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Ibogaine affects brain energy metabolism

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Abstract

Ibogaine is an indole alkaloid present in the root of the plant *Tabernanthe iboga*. It is known to attenuate abstinence syndrome in animal models of drug addiction. Since the anti-addiction effect lasts longer than the presence of ibogaine in the body, some profound metabolic changes are expected. The aim of this study was to investigate the effect of ibogaine on protein expression in rat brains. Rats were treated with ibogaine at 20 mg/kg body weight i.p. and subsequently examined at 24 and 72 h. Proteins were extracted from whole brain and separated by two-dimensional (2-D) electrophoresis. Individual proteins were identified by matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI–TOF MS). Enzymes of glycolysis and tricarboxylic acid (TCA) cycle namely glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase were induced. The results suggest that the remedial effect of ibogaine could be mediated by the change in energy availability. Since energy dissipating detoxification and reversion of tolerance to different drugs of abuse requires underlying functional and structural changes in the cell, higher metabolic turnover would be favourable. Understanding the pharmacodynamics of anti-addiction drugs highlights the subcellular aspects of addiction diseases, in addition to neurological and psychological perspectives.

Keywords: Ibogaine; Addiction; Energy metabolism; Glycolysis; Tricarboxylic acid cycle; Proteomics; Rat brain

1. Introduction

Ibogaine is an indole alkaloid present in the root of plant *Tabernanthe iboga*. The attention it has received in recent decades from scientists and laypersons alike is due to its anti-addiction properties against opiates, stimulants, alcohol and nicotine (Alper et al., 1999; Glick and Maisonneuve, 2000), and its anti-depressive, anti-epileptic and stimulant properties (Schneider and Sigg, 1957; Leal et al., 2000; Alper, 2001).

The ritual use of the plant for spiritual reasons has been practised in tribal communities in Africa for centuries (De Rios et al., 2002). There it is considered as a perception expanding drug that enables the user to reach depths of the subconscious or, in smaller doses, that acts as a stimulant. These "fantasy-enhancing" properties are favoured by some psychotherapists (Naranjo, 1973). This effect is acute, lasting from 12 to 24 h and can by explained by binding of ibogaine to receptors or enzymes.

Besides numerous anecdotal reports of spiritual users and addicts on the Internet and in lay articles, multiple actions of ibogaine have been described in the scientific literature: monoamine oxidase (MAO) inhibition, agonism on 5-Hydroxytryptamine 2A (5-HT_{2A}), opioid kappa, sigma-1 and sigma-2 receptors, modulation of ligand binding to mu opioid receptor, antagonism on dopaminergic and 5-HT transporters, antagonism on *N*-methyl-D-aspartate (NMDA) and nicotinic receptors (Alper, 2001; Glick et al., 2002; Leal et al., 2003).

Preclinical studies on laboratory animals showed attenuation of withdrawal symptoms in opiate dependent animals, attenuation of morphine and cocaine self-administration after application of ibogaine, synergism with morphine on antinociception, modulation of anxiety, amelioration of alcohol drinking disorders and positive influence on learning and memory (Cappendijk and Dzoljic, 1993; Rezvani et al., 1995; Popik, 1996; Alper et al., 1999).

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What is interesting in the pharmacodynamics of ibogaine is that some of the effects last much longer than a pharmacokinetic model could support. The main, mood elevating effect usually appears a day or two after application, when tissue concentrations of ibogaine are already reduced to minute levels. The effect also lasts from days to weeks, when the substance itself and its metabolites, are no longer present in measurable quantities (Hough et al., 1996). The proposed explanation in terms of a very long half-life for ibogaine or its active metabolite, noribogaine, caused by high lipid solubility and two compartment kinetics, cannot support the observed duration of effects for weeks after a single dose (Baumann et al., 2000).

Thus, besides acute effects on receptor and enzyme sites, more complex biochemical, neuroendocrine and possible structural and functional changes in terms of brain plasticity have been suggested (Ali et al., 1996; He et al., 2005). Signal transduction and modulation of gene expression could be the basis for such adaptations (Ali et al., 1999; Onaivi et al., 2002).

Since the anti-addiction effect lasts longer than the presence of ibogaine in the body, some profound metabolic changes on the protein expression level are expected. In this study we analyzed proteome changes in the brain of ibogaine-treated rats by 2-D electrophoresis, and identified certain proteins whose expression was changed. This is the first approach, to our knowledge, to study ibogaine action on animal model using the proteomic approach, which is thought to be more relevant to function than changes in gene expression.

2. Materials and methods

2.1. Animals

12 male Wistar rats weighing 200–250 g were maintained on a 12 h light–dark cycle (light on: 07:00 AM–19:00 PM) in a temperature-controlled colony room at 22–24 °C, with free access to rodent pellets and tap water. Each rat was housed in a separate cage. They were handled according to the European Communities Council Directive of 24th of November 1986 (86/609/EEC) and the National Veterinary Institute Guide for the Care and Use of Laboratory Animals.

