Meanwhile, the great majority of Brazilian ayahuasca contains, on average: DMT (0.6 mg/mL); HRM (1.2 mg/mL) HRL (0.2 mg/mL) and THH (1.07 mg/mL)[10]. In this context, our data are in accordance with those described by other authors since we found DMT = 0.59 mg/mL, THH = 0.99 mg/mL, HRL = 0.19 mg/mL and HRM = 5.09 mg/mL in the ayahuasca sample.

The present study aimed to investigate the effects of the ingestion of different concentrations of the ayahuasca infusion upon the levels of some neurotransmitters (monoamines and amino acids), as well as the utilization rate of monoamines in the hippocampus and amygdala of naive rats.

No changes were found in the glutamate concentrations, either in the hippocampus or the amygdala of treated animals, which indicates that this excitatory amino acid is not involved in ayahuasca-induced behavioral changes. However, a reduction in GLY levels in the amygdala was observed with the administration of 250, 500 and 800 mg/kg of infusion, and a reduction in GABA levels was seen after administration of 500 and 800 mg/kg, suggesting increased release of both inhibitory amino acids in this brain structure. In contrast, in the hippocampus,
an increased concentration of GABA was found in all ayahuasca-treated groups, suggesting decreased release of this neurotransmitter, compatible with increased excitation in the hippocampus and increased inhibition in the amygdala of ayahuasca-treated rats.

In this context, we showed here, for the first time, that ayahuasca induces changes in the concentration of inhibitory amino acids in two limbic structures. The opposite effects found in the hippocampus and amygdala could support the activation and/or inhibition of several pathways involved in important processes, such as memory and learning and emotional behavior. Furthermore, the GABAergic system has also been linked to an amnesic function in the hippocampal-dependent declarative memory[17] and a change in this pathway could represent modification of this function. In direct opposition to this idea, data from Doering-Silveira et al[18] showed that ayahuasca-user adolescents did not differ from control subjects when neuropsychological tests were applied to both groups. Thus, more studies are needed to elucidate these findings.

The determination of monoamine content showed that among the analyzed structures, the amygdala was most affected by ayahuasca treatment; all the concentrations used in this work increased the levels of NA, DA and 5-HT in this limbic structure. The utilization rates of these monoamines were also reduced, suggesting MAO inhibition or increased synthesis of these neurotransmitters, or both.

In the hippocampus, 5-HT levels were also increased in rats that received 500 and 800 mg/kg of ayahuasca. However, the comparison between drug-treated and saline-treated groups showed a decreased utilization rate for 5-HT in the hippocampus only when the higher dose of ayahuasca was employed. These data show that, although with some variation, ayahuasca increases the concentration of monoamines in limbic structures, mainly in the amygdala. Furthermore, previous studies have shown an increased stimulation of 5-HT receptors by the individual ayahuasca components[5,6] showing an over stimulation of monoamine pathways in the brain.

According to Schwarz et al[59], HRM and HRI also stimulate DA release from striatal slices and these data, in
association with MAOA inhibition, suggest that B. caapi could be tested in the treatment of Parkinson disease. In addition, Schmoldt et al. observed that MAO inhibition is able to reduce oral ethanol self-administration, due to high levels of DA and 5-HT in the synaptic cleft. In this context, ayahuasca could also be useful in the treatment of alcohol dependence. Recent data showed an important application in therapies for addiction [9,11,21] and anxiety disorders [23,25] and Parkinson disease [19,21,24], and modulates REM and slow-wave sleep in healthy volunteers [25]. HRM is also related to inhibition of angiogenesis and suppression of tumor growth through activation of p53 in endothelial cells [25].

Taken together, these data show that ayahuasca components have different actions on different brain structures, involving changes in monoamines and amino acid concentrations.

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**COMMENTS**

**Background**

Ayahuasca is an infusion containing psychoactive compounds, such as N,N-

dimethyltryptamine (DMT), is structurally similar to serotonin (5-hydroxytrypt-

tamine) and also has similarities with lysergic acid and mescaline. This infusion also has beta-carbolines, such as harmine, harmaline and tetrahydroharmine. DMT is a short-acting hallucinogenic tryptamine, which is present in several plants used as admixtures to the Banisteriopsis caapi vine in ayahuasca prepa-

**Research frontiers**

As ayahuasca use has been growing in the world, mainly in South and recently in North America, the knowledge of how it can modify normal neurotransmitters in limbic structures is important since this infusion could be employed in the treatment of other brain pathologies.

**Innovations and breakthroughs**

This work shows, for the first time, detailed changes in amino acids and mono-

**Applications**

Clinical use of ayahuasca could be important as an additional drug in treatment of major depression and/or in other central pathologies.

**Terminology**

Utilization rate of a monoamine is used to visualize the release of this neu-

**Peer review**

Ayahuasca is a psychoactive plant preparation which contains a serotonin ana-

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