UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

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FARMAKODINAMIKA ENTEOGENIH DROG – VPLIV NA IZRAŽANJE GENOV

DOKTORSKA DISERTACIJA

PHARMACODYNAMICS OF ENTHEOGEN DRUGS – INFLUENCE ON GENE EXPRESSION

DOCTORAL DISSERTATION

Ljubljana, 2012

Bojevnik se pravzaprav sploh ne uči šamanizma, pač pa se uči shranjevati energijo. Ta prihranjena energija mu bo omogočila rokovanje z nekaterimi izmed energijskih polj, ki so mu običajno nedostopna. Šamanizem je stanje zavesti, sposobnost izrabe tistih energijskih polj, ki ne sodelujejo pri zaznavanju vsakodnevnega sveta, kot ga poznamo.

(Carlos Castaneda)

Na podlagi Statuta Univerze v Ljubljani ter po sklepu senata Biotehniške fakultete in po sklepu Senata Univerze v Ljubljani z dne 20.11.2007 je bilo potrjeno, da kandidat izpolnjuje pogoje za neposreden prehod na doktorski Podiplomski študij bioloških in biotehnoloških znanosti ter opravljanje doktorata znanosti s področja biotehnologije. Za mentorja je bil izbran prof. dr. Borut Štrukelj.

Doktorska disertacija je rezultat dela na OMI Inštitutu, zavodu za antropološko medicino, na Katedri za farmacevtsko biologijo, Fakultete za farmacijo, Univerze v Ljubljani in na Katedri za biotehnologijo, Oddelka za živilstvo, Biotehniške fakultete Univerze v Ljubljani iz lastnih sredstev in delno v sklopu raziskovalnega projekta (J4–7062) Javne agencije za raziskovalno dejavnost Republike Slovenije.

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Datum zagovora: 4.7.2012

Delo je rezultat lastnega raziskovalnega dela. Izjavljam, da so vsa vključena znanstvena dela identična objavljeni verziji.

Roman Paškulin

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

ŠD Dd
DK UDK 577.2:615.2:606(043.3)
KG ibogain / *Tabernanthe iboga* / enteogeni / energetski metabolizem / antioksidanti / proteomika / adaptogeni
AV PAŠKULIN, Roman, dr. med.
SA ŠTRUKELJ, Borut (mentor)
KZ SI-1000 Ljubljana, Jamnikarjeva 101
ZA Univerza v Ljubljani, Biotehniška fakulteta, Podiplomski študij bioloških in biotehniških znanosti, področje biotehnologije
LI 2012
IN FARMAKODINAMIKA ENTEOGENIH DROG – VPLIV NA IZRAŽANJE GENOV
TD Doktorska disertacija
OP X, 59 str., 5 pregl., 16 sl., 113 vir.
IJ sl
JI sl/en
AI Tropska rastlina *Tabernanthe iboga* se v Centralni Afriki uporablja kot tradicionalno zdravilo in obredna droga. V manjših odmerkih deluje kot poživilo in pospešuje okrevanje po bolezni, v višjih odmerkih pa sproži stanje transa. Zahod je ibogo spoznal kot sredstvo za prekinjanje zasvojenosti. Preseneča večplastno delovanje iboge t.j. olajšano razstrupljanje telesa, odpravljanje prisilnih vedenjskih vzorcev in doseganje duhovnega blagostanja. Vstopno vprašanje teze se je glasilo: »Ali se duhovna izkušnja odraža na materiali ravni; kaj jo v biokemijskem smislu posreduje in kakšne so njene presnovne posledice?« Z metodo dvodimenzionalne elektroforeze in masne spektrometrije smo identificirali spremembe v proteomu podganjih možgan in kvasovke po aplikaciji ibogaina. Rezultati so pokazali povečanje količine oz. indukcijo encimov energetskega metabolizma in antioksidativne obrambe. Pri podganjih možganih so bili 72 ur po intraperitonealni aplikaciji 20 mg/kg t.t. ibogaina inducirani encimi gliceraldehid-3-fosfat dehidrogenaza, aldolaza A, piruvatna kinaza in malatna dehidrogenaza, pri kvasovki po 5 urah kultivacije v mediju z 1 mg/L ibogaina pa encimi gliceraldehid-3-fosfat dehidrogenaza, fosfoglicerat kinaza, enolaza in alkoholna dehidrogenaza ter superoksidna dismutaza. Pri kvasovki smo zaznali tudi od odmerka odvisen prehodni upad ATP ravni ob sočasno povečani proizvodnji CO_2. Pod vplivom ibogaina se sproži preoblikovanje hišne presnove. Ob uvodni energetski obremenitvi pride do povečanja učinkovitosti fizioloških antioksidativnih sistemov, ki zmanjšujejo oksidativno obremenitev in s tem povezane energetske izdatke. Ob sočasni indukciji katabolnih encimov se vzpostavi novo metabolno ravnovesje, ki varčuje z energijo, v primeru dodatnih potreb pa omogoča njeno povečano razpoložljivost. Zdrav organizem lahko tako vzdrži večje fizične in mentalne napore brez tveganja stresne preobremenitve. Po istem načelu iboga omogoča hitrejše okrevanje v primeru bolezni, vključno z motnjo odvisnosti.