2.2. Drug treatment and brain preparation

Ibogaine hydrochloride (courtesy of Sacrament of Transition, Maribor, Slovenia; purity checked by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) and estimated 98.93%) was dissolved in sterile water to 10 mg/ml. Ibogaine is rather hydrophobic and dissolves poorly in saline. Rats were randomly sorted in four groups, each of three rats. Two groups of rats were treated with ibogaine 20 mg/kg body weight i.p. and were sacrificed 24 h and 72 h respectively after the treatment. The two control groups received injections of water i.p. and were sacrificed at the same times as the test animals. Rats were decapitated under CO_2 anaesthesia. Whole brains were rapidly removed and quickly frozen on dry ice and stored at −80 °C until used.

2.3. Sample preparation

Whole brain tissue (0.5 g) had been cooled with liquid nitrogen and ground by a mortar and pestle to a fine powder which was added to a 2.5 ml of sample buffer (20 mM Tris, 9 M urea, 4% (w/v) 3-[(3-Cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate (CHAPS), 10 mM dithiothreitol (DTT), 1 mM ethylenediaminetetraacetic acid (EDTA)) containing a protease inhibitor cocktail (Complete, Mini; Roche) (1 tablet per 10 ml of buffer). The homogenate was sonicated for 30 s and then centrifuged at 25,000 ×*g* for 1 h in order to collect the cytosolic fraction (Lubec et al., 2003). The protein concentration of the supernatant was determined by the method of Bradford (1976).

2.4. Two-dimensional electrophoresis

Two-dimensional electrophoresis was performed according to Görg (1991) with minor modifications. Samples (150 μg protein) were mixed with rehydration solution (9 M urea, 2% (w/v) CHAPS, 2% (v/v) immobilized pH gradient (IPG) buffer, 18 mM DTT, a trace of bromophenol blue) and applied on 13-cm immobilized pH 3 to 10 non-linear gradient (IPG) strips (Amersham Pharmacia Biotech). Rehydration of IPG strips was carried out for 13 h employing an Immobiline Dry Strip Reswelling Tray (Amersham Pharmacia Biotech). The rehydrated strips were then subjected to isoelectric focusing (IEF), which was carried out at 20 °C on a Multiphor II (Amersham Pharmacia Biotech) with the following voltage program: 300 V (gradient over 1 min), 3500 V (gradient over 1.5 h) and 3500 V (fixed for 4 h). Prior to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) IPG strips were equilibrated in SDS equilibration buffer (50 mM Tris–HCl, pH 8.8; 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, a trace of bromophenol blue) containing 1% DTT for 15 min, and containing 4.8% iodoacetamide for an additional 15 min. SDS-

Table 1
Identification of proteins that were induced 24 and 72 h after ibogaine treatment

Spot/enzyme	Accession number	Fold over control 24 h	Fold over control 72 h	Theoretical M_r (Da)/p*I*	Score	Matched peptides	Sequence coverage (%)
1 Glyceraldehyde-3-phosphate dehydrogenase	Q9QWU4	1.13	3.21	36,090/8.14	62	9	36
2 Malate dehydrogenase	42476181	1.42	3.64	36,117/8.79	54	9	30
3 Aldolase A	6978487	1.23	2.45	39,783/8.05	60	9	24
4 Pyruvate kinase	206205	1.38	2.94	58,314/7.19	70	10	26

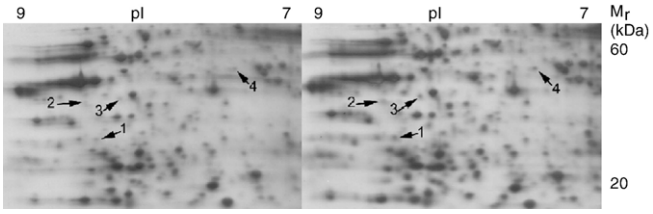

Fig. 1. Partial 2-D gel images of rat brain proteome that are representative of a single control (left) and single 72 h after treatment of the animal (right). Details for each spot are listed in Table 1.

PAGE as the second dimension was carried out with a 12% running gel on the vertical discontinuing electrophoretic system SE 600 (Hoeffer Scientific Instruments) at constant 20 mA/gel 15 min and then at constant 40 mA/gel until the bromophenol blue reached the bottom of the gel. 2-D gels were silver stained using a protocol compatible with matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) (Yan et al., 2000).

2.5. Protein visualization and image analysis

2-D gels were recorded using an Artixscan 1800f scanner (Microtek). Gel image analysis was done with 2-D Dymension software version 2.02 (Syngene).

Gels, which were done in triplicates, were matched to create an average gel using software tools. The average gels of treated and control groups were compared. For all spot intensity calculations, normalised volume values were used. The results are expressed as the ratio of the normalised volume of a protein spot in ibogaine-treated rats divided by the normalised volume of matched protein spot in control rats.

2.6. Protein identification

The protein spots that showed significant changes in intensity compared to controls were excised from the separate gels and analyzed by MALDI–TOF MS using Voyager DE-STR instrument at the Aberdeen Proteome Facility (University of Aberdeen, Aberdeen, Scotland). The Mascot software was used to search NCBInr database. The following search parameters were applied: *Rattus norvegicus* as species; appropriate isoelectric point and molecular weight range depending on the region of the gel; tryptic digest with a maximum number of one missed cleavage and monoisotopic peptide masses. The mass tolerance was set to 100 ppm after internal calibration. Additionally, carbamidomethylation and oxidation of methionine were considered as possible modifications. The criteria used to identify proteins included comparison of the theoretical and observed molecular weights and isoelectric points, the probability based score, the number of matched peptides and sequence coverage.

3. Results

Brain proteins from ibogaine-treated rats and control rats were separated on immobilized pH 3–10 non-linear gradient strips followed by 12% SDS-PAGE gel. The gels were silver stained and then analyzed using 2-D Dymension software. The twelve protein spots that showed significant change in intensity compared to that of control samples had been excised and analyzed by MALDI–TOF mass spectrometry, which gave sufficient confirmation of protein identity for four spots.

Proteins that were induced in rat brains treated with ibogaine relative to control samples were identified as metabolic enzymes involved in glycolysis and the TCA cycle.

Changes in protein expression were most significant 72 h after ibogaine administration. Spot intensities of the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase A, and pyruvate kinase were increased about 3.2-, 2.5-, and 2.9-fold, respectively. The amount of one enzyme from the TCA cycle, malate dehydrogenase, was increased about 3.6-fold (Table 1, Fig. 1). 24 h after ibogaine administration the levels of these enzymes were only slightly above the control values, between 1.1- and 1.4-fold increases (Table 1).

4. Discussion

Ibogaine is known to attenuate abstinence syndrome in animal models of drug addiction (Alper, 2001). Since the anti-addiction effect lasts longer than the presence of ibogaine in the body, it is reasonable to recognize any alteration of protein expression in brain cells after application. Brain proteins were separated by two-dimensional gel electrophoresis and mass spectrometry was used for their identification. Comparative analysis of protein spots between 2-D images of control and ibogaine-treated rat brains was carried out which showed induction of energy metabolism related enzymes.

The most significant alterations in protein expression were observed in rat brains 72 h after ibogaine administration, while at 24 h time point there was only a minor change. This could explain its prolonged action. Spots that were significantly up-regulated were identified as metabolic enzymes involved in glycolysis and TCA cycle. These are glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase (Table 1).

These enzymes participate in a central, key metabolic pathway dealing with the production of energy-rich compounds and therefore interfering with complete metabolic turnover. Namely, in a number of physiological and pathological conditions the organism must adjust the rate of flux through its catabolic pathways in order to cover the need for energy. Allosteric modulation of regulatory enzyme activity was considered in literature to be the key event in the regulation of the rate of flux. In addition to that functional genomics highlights new

regulatory principles mediated by control of gene expression resulting in a control of the quantity of the enzyme.

Higher levels of enzymes have little effect on the steady state equilibrium since saturation, zero order kinetics are not involved in this case. Rather, their influence is best seen in phases of high energetic demands, in which they can support the constant level of the product, in the present case maintaining normal ATP/ADP ratio instead of its decrease.

Whether the elevated energy availability 72 h after application is secondary as a compensation of possible elevated demand on energy during acute phase in the first hours, or is it event per se, remains unclear. Ibogaine indeed acutely elevates cerebral glucose utilization in drug naive, but reduces it in morphine depended animals (Levant and Pazdernik, 2004).

The induced cluster of energy metabolism enzymes indicates that the remedial effect of ibogaine is mediated, at least partially, through an influence on the brain energy metabolism. It is noteworthy in this context that chronic exposure to morphine has the opposite effect in rat brains of reducing levels of glycolysis and TCA cycle intermediates (Sherman and Mitchell, 1973). Chronic morphine treatment stimulates anaerobic metabolism and elevates lactate (Sharma et al., 2003). General fatigue and especially yawning as an early sign of withdrawal, which is a physiological misinterpretation of low energy availability as hypoxia are in coherence with this concept. If at least a part of the abstinence syndrome is mediated through cell energy depletion, the opposite mechanism of action of ibogaine explains its attenuation of withdrawal and craving and its anti-addictive properties.

Since energy dissipating detoxification and reversion of tolerance to drugs of abuse requires underlying functional and structural changes in the cell, higher metabolic turnover is favourable (Squire, 2002). Additional requirement for energy is also expected, since morphological changes in the brain are involved, as suspected on the basis of glial neurotrophin release (He et al., 2005). Consequent brain plasticity changes could be the basis for lifetime behavioural changes.

Also, it is reasonable to assume that the induction of energy metabolism influences mental agility, learning and retrieval of repressed memory (Popik, 1996). This facilitates insight into one's own psychical status and improves efficiency of psychotherapeutic approach to addiction diseases (Naranjo, 1973; De Rios et al., 2002).

Understanding the pharmacodynamics of anti-addiction drug ibogaine highlights some molecular aspects of addiction diseases, in addition to neurological and psychological perspectives, but further work is needed to bring together the diverse explanations of ibogaine action on human beings.

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