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Metabolic plasticity and the energy economizing effect of ibogaine, the principal alkaloid of *Tabernanthe iboga*

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ABSTRACT

Ethnopharmacological relevance: The root bark of iboga plant—*Tabernanthe iboga* has been used traditionally in Central Africa as a psychoactive substance in religious rituals, while in smaller doses it is appreciated due to its stimulant properties. The iboga root bark, iboga extract or pure ibogaine are being recognized in the West as an anti-addiction remedy and their use is increasing.
Aim of the study: Our previous studies have demonstrated a transient ATP pool reduction under ibogaine accompanied by the induction of energy metabolism related enzymes. The present study aimed to find the cause of this energy deprivation and to foresee its immediate and long-term impact on metabolism.

The overall project is designed to disclose the common mechanism of action at these seemingly diverse indications for iboga use, to predict eventual adverse effects and to build the grounds for its safe and beneficial utilization.
Materials and methods: The rate of carbon dioxide (CO_2) as a marker of energy metabolism in stationary yeast model under aerobic conditions in the presence of ibogaine at concentration of 1, 4 and 20 mg/l was measured for 5 h by gas chromatography. The overall oxidative load was determined fluorimetrically by 2′,7′-dichlorofluorescein diacetate (H_2DCFDA) and *in vitro* antioxidant properties of ibogaine were defined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test.
Results: The CO_2 production under ibogaine was temporarily increased in a dose dependent manner.

The increased energy consumption as an early effect of ibogaine was proven by the fact that in spite of energy mobilization, the ATP pool has been simultaneously decreased.

Although increased cellular respiration co-produces reactive oxygen species (ROS), the overall oxidative load was significantly lowered by ibogaine. Since ibogaine does not show any significant *in vitro* antioxidant properties, the results indicate its stimulating influence on physiological oxidative stress defence system.
Conclusion: Ibogaine triggers remodeling of the housekeeping metabolism. Under the initial energy cost it results in increased efficacy of physiological antioxidative systems, which reduce oxidative damage and lowers basal metabolic needs. Together with induced catabolic enzymes they set a new metabolic equilibrium that saves energy and makes it easily available in case of extra needs. While healthy organism profits from improved fitness and mental performance and can withstand higher stress without risking a disease, due to the same principle ibogaine provides beneficial support at the recovery after diseases including addiction syndrome.

1. Introduction

Ibogaine is an indole alkaloid naturally found in the root bark of tropical rainforest shrubby plant iboga—*Tabernanthe iboga* Baill. (Apocynaceae family) and to a lesser extend in some other species of Tabernaemontana tribe. Iboga (tabernanthe radicis

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cortex) has been traditionally used in tribes of the Congo basin in Central Africa as a psychoactive sacrament used in the ceremony of initiation into adulthood. It induces trance and is considered to reveal one's purpose of life and his role in a society (Fernandez, 1982). In smaller doses it is appreciated due to its stimulant and aphrodisiac properties (Naranjo, 1969; Schultes, 1970). Hunters use it to promote vigilance while stalking pray (Fernandez, 1982). Its use is highly valued on long, tiring marches, on lengthy canoe voyages, and on difficult night watches (Schultes et al., 2001).

In the former century iboga extract under trademark Lambarene was sold in France and recommended as a tonic against fatigue, asthenia and depression and for recovery after infectious diseases (Goutarel et al., 1993). Other compositions containing ibogaine had been on the marked named Bogadin, Iperton, Endabuse (Ratsch, 1998).

In the last four decades the urban traditional use of iboga root bark, iboga extract or pure ibogaine is on the increase as an anti-addiction therapy (Alper et al., 2008). In the so called Ibogaine medical subculture it is used to ease the detoxification of drugs, for abstinence syndrome alleviation and to speed up the tolerance reversion. In long-term abstinence, it reduces craving for drugs by anxiety reduction and improvement of mood (Mash et al., 2000) and one of the explanations for this is psychoanalytical catharsis with resolution of inner conflicts (Naranjo, 1973). Existential insights resulting in social (re)integration of an individual are recognized as important consequence of iboga initiation in both native and Western societies (Fernandez and Fernandez, 2001). Besides, descriptions as spiritual revelation and religious redemption are not uncommon (www.ibogaine.co.uk/experience.htm) and interest for bare psycho-spiritual and religious use of iboga is so taking roots also in the West (www.sacrament.kibla.si). On the other hand *in vitro* and *in vivo* studies in animal models expose diverse biochemical impacts of ibogaine application (Alper, 2001; Maciulaitis et al., 2008).

Our recent work (Paškulin et al., 2010) showed that the induction of energy related enzymes in the yeast *Saccharomyces cerevisiae* accompanies the dose dependant decrease in ATP energy pool caused by ibogaine at concentrations of 1, 4 and 20 mg/l during 5 h. Yeast in stationary growth phase under aerobic conditions is an accepted model for studies of basic metabolic pathways of higher eukaryotes, including mammalian cells (Ma, 2001).

The aim of present study was to identify the cause and to foresee the consequences of ATP energy pool deprivation observed under ibogaine exposure, especially to confirm whether this energy shortage is a consequence of increased ATP consumption or it might be due to its silenced production. The rate of carbon dioxide (CO_2) production in yeast *Saccharomyces cerevisiae* in aerobic stationary growth phase was measured to define the level of oxidative catabolism and ATP production, under the concentrations of ibogaine that mirror those in the blood at different use—up to 1 mg/l corresponds to moderate stimulant effect, raising the dose brings psychoactive range and approaching 4 mg/l relates to the anti-addictive properties, while above are the traditional initiation doses (Fernandez and Fernandez, 2001; Mash et al., 2000). Parallel work on potential energy consumers like toxicity, oxidative stress and kinetics of ibogaine were conducted.

Our hypothesis was that ibogaine triggers energy consuming process and that there is a common denominator at diverse outcomes of iboga use.

2. Material and methods

2.1. Material

Ibogaine HCL was donated by Sacrament of Transition, Maribor, Slovenia. Ibogaine was used in our series of experiments since it is directly related to the iboga plant as its principal alkaloid. Besides, majority of literature concerns this pure form. The effect and after-effect of iboga root bark, its extract or pure ibogaine is except for kinetics reported as subjectively indiscriminative.

2.2. Yeast cultivation

Yeast *Saccharomyces cerevisiae* was cultivated in YEPD growth medium with the following composition: 10 g/l glucose (Kemika), 5 g/l yeast extract (Biolife), 5 g/l peptone (Oxoid), at 28 °C and 220 rpm to the stationary growth phase. Then cells were centrifuged for 5 min at 4000 rpm, washed with and resuspended in 50 mM potassium phosphate buffer, pH 7.0 to density of 1×10^8 cells/ml. The yeast culture was incubated at 28 °C and 220 rpm.

2.3. Cell CO_2 production

To determine cell respiration, 5 ml of 1×10^8 cells/ml yeast culture in 50 mM potassium phosphate buffer were transferred in sterile 15-ml serum bottles covered with airtight rubber stoppers. The suspension was incubated with ibogaine in concentrations of 0, 1, 4 and 20 mg/l at 150 rpm at 28 °C in the dark. The amount of CO_2 produced was measured at 0, 0.25, 0.5, 1, 2, 3, 4, and 5 h of incubation with gas chromatograph Hewlett Packard HP5890, as described by Odić et al. (2007). The chromatograph settings were as follows: column Porapak R mesh 100/120 (180 cm/1.8 in), oven temperature 50 °C, injector temperature 100 °C, TCD detector temperature 100 °C, carrier gas helium (180 ml min^{-1}), integrator HP3392A. The chromatograph was calibrated with an external standard having known CO_2 concentration. For each time point the results are expressed as relative difference in production of CO_2 by yeast cells under ibogaine compared to the control.

2.4. Estimation of oxidative stress

Intracellular oxidation was defined by using 2′,7′-dichlorofluorescein (H_2DCF), which is able to react with oxidants—reactive oxygen species (ROS) (Jakubowski and Bartosz, 1997).

Stationary phase yeast cells at concentration of 1×10^8 cells/ml were added H_2DCFDA as a stock of 1 mM ethanol solution to the final concentration of 10 μM. After incubation for 20 min at 28 °C, 220 rpm cells were treated with ibogaine in concentrations of 0, 1, 4 and 20 mg/l or ascorbic acid in *in-vitro* equipotent concentrations of 0, 1, 2 and 4 μM and samples were taken at the end of accelerated energy metabolism period. 200 μl of the cell suspension was transferred to the microplate and fluorescence was measured using Tecan microplate reader Safire II (excitation and emission wavelengths of 488 and 520 nm, respectively). The results are expressed as a relative difference in overall ROS load compared to the control:

$$\text{Ratio}\,[\%] = \left[E_{\text{treated}}/E_{\text{control}}\right] \times 100$$

where E is emission of ibogaine treated or control solution.

2.5. DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 nm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment (Yamaguchi et al., 1998).

The reaction mixture (1 ml) contained 500 μl of daily prepared (1,1-diphenyl-2-picrylhydrazyl) DPPH solution (250 μM), 400 μl of Tris–HCl buffer pH 7.4 (100 mM) and 100 μl of various concentrations (10, 20, 40 and 80 μM) of ibogaine dissolved in distilled water. After thorough mixing, the solutions were kept in