KEY WORDS DOCUMENTATION

ND Dd
DC UDC 577.2:615.2:606(043.3)
CX ibogaine / *Tabernanthe iboga* / entheogens / energy metabolism / antioxidants/ proteomics / adaptogens
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PY 2012
TI PHARMACODYNAMICS OF ENTHEOGEN DRUGS – INFLUENCE ON GENE EXPRESSION
DT Doctoral Dissertation
NO X, 59 p., 5 tab., 16 fig., 113 ref.
LA sl
AL sl/en
AB Tropical plant *Tabernanthe iboga* has been used traditionally in Central Africa as a remedy and ritual substance. In low doses it acts as a stimulant and accelerates recovery after disease. In higher doses it induces trance. West recognizes iboga through its anti-addictive properties. It is the multilevel action of iboga that surprises; its detoxifying properties, elimination of compulsive behavioral patterns and introduction of spiritual well-being. The basic question is: »Does the spiritual experience manifests itself on a material level; where are its biochemical pathways and what are its metabolic consequences?« With the method of two-dimensional electrophoresis and mass spectrometry we have identified proteome changes in rat brain and yeast cells after the application of ibogaine. The results have shown the induction of energy metabolism and antioxidative defence enzymes. In rat brain 72 hours after intraperitoneal application of 20 mg/kg per body weight of ibogaine the enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase had been induced. Yeast after 5 hours of cultivation in media with ibogaine 1 mg/L showed induction of glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase, alcohol dehydrogenase and superoxide dismutase. In the yeast model we have also observed transitory fall in ATP pool accompanied by enhanced CO_2 production. Ibogaine triggers adaptation of house keeping metabolism. Under the initial energy load it results in increased efficacy of physiological antioxidative systems, which reduce oxidative damage and related energetic costs. Together with induced catabolic enzymes this sets 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.

KAZALO VSEBINE

KAZALO PREGLEDNIC

KAZALO SLIK

OKRAJŠAVE IN SIMBOLI

ADP/ATP	adenozin difosfat/adenozin trifosfat
CHAPS	3- [(3-holamidopropil) dimetilamonij] -propansulfonat
CYP	citokrom P450
2-D elektroforeza	dvodimenzionalna elektroforeza
DCF	diklorofloresceín
DPPH	1,1-difenil-2-pikrilhidrazil
DTT	ditioteritol
EDTA	etilendiamintetraocetna kislina
EEG	elektroencefalogram
GDNF	nevrotrofični dejavnik glialnega izvora
H_2DCFDA	2,7-diklorofloresceín diacetat
HPCL	tekočinska kromatografija visoke ločljivosti
5-HT	serotonin
IEF	izoelektrično fokusiranje
IPG	imobilizirani pH gradient
LC-MS/MS	tekočinska kromatografija z masno spektrometrijo
MALDI-TOF MS	masna spektrometrija z ionizacijo v matriksu z desorpcijo z laserjem in merjenjem časa preleta ionov
MAO	monoaminska oksidaza
NMDA	N-metil-D aspartat
SDS-PAGE	natrijev dodekil sulfat - poliakrilamidna gelska elektroforeza
ROS	reaktivne kisikove zvrsti
SOD	superoksidna dismutaza
SZO	svetovna zdravstvena organizacija
TLC	tankoplastna kromatografija
YEPD	kvasni ekstrakt, pepton, dekstroza
ZDA	Združene države Amerike

SLOVAR

enteogen - psihoaktivna substanca, ki se uporablja v duhovne namene; neologizem iz grških besed *entheos* (□νθεος) – »poln boga, navdiha, zanosa« in *genesthai* (γενέσθαι) – »vznikniti, vstopiti, postati«

adaptogen – substanca, ki poveča odpornost na stres, izboljša psihofizično vzdržljivost in olajša prilagajanje organizma na spremembe okolja

pro-antioksidant – substanca, ki vzpodbuja fiziološki antioksidativni sistem, sama pa nima antioksidativnih lastnosti

ibogain – indolni alkaloid, ki se nahaja v skorji korenine rasline *Tabernanthe iboga*

iniciacija – obred prehoda, preporod v novo družbeno vlogo in sprejem v skupnost

šamanizem – uporaba spremenjenih stanj zavesti za posvetitev in/ali zdravljenje

redukcionizem – enostranska, ozka obravnava problemov

1 UVOD

Enteogene droge si z drogami, ki povzročajo odvisnost, delijo le slabšalni izraz. Morda niti to ne, če upoštevamo, da je droga v farmakološkem smislu le rastlinski produkt in ne nujno opojna snov. Nekatere iz te skupine sicer domujejo na listah kontroliranih substanc, čeprav bolj po človeški krivdi, kot po lastni naravi. Napačna uporaba je plod neznanja, ta pa spet produkt strahu in odrinjenosti v subkulturo. Temu botruje prej nepoznavanje kot pa slabi nameni in cilj pričujočega dela je z znanstvene perspektive osvetliti farmakološko dogajanje pred, med in po zaužitju take obredne substance.

Ker so tovrstni rastlinski, včasih tudi živalski pripravki številni, se raziskava osredotoča na prominenten primer afriške rastline iboga (*Tabernante iboga* Baill.), ki v Gabonu uživa ugled narodnega bogastva (Ratsch, 1998; Schultes et al., 2001).

Iboga ima tako kot številne druge učinkovine naravnega izvora kar pester nabor indikacij in namenov uporabe. V Centralni Afriki se uporablja kot tradicionalno zdravilo in obredna droga. V manjših odmerkih deluje kot poživilo za premagovanje naporov, povečuje odpornost na stres in pospešuje okrevanje po bolezni (Goutarel et al., 1993). V višjih odmerkih sproži stanje transa, ki posreduje uvid v duhovnem smislu in odgovarja na eksistencialna vprašanja (Naranjo, 1973). Iniciacija z zaužitjem iboge je družbeno sprejet obred prehoda mladostnika v odraslost s polno močjo svobode odločanja in odgovornosti in je analogija zahodnjaški maturitetni simboliki (Fernandez, 1982). Zaužitje substance ima tako fiziološko, psihološko, vzgojno, socialno, simbolno in duhovno komponento.

Zahod je ibogo spoznal kot sredstvo za prekinjanje zasvojenosti (Alper et al., 2008, Maciulaitis et al., 2008). Deluje na številne receptorje, encime in transporterje, ibogain pa sproži tudi sproščanje nevrotrofinov (Alper, 2001, He et al., 2005). Preseneča večplastno delovanje iboge t.j. olajšano razstrupljevanja telesa s ponovno vzpostavitvijo metabolnega ravnovesja, odpravljanje prisilnih vedenjskih vzorcev ter doseganje psihičnega in duhovnega blagostanja.

Vstopno vprašanje teze se glasi: »Ali se duhovna izkušnja odraža na materiali ravni; kaj jo v biokemijskem smislu posreduje in kakšne so njene presnovne posledice?«.

1.1 CILJI RAZISKOVANJA IN DELOVNE HIPOTEZE

Cilj raziskovanja je dobiti vpogled v mehanizme delovanja iboge na človeka; na njegovo telo, osebnost in duha. S tem se pridobi potrebno razumevanje, ki odstrani obči strah pred neznanim, hkrati pa zadevo približa stroki, kateri izkustveni dokaz ne zadostuje in za posvojitev potrebuje razumske razlage in razloge.
Poglabljanje znanja nakaže nove možnosti koristne uporabe, omogoča pa tudi previdevanje možnih neželenih učinkov, njihovo zmanjševanje in izogibanje nepotrebnim tveganim situacijam.

Delovna hipoteza je bila:

- da je vsaj del delovanja iboge pripisati spremembi genskega ekspresijskega vzorca in posledičnim spremembam proteoma in metaboloma, kar rezultira v trajnih strukturnih in funkcionalnih spremembah na vseh ravneh od celice do skupnosti
- da ti učinki presegajo okvire medicine in so uporabni tudi na drugih področjih, kot so psihologija, vzgoja, šport, socialno delo, duhovnost…
- da se da te učinke koristno izrabljati, da pa je potrebna izdelava varnih protokolov, saj je smiselno pričakovati tudi pasti pri uporabi

2 ZNANSTVENA DELA

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2.1 IBOGAIN VPLIVA NA ENERGETSKI METABOLIZEM MOŽGAN

IBOGAINE AFFECTS BRAIN ENERGY METABOLISM

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European Journal of Pharmacology, 2006, 552: 11-14

POVZETEK

Ibogain je indolni alkaloid, ki se nahaja v skorji korenine rasline *Tabernanthe iboga*. Zmanjšuje abstinenčni sindrom v živalskem modelu odvisnosti. Ker je učinek odpravljanja odvisnosti daljši od prisotnosti ibogaina v telesu, je po aplikaciji pričakovati temeljite metabolne spremembe. Cilj študije je bil definiranti vpliva ibogaina na proteinsko izražanje v podganjih možganih. Podganam smo intraperitonealno vbrizgali 20 mg/kg telesne teže ibogaina in jih raziskali po 24 in 72 urah. Izvleček proteinov celih možgan smo ločili z dvodimenzionalno (2-D) elektroforezo. Istovetnost posameznih proteinov smo določili z masno spektrometrijo z ionizacijo v matriksu z desorpcijo z laserjem in merjenjem časa preleta ionov (MALDI-TOF MS). Odkrili smo povečano količino encimov glikolize in cikla trikarboksilnih kislin; gliceraldehid-3-fosfat dehidrogenaze, aldolaze A, piruvatne kinaze in malatne dehidrogenaze. Rezultati nakazujejo možnost, da je zdravileni učinek iboge povezan s povečanjem energetske razpoložljivosti. Povečan metabolni obrat olajša detoksifikacijo in odpravo tolerance na različne droge, saj je ta proces povezan s funkcijskimi in strukturnimi spremembami znotraj celic in je energetsko potraten. Razumevanje farmakodinamike sredstev za odpravo odvisnosti osvetljuje poleg nevroloških in psiholoških tudi celične vidike odvisnosti.

Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 552 (2006) 11–14
www.elsevier.com/locate/ejphar

Ibogaine affects brain energy metabolism

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Received 23 March 2006; received in revised form 31 August 2006; accepted 5 September 2006
Available online 16 September 2006

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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0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.09.008

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)/*pI*	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

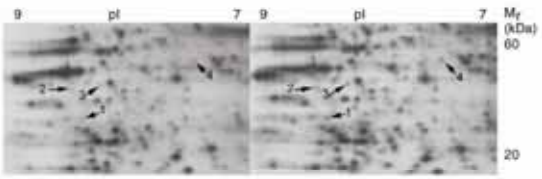

Fig. 1. Partial 2-D gel images of rat brain proteome that are representative of a single control (left) and single 72 h after treatment of the animal (right). Details for each spot are listed in Table 1.

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.

Acknowledgements

Ibogaine was donated by the Sacrament of Transition, Maribor, Slovenia.

The Aberdeen Proteome Facility is supported by grants from SHEFC, BBSRC and Aberdeen University.

Thanks are given to Prof. Dr. Roger Pain for his critical reading of the paper.

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2.2. IBOGA – MED MITOM IN RESNIČNOSTJO

IBOGA – ENTRE MYTHE ET REALITE

Roman Paškulin

Phytotherapie, 2009, 7: 15-19

POVZETEK

Iboga (*Tabernanthe iboga*) je centralnoafriška tropska rastlina, ki je v uporabi kot tradicionalno zdravilo pri rekonvalescenci po infekcijskih boleznih, proti oslabelosti in kot krepčilo pri splošni izčrpanosti. Poleg tega ima iboga pomembno mesto v socialnih in religioznih obredih, saj omogoča komunikacijo s podzavestjo. V zadnjem času je pozornost zahodne medicine in znanosti pritegnila uporaba iboge za razstrupljevanje in prekinjanje odvisnosti od nikotina, alkohola, opiatov in poživil. Ti učinki so potrjeni na živalskih modelih, klinična testiranja pa še čakajo na izpeljavo.

Phytothérapie (2009) 7: 15–19

DOI 10.1007/s10298-009-0366-y

Article original

Phytothérapie expérimentale

Iboga : entre mythe et réalité*

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Résumé : L'iboga (*Tabernanthe iboga*) est une plante de la forêt tropicale de l'Afrique centrale utilisée en tant que remède lors d'une convalescence, après une infection pour combattre la cachexie et comme stimulant lors d'une fatigue générale. L'iboga a, par ailleurs, une place importante dans le contexte social dans des rituels spirituels, facilitant l'introspection du subconscient. Dans les décennies récentes, les propriétés anti-addictives et détoxifiantes de l'iboga vis-à-vis de la nicotine, de l'alcool, des opiacées et des médications stimulantes ont attiré l'intérêt des professionnels et du corps scientifique. Ces effets ont été confirmés sur des animaux de laboratoire, mais des essais cliniques contrôlés n'ont pas encore pu être conduits.

Mots clés : Iboga – *Tabernanthe iboga* – Détoxification – Addiction – Psychothérapie – Métabolisme énergétique

Iboga: between mythe and reality

Abstract: Iboga (*Tabernanthe iboga*) is a rainforest plant from Central Africa that has been used for centuries as a remedy in convalescence, after an infection, for fighting cachexia and as a stimulant in times of general fatigue. In addition, iboga has an important place socially in spiritual rituals, facilitating communication with the subconscious. In recent decades, the anti-addictive and detoxifying properties of iboga with respect to nicotine, alcohol, opiates and stimulant drugs has attracted the interest of health professionals and scientists in the West. These effects have been confirmed on laboratory animals, but controlled clinical trials have yet to take place.

Keywords: Iboga – Detoxification – Addiction – Psychotherapy – Energy metabolism

Introduction

L'ibogaïne est un alcaloïde indolique présent dans les racines du *Tabernante iboga*. L'attention qui lui a été portée ces dernières décades, par les scientifiques autant que par les non scientifiques, est due à son effet anti-addictif contre la nicotine, l'alcool, les opiacés et les stimulants [5, 10].

Les communautés tribales d'Afrique l'utilisent lors de rites pour des raisons spirituelles depuis des siècles [9]. Localement, on considère que c'est une substance accroissant la perception qui rend l'utilisateur capable d'atteindre les profondeurs du subconscient.

Certaines de ces propriétés, catalysant l'introspection, sont utilisées par des psychothérapeutes [19]. L'effet est aigu, de 12 à 24 heures, et peut être expliqué par la liaison de l'ibogaïne sur des récepteurs ou des enzymes.

On retrouve sur le Net de nombreux rapports anecdotiques d'utilisateurs spirituels et de sujets souffrant d'addiction ainsi que des articles écrits par des non-professionnels, mais de multiples actions de l'ibogaïne ont également été décrites dans la littérature scientifique : inhibition de la monoamine-oxydase (MAO), action agoniste sur l'hydroxytryptamine 2A (5-HT2A), récepteurs opioïde kappa, sigma-1 et 2, modulation du ligand liant au récepteur opioïde micron, antagonisme des transporteurs de la dopamine et du 5-HT, antagonisme du N-méthyl-D-aspartate (NMDA) et des récepteurs nicotiniques [4, 11, 16]. La pharmacodynamique de l'ibogaïne est intéressante, car certains de ses effets persistent plus longtemps qu'un modèle pharmacodynamique est capable de le supporter. Le plus important, l'effet d'élévation de l'humeur, apparaît un ou deux jours après la prise, à un moment où les concentrations tissulaires d'ibogaïne sont réduites à des taux déjà minimes. L'effet

* Conférence présentée lors du dixième Symposium de phytoaromathérapie et plantes médicinales, Grasse 2008, et traduite par le docteur Paul Goetz.

persiste de plusieurs jours à des semaines, quand la substance ou ses métabolites ne sont plus présents à des quantités mesurables [14]. Une longue demi-vie de l'ibogaïne ou de son métabolite actif, la noribogaïne, qui serait causée par une solubilité haute aux lipides et deux compartiments cinétiques, en serait responsable, mais elle n'est pas en mesure de supporter la durée d'action observée sur des semaines par une prise unique d'une dose [6].

À côté d'effets aigus sur les sites de récepteurs ou les sites enzymatiques, plus de modifications des complexes biochimiques ou neuroendocriniens et des modifications structurales et fonctionnelles, en termes de plasticité du cerveau, ont été suggérées [1, 13]. Des transductions ou modulations notables de l'expression des gènes pourraient être la base de telles adaptations [2, 20]. Depuis que l'on sait que l'effet anti-addictif dure plus longtemps que la présence d'ibogaïne dans le corps, de profondes modifications métaboliques du niveau d'expression des protéines sont attendues.

Matériel et méthodes

Douze rats Wistar mâles d'un poids de 200 à 250 g ont été maintenus dans un cycle de lumière sombre (lumière allumée de 7 heures du matin à 19 heures le soir) avec une chambre à température contrôlée de 22 à 24° C, avec un accès libre à des boulettes pour rongeurs et à l'eau courante. Chaque rat avait une cage séparée. Ces animaux ont été traités selon la directive European Communities Council Directive du 24 novembre 1986 (86/609 (86/609/EEC) et le National Veterinary Institute Guide for the Care and Use of Laboratory Animals.

L'hydrochloride d'ibogaïne (fourni par Sacrament of Transition, Maribor, Slovénie) a été dissous dans de l'eau stérile à 10 mg/ml. Les rats ont été répartis de manière randomisée en quatre groupes, de trois rats chacun. Deux groupes de rats ont été traités avec 20 mg/kg de poids et 72 heures après administration. Les deux groupes témoins ont reçu des injections i.p. d'eau et sacrifiés au même moment que les rats traités. L'ensemble du cerveau a été prélevé rapidement, congelé immédiatement avec de la glace sèche et conservé à une température de −80° C avant utilisation. L'ensemble du tissu cérébral (0,5 g) a été refroidi avec de l'azote liquide, moulu dans un mortier et réduit en poudre fine avant d'être ajouté à un échantillon de substance tampon : 20 mM de tris, 9 M d'urée, 4 % de (w/v) 3-[(3-cholamidopropyl) diméthyl-ammonio]-1-propanesulfonate (CHAPS), 10 mM de dithiotréitol (DTT), 1 mM d'acide éthylènediamine-tétraacétique (EDTA) contenant un cocktail d'inhibiteurs de la protéase (Complete-Mini-Roche) [une tablette par 10 ml de tampon]. L'homogénat a été soumis à une sonication en 30 secondes et centrifugé à 25 000 g pendant une heure pour récupérer la fraction cytosolique [18]. La concentration en protéine du supernatant a été déterminée par la méthode de Bradford [7].

Une électrophorèse bidimensionnelle a été réalisée selon la méthode de Görg (1991).

Les gels 2-D ont été colorés en argent, selon un protocole compatible, avec un spectromètre de masse par temps de volume (MALDI-TOF MS^{TM}) [27]. Les gels 2-D ont été enregistrés en utilisant un scanner Artixscan 1800 f (*Microtek*). L'analyse de l'image du gel a été réalisée avec un logiciel 2-D *Dimension version* 2,02 (*Syngene*). Des gels, qui sont réalisés en triplicatas, ont été conformés pour créer un gel moyen avec des outils logiciels. Les gels moyens des groupes témoins ou testés ont été comparés.

Pour le calcul de chaque repère d'intensité, des valeurs de volume normalisé ont été utilisées. Les résultats sont exprimés comme étant le rapport du volume de repère de protéine pour les rats traités par l'ibogaïne, divisé par le volume de repère de protéine normalisé pour les rats témoins. Les amas de protéine qui montrent des changements significatifs d'intensité comparés à ceux des témoins ont été excisés des gels et analysés par un spectromètre MALDI-TOF MS^{TM}, à l'aide d'un instrument Voyager-DE^{TM} STR au centre Aberdeen Proteome Facility (*university of Aberdeen, Aberdeen, Scotland*). Le logiciel Mascot a été utilisé pour la banque de données NCBInr. Les paramètres de recherche suivants ont été appliqués :

- *rattus norvegicus* sp ;
- les points isoélectriques appropriés ;
- la ligne de poids moléculaire qui dépendent :
  - de la localisation dans le gel ;
  - du digeste triptyque avec un nombre maximal d'un clivage manquant ;
  - des masses de peptide mono-isotopique.

Le critère utilisé pour identifier les protéines incluait la comparaison entre le poids moléculaire théorique et celui observé, et les points isoélectriques, le score probable, le nombre de peptides conformés et la couverture séquentielle.

Résultats

Les protéines induites dans les cerveaux des rats traits à l'ibogaïne, en relation avec les échantillons de contrôle, ont été étudiées avec des enzymes métaboliques que l'on trouve dans la glycolyse et le cycle de Krebs.

Les modifications de l'expression protéinique ont été, pour la plupart, significatives 72 heures après l'administration d'ibogaïne. L'intensité des spots des enzymes glycolytiques : glycéraldéhyde-3-phosphate déhydrogénase, aldolase A et pyruvate kinase a été augmentée, respectivement de 3,2 ; 2,5 et 2,9 fois. La quantité d'une enzyme du cycle de Krebs, le malate déhydrogénase, a été augmentée de 3,6 fois (Tableau 1, Fig. 1). Vingt-quatre heures après l'administration d'ibogaïne, les niveaux de ces enzymes ne se sont révélés que faiblement au-dessus du niveau des valeurs du contrôle, entre 1,1 et 1,4 fois (Tableau 1).

Discussion

L'ibogaïne est connue pour atténuer le syndrome de sevrage dans les modèles animaux d'addiction aux morphiniques [4]. Puisque les effets anti-addictifs durent plus longtemps que la

Tableau 1. Identification de protéines induites 24 et 72 heures après l'administration d'ibogaïne

Spot enzyme	*Nombre d'accession*	*Perte de contrôle 24 heures*	*Perte de contrôle 72 heures*	*Théorique Mr (Da)/pI*	*Résultats*	*Peptides assorties*	*Couverture de séquence (%)*
1. Glycéraldéhyde-3-phosphate déhydrogénase	Q9QWU4	1,13	3,21	36 090/8,14	62	9	36
2. Malate déhydrogénase	42 476 181	1,42	3,64	36 117/8,79	54	9	30
3. Aldolase A	6 978 487	1,23	2,45	39 783/8,05	60	9	24
4. Pyruvate kinase	206 205	1,38	2,94	58 314/7,19	70	10	26

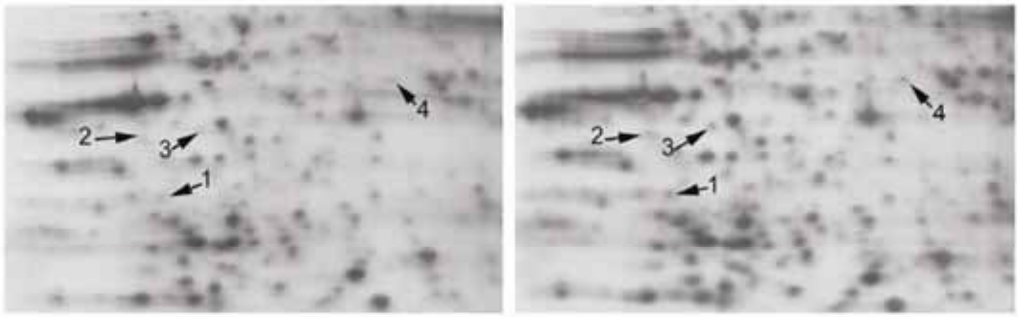

Fig. 1. Images partielles de gel 2-D de protéome du cerveau de rat, représentatives d'un contrôle simple (à gauche) et 72 heures après traitement de l'animal (à droite). Détails pour chaque spot listé dans le Tableau 1

présence dans l'organisme de l'ibogaïne, il est raisonnable de vérifier s'il y a des altérations de l'expression des protéines dans les cellules cérébrales, après son administration. Les protéines cérébrales ont été séparées par une électrophorèse en gel bidimensionnelle. La spectrométrie de masse a été utilisée pour les différencier. L'analyse comparative montre des amas protéiniques entre les images 2-D du contrôle et celles des animaux traités à l'ibogaïne qui a été réalisée et démontre l'induction des enzymes liées au métabolisme énergétique.

La plus importante altération de l'expression des protéines a été observée dans les cerveaux de rats, 72 heures après l'administration de l'ibogaïne, alors qu'à 24 heures, il n'y avait qu'un changement mineur. Cela peut expliquer son action prolongée. Les amas de protéines significativement augmentés ont été identifiés comme étant des enzymes métaboliques qui font partie de la glycolyse et du cycle de Krebs. Ce sont les glycéraldéhyde-3-phosphate déhydrogénase, aldolase A et pyruvate kinase (Tableau 1). Ces enzymes participent à une voie métabolique clé centrale par la production de constituants riches en énergie et interférant donc avec le *turnover* métabolique complet.

Plus explicitement, un certain nombre d'états physiologiques ou pathologiques de l'organisme doit ajuster la proportion du flux, à travers des voies cataboliques, pour couvrir la demande en énergie. Une modulation allostérique de l'activité d'enzymes régulatrices a été considérée dans la littérature comme pouvant être un événement clé dans la régulation de la proportion du flux. Il faut ajouter que l'étude des génomes fonctionnels met en lumière des principes nouveaux de régulation, dont la médiation se fait par le contrôle de l'expression de gènes dont il résulte un contrôle de la quantité d'enzymes. Des niveaux plus élevés d'enzymes ont un effet minime sur l'état d'équilibre stable, puisque la saturation à cinétiques d'ordre zéro n'est pas engagée dans ce cas. Plus encore, leur influence est la mieux perçue dans des phases de demandes à énergie élevée, au cours desquelles elles peuvent soutenir un niveau constant de production, et, dans notre cas, en maintenant une proportion normale d'ATP-ADP au lieu d'une diminution.

Déterminer si la disponibilité d'énergie élevée 72 heures après l'administration est secondaire, comme une compensation d'une possible demande élevée en énergie, durant la phase aiguë des premières heures ou si c'est un phénomène en soi, reste incertain.

L'ibogaïne, en fait, augmente de manière aiguë l'utilisation de glucose au niveau cérébral chez des animaux n'étant pas habitués à une drogue, alors qu'elle le réduit chez les animaux dépendant à la morphine [17]. L'amoncellement d'enzymes du métabolisme énergétique indique que l'effet bénéfique de l'ibogaïne est sous la médiation, du moins en partie, d'une influence sur le métabolisme énergétique du cerveau. Il est

important de noter dans ce contexte que l'exposition chronique à la morphine a, dans le cerveau des rats, un effet opposé de réduction des niveaux de la glycolyse et du cycle de Krebs. Un traitement chronique à la morphine stimule le métabolisme anaérobique et fait augmenter le taux de lactate [23]. La fatigue générale et, spécifiquement, le bâillement comme signe précoce de sevrage, qui est une interprétation physiologique fallacieuse d'une basse disponibilité en énergie comme étant une hypoxie, sont en réalité en cohérence avec ce concept. Si au moins une partie du syndrome de sevrage passe par une déplétion cellulaire en énergie, le mécanisme d'action opposé de l'ibogaïne explique l'atténuation du syndrome de sevrage et du besoin irrésistible ainsi que ses autres effets anti-addictifs. Puisqu'une détoxification par dissipation de l'énergie et une réversion de la tolérance à la drogue demande des modifications sous-jacentes de fonction et de structure dans les cellules nerveuses, un turnover métabolique augmenté les favorise [25].

Une exigence additionnelle d'énergie est aussi attendue puisque des modifications morphologiques dans le cerveau sont concernées, comme le fait suspecter la sécrétion basale de neurotrophine gliale [13]. Les modifications conséquentes de la plastique cérébrale pourraient être la base de changements du comportement de style de vie. De même, il est raisonnable de supposer que l'induction du métabolisme énergétique influence l'agilité mentale, l'apprentissage et le recouvrement de la mémoire refoulée [21]. Cela facilite la révélation du statut psychique propre à chacun et augmente l'efficacité de l'approche psychothérapique des maladies addictives [9, 19].

Conclusion

Certaines médications, en particulier celles issues de la flore ou du monde animal, semblent avoir un effet de panacée aspécifique qui influence de nombreuses fonctions biochimiques et physiologiques conséquentes, comme également l'état psychologique et l'intégration sociale d'un individu. Un turnover métabolique facilité, soutenu par une disponibilité en énergie agrandie, peut être bénéfique dans différents états pathologiques, et l'iboga pourrait être un adjuvant pharmaceutique utilisé en synergie avec des médications spécifiques de certaines maladies.

Selon l'évaluation actuelle de cette discipline scientifique qu'est la médecine, l'ibogaïne fait partie d'une « sous-culture médicale » qui est reconnue comme une réponse spontanée à un besoin, une offre réactionnelle à une demande d'une population non satisfaite avec un traitement par une substance courante et une tactique de réadaptation. L'ibogaïne est de façon déméritoire connectée avec un réseau de fournisseurs qui n'ont pas de fondement professionnel.

Dans les cinq dernières années, 3 414 traitements ont été documentés en Occident, sans tenir compte des expériences traditionnelles d'Afrique Centrale, ce qui représente une augmentation multipliée par quatre en comparaison

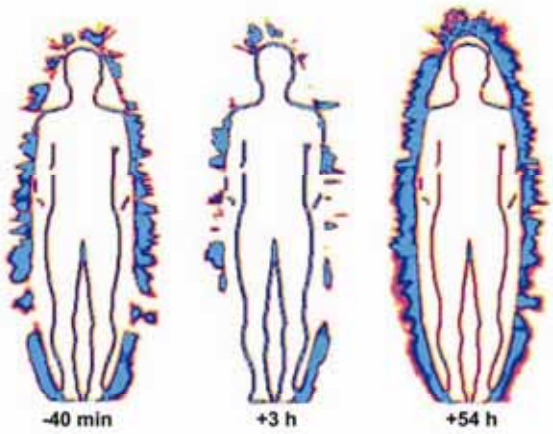

Fig. 2. Effet de l'iboga sur l'aura humaine (avant, entre et après traitement à l'iboga)

des cinq années précédentes ! [3]. La motivation des expérimentateurs est médicale ou spirituelle (si on estime qu'il y a une distinction à faire entre les deux). La communauté religieuse Sacrament of Transition (basée en Slovénie : N.D.L.R) fournit de la matière première, et cette organisation représente une synthèse éclectique entre ces deux attitudes.

Au lieu de placer la « plante des dieux » sur la liste des substances prohibées, démarche souvent politique sans fondement évident en ce qui concerne la nuisance, il faudrait faire un effort dans la direction de l'estimation et l'évitement des risques possibles, sur la base de protocoles sécurisés, d'un monitoring de la situation, d'une information du public et d'une éducation mise à jour des protagonistes de traitements. Des recherches plus avancées sont nécessaires pour mettre en lumière divers aspects de la plante avec son interaction humaine (Fig. 2). L'iboga et les plantes qui lui sont associées ont leur place dans la pharmacopée.

Remerciements / *Acknowledgment*

The research has been done in collaboration with Faculty of Pharmacy and Biotechnical Faculty, University of Ljubljana and Faculté de Pharmacie, Université Montpellier I Special thanks to Pr. Francoise Bressolle and Pr. Yves Pelissier for their help at the research. Ibogaine was donated by Sacrament of Transition, Slovenia.

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2.3. IBOGAINSKA MEDICINSKA SUBKULTURA KOT SESTAVNI DEL GIBANJA ZA ZMANJŠEVANJE ŠKODE NA PODROČJU DROG

IBOGAINSKA MEDICINSKA SUBKULTURA KOT SESTAVNI DEL GIBANJA ZA ZMANJŠEVANJE ŠKODE NA PODROČJU DROG

Roman Paškulin
Časopis za kritiko znanosti, 2009, 239: 253-260

POVZETEK

Prispevek obravnava etične poglede na življenje, zdravje, varnost in svobodo. Oriše trenutno stanje na področju motnje odvisnosti, odnos do odgovornosti zanjo ter možnosti terapije. Umešča duhovni pristop ob uporabi iboge. S pretresanjem možnih dobrobiti in tveganj nakaže praktične možnosti za sistematizirano izrabo njenega potenciala; tako v zdravstvene, kot tudi v namene osebnostne in družbene rasti.

Roman Paškulin

Ibogainska medicinska subkultura kot sestavni del gibanja za zmanjševanje škode na področju drog

Svetovna zdravstvena organizacija definira zdravje kot stanje popolne telesne, psihološke in socialne blaginje. Opisuje torej trenutno stanje posameznika na neki točki v času. Tako predvideva menjajoča se obdobja zdravja in bolezni skozi življenje.

Ne predvideva pa vpetosti posameznika v kontinuumu časa, njegovega odnosa do preteklosti in še pomembnejše: pričakovanj do prihodnosti. Ta so kot nekaj nepredvidljivega, a vseeno odzivajočega se na naše delovanje v domeni duhovnosti; upanja, želja, priprošnje, vere ali namere.

Definicija zdravja SZO torej ne vključuje stopnje optimizma[1] kot glavne karakteristike duhovne blaginje in je kot taka pomanjkljiva.

Človek je končno bitje. Smrt je naravna. Je zdrava. Je normalna. Zato iztekajoči se človek, ki do svojega stanja goji tvoren odnos, pa naj bo to upor ali potrpljenje po odločitvi, ni bolan človek. Je le umirajoči ...

Zdravje je aktivnost. Zdravje je lahko tudi pasivnost, če se zanjo aktivno odločimo. Zdravje je sposobnost prevzemanja odgovornosti. Zdravje je odločitev. In zanj se namesto nas ne more odločiti zdravnik, on nam pri tem lahko samo pomaga.

Gre za tisti odtenek, ki prehlajenega posadi na kolo, ki šepavega požene na plesišče in ki nekatere umirajoče naredi blažene in bolj mladostne, kot je prenekateri starec v mladem telesu. In obstaja nasprotni odtenek, ki nekatere žene v beg, omamo, spanje ...

Problematika odvisnosti[2] je kompleksno področje, ki sega od organskega prek mentalnega na duhovno sfero, po pristopu od humanistike do skrajnosti naravoslovja, od tolerance do nestrpnosti na področju etike in morale. Korenine se dotikajo vsakega izmed teh težišč in nikdar izolirano.

Zato po načelu relativnosti ni končnega, pravega pristopa, temveč je pravilni tisti, ki je prirejen potrebam in pričakovanjem uporabnika. Pluralizem in deinstitucionalizacija sta za dobro politiko na področju drog tako v preventivi kot kurativi nujna.

[1] Tudi; vedrost, rožnoglednost, vizija, smisel, zaupanje ...

[2] Odvisnost v najširšem pomenu, ne le odvisnost od kemičnih substanc, pri kateri najpogosteje pomislimo na sliko odvisnika od heroina. Pri tem pa pozabljamo, da gledamo le vrh ledene gore in da se pod gladino skriva velikanski nabor različnih bolj ali manj nesvobodnih posameznikov, ki se brez svojega brezplodno ponavljajočega se vedenjskega vzorca ne počutijo uravnoteženi.

Pluralizem ponudbe kurativne obravnave razvitega sindroma odvisnosti je pravica državljanov, kajti družbeno okolje je soodgovorno za nastanek tovrstnih motenj; z izbiro pa uporabnik vstopi v proces kot sotvorec zdravstvene obravnave.

Pojmovanje odvisnosti kot bolezni ima dve plati: prva je vsaj delna razbremenitev posameznika odgovornosti za stanje, v katerem se nahaja, in tako vzpostavitev okoliščin za rehabilitacijo s prilagojenim tempom, ki ga ta zmore. Seveda pričakujemo postopno naraščanje odgovornosti, vzporedno in sopomensko z ozdravitvijo (podobno kot vloga mavca pri zlomu).

Ker pa težnja k prepoznavi odvisnosti kot bolezni predvideva in zapoveduje njeno kroničnost, vzpostavlja začaran krog odvisnosti. Ne samo to, dotedanjo kemično odvisnost razširi še na odvisnost od zdravnika oz. zdravstva. Bodimo bolj natančni: toksikoman ni odvisen od droge, odvisen je od njenega dobavitelja.[3]

Tu ima označevanje odvisnosti kot kronične in ponavljajoče se bolezni tudi politično in ekonomsko noto. Odvisnik (zasvojenec, morda samo uživalec) naj ne bi bil sposoben odgovornosti in bolj ali manj potrebuje skrbnika. Sam koncept dekriminalizacije uživanja drog ni zgrešen, neprimerno pa je, da se namesto svobodne presoje posameznika postavi nov čuvaj – zdravnik. Gre za vzpostavitev kontrole nad populacijo, ki, če je pri njej kaj kroničnega, je to nepripadnost main-streamu.[4]

Neselektivna medikalizacija substitucijsko naravnane medicine odvisnosti spominja na apatičnost in nihilizem tistih, ki naj bi bili usposobljeni za boj proti resignaciji. Definicija odvisnosti kot kronične in ponavljajoče se bolezni in njeno razglašanje je le korak od nagovarjanja k uživanju drog. Sklicevanje nanjo spominja na opravičevanje za lastne neuspehe in je očiten dokaz pomanjkanja vere v lastno delo.

Medicinska doktrina substitucijske terapije se je izkazala za zelo učinkovito pri zmanjševanju škode, ki jo uporaba drog povzroči družbi, bistveno manj pa kot dolgoročna rešitev za posameznika.

Motnja odvisnosti ne izvira iz biokemičnega ali nevrotransmiterskega neravnovesja »per se« po zapovedi genetske konstitucije. Če bi, bi bil do danes že znan gen in potomci odvisnikov bi bili skoraj praviloma odvisniki, sledeč Mendlovemu dedovanju. Odvisnost se ne deduje, govorimo lahko kvečjemu o predispoziciji, pa še za to ne vemo, ali ni priučena v družinskem krogu.[5]

Drži pa, da trajna uporaba drog spremeni genski ekspresijski vzorec in s tem snovni ustroj kot adaptacijo na prisotnost tuje snovi. Vsekakor pa sama kal predispozicije za razvoj odvisnosti ne tiči v telesu samem, temveč v odnosu posameznika do okolja.[6]

Dalj časa trajajoča stresna stanja, še zlasti v obdobju razvoja, lahko vodijo do odklonov v osebnostnem razvoju. Pri tem lahko razumemo kemično iztirjenje kot fizično manifestacijo psiholoških motenj in ne obratno. Proces je praviloma reverzibilen, a dolg in drag. Substitucija nezadostnih endorfinov je daleč cenejša ...

[3] Prisvojitev večine aspektov odvisnosti pod okrilje medicine odvisnosti sicer pravilno varuje odvisnika kot bolnika pