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Ex vivo effects of ibogaine on the activity of antioxidative enzymes in human erythrocytes

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ABSTRACT

Ethnopharmacological relevance: Ibogaine is a naturally occurring alkaloid with psychotropic and metabotropic effects, derived from the bark of the root of the West African *Tabernanthe iboga* plant. The tribes of Kongo basin have been using iboga as a stimulant, for medicinal purposes, and in rite of passage ceremonies, for centuries. Besides, it has been found that this drug has anti-addictive effects.

Aim of the study: Previous studies have demonstrated that ibogaine changed the quantity of ATP and energy related enzymes as well as the activity of antioxidant enzymes in cells thus altering redox equilibrium in a time manner. In this work, the mechanism of its action was further studied by measuring the effects of ibogaine in human erythrocytes in vitro on ATP liberation, membrane fluidity and antioxidant enzymes activity.

Materials and methods: Heparinized human blood samples were incubated with ibogaine (10 and 20 μM) at 37°C for 1 h. Blood plasma was separated by centrifugation and the levels of ATP and uric acid were measured 10 min after the addition of ibogaine using standard kits. The activity of copper–zinc superoxide dismutase (SOD1), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were measured in erythrocytes after incubation period. The stability of SOD1 activity was further tested through in vitro incubation with H_2O_2 and scanning of its electrophoretic profiles. Membrane fluidity was determined using an electron paramagnetic resonance spin-labelling method.

Results: Results showed that ibogaine treatment of erythrocytes in vitro increased ATP concentration in the blood plasma without changes in neither erythrocytes membrane fluidity nor uric acid concentration. Ibogaine also increased SOD1 activity in erythrocytes at both doses applied here. Treatment with 20 μM also elevated GR activity after in vitro incubation at 37 °C. Electrophoretic profiles revealed that incubation with ibogaine mitigates H_2O_2 mediated suppression of SOD1 activity.

Conclusion: Some of the effects of ibogaine seem to be mediated through its influence on energy metabolism, redox active processes and the effects of discrete fluctuations of individual reactive oxygen species on different levels of enzyme activities. Overall, ibogaine acts as a pro-antioxidant by increasing activity of antioxidative enzymes and as an adaptagene in oxidative distress.

1. Introduction

Ibogaine is a naturally occurring alkaloid with psychotropic and metabotropic effects, derived from the bark of the root of the West African *Tabernanthe iboga* plant. Iboga (*Tabernanthe radicis* 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

Abbreviations: 12-DS, 12-doxyl stearate; 5-DS, 5-doxyl stearate; ATP, adenosine triphosphate; AD, antioxidative defense; CO_2, carbon dioxide; CAT, catalase; SOD1, copper–zinc superoxide dismutase; EPR, electron paramagnetic resonance; GSH-Px, glutathione peroxidase; GR, glutathione reductase; Hb, hemoglobin; H_2O_2, hydrogen peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NBT, nitro blue tetrazolium; ROS, reactive oxygen species; RBC, red blood cells; TCA, tricarboxylic acid; UA, uric acid

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doses it is appreciated due to its stimulant and aphrodisiac properties or as a tonic during convalescence after diseases (Naranjo, 1969; Schultes, 1970). Hunters use it to promote vigilance on long, tiring marches, on lengthy canoe voyages, and on difficult night watches while stalking pray (Fernandez, 1982; Schultes et al., 2001). Youthfulness and longevity are claimed effects of low daily iboga supplementation (personal communication with Fang tribe – Paškulin, Gabon 1997).

More recently, based on human case reports and experimental data from animal studies it has been found that this drug has anti-addictive effects. The administration of ibogaine reduced self-administration of cocaine, morphine, heroin, alcohol, and reduced nicotine preference (Alper, 2001). Doses up to 1 mg/L (3,22 μM) correspond to moderate stimulant effect, raising the dose brings psychoactive range and approaching 4 mg/L (12,89 μM) relates to the anti-addictive properties, while above are the traditional initiation doses (Fernandez and Fernandez, 2001; Mash et al., 2000). Although ibogaine possess affinity for different types of receptors, its effects appear not to be mediated via any single type of receptor (Alper, 2001). Paškulin et al. (2006) showed the stimulating influence of ibogaine on rat brain energy metabolism. The results pointed out the elevation of the enzymes of glycolysis and tricarboxylic acid (TCA) cycle and subsequent higher metabolic turnover. Separate experiment on yeast *Saccharomyces cerevisiae* showed that induction of energy metabolism-related enzymes was not mediated via receptor bindings and it is not linked to cell differentiation or organization in tissue. Yeast incubation with ibogaine at doses from 1 to 20 mg/L (approximately 3 to 60 μM) is followed by a fall in cellular ATP level and an increase in CO_2 production in the first hour of exposure to ibogaine in a dose dependant manner, suggesting that ibogain promotes ATP consumption (Paškulin et al., 2010, 2012). A transient and reversible fall in ATP pool happens immediately (10 min) after addition of ibogaine. Consequently increased cellular respiration is associated by the production of reactive oxygen species (ROS) and, surprisingly, significant drop in the total oxidative load. After 5 h of exposure, energy metabolism related enzymes (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase and alcohol dehydrogenase) were induced, as well as SOD1 protein suggesting increased energy production and antioxidant cellular protection (Paškulin et al., 2010). Since ibogaine did not show any significant in vitro antioxidant properties per se (Paškulin et al., 2012), the results indicate its stimulating influence on physiological oxidative stress defense system in a pro-antioxidant manner. It seems that ibogaine triggers remodeling of the housekeeping metabolism and under the initial energy cost it results in increased metabolic efficacy, enhanced free energy availability and sustainability.

Since the ATP depletion affects the cellular mitochondrial metabolism, here we used erythrocytes as a model system that is not dependant on mitochondrial energetics. Furthermore, it was shown that erythrocytes deliberated ATP from membrane bound ATP pools (Chua et al., 2012) after different kind of stimuli (Ellsworth et al., 2009). Therefore, we measured the concentration of ATP in blood plasma after ibogaine treatment and its influence on membrane fluidity. Because ATP in the blood plasma is dephosphorylated (Coade and Pearson, 1989) and further decomposed by adenosine deaminase to hypoxanthine (Plagemann et al., 1985) and finally uric acid (UA), here we also measured the content of UA.

In physiological settings, erythrocytes show a self-sustaining activity of antioxidative defense (AD) enzymes, such as: copper–zinc superoxide dismutase (SOD1, EC 1.11.16), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSHPx, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2). Their coordinate actions protect the erythrocyte's bio-macromolecules from free radical-mediated damage. Since there is no de novo synthesis of AD enzymes in mature erythrocytes, their defense capacity is limited (Nikolić-Kokić et al., 2010). According to previous results that ibogaine treatment led to elevated anti-oxidant defense (Paškulin et al., 2006, 2010), the aim of our study was also to explore in vitro effects of ibogaine on the activity of the key antioxidant enzymes: SOD1, CAT, GSHPx and GR in the model of erythrocytes where de novo enzyme synthesis is absent.

2. Materials 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 the literature concerns this pure form.

2.2. Subjects and blood sampling

Fresh blood was obtained from 13 normal weights, non-smoking, male volunteers (ages 23–39). They were healthy and free of clinical evidence of any chronic illness. All study participants provided written informed consent and the study protocol was reviewed and approved by principal institution's ethical review boards. After 12–14 h overnight fast, blood samples were obtained by venipuncture and pooled in tubes containing heparin (1 g/L).

2.3. Experimental procedures

Fresh whole blood aliquots were incubated in vitro for 1 h at 37 °C without (control) or with ibogaine (doses of 10 and 20 μM according to Paškulin et al., 2010). ATP and uric acid concentrations in blood plasma were measaured after only 10 min of incubation. After 1 h erythrocytes and plasma were immediately separated by centrifugation (10 min, 5000 rpm, 4 °C).

Aliquots of three-times washed erythrocytes with saline (0.9% w/w) were centrifuged and separated erythrocytes were hemolysed in ice-cold distilled water. Antioxidant defense enzyme activities were measured in lysate. For SOD1 activity determination hemoglobin was removed by the method of Tsuchihashi (1923). Hemoglobin was estimated by the method of Drabkin and Austin (1935).

2.4. ATP assay

ATP was measured in human plasma samples as suggested by Gorman et al. (2007). Whole blood was separated in 3 aliquots: control (1) and treated with 10 (2) and 20 μM (3) of ibogaine. Samples were centrifuged at 5000 g/10 min/4 °C to extract plasma. ATP concentration was measured using an ATP bioluminescence assay kit. Luciferin–luciferase agent (100 μL) was added to samples (100 μL), and ATP-dependent luminescence was measured with a luminometer (CHAMELEON™V, Hidex, Turku, Finland). The standard curve was obtained by serial dilutions of 2 nM ATP stock solution. ATP concentrations were expressed as pmol/ml of blood plasma.

2.5. Uric acid determination

Uric acid (UA) concentration in human plasma samples was determined using commercially available enzymatic colorimetric assay according to the manufacturer's instructions (Randox-analyzer Monarch, Milan, Italy). The reference values for UA are 142–339 μmol/L for females and 202–416 μmol/L for males.

2.6. Indication of membrane fluidity

Fresh blood was obtained from four healthy volunteers (ages 23–39) using tubes containing 0.072 mL of 7.5% K_3EDTA as the anticoagulant per 3 mL of blood (Vacuette EDTA, Greiner Bio-One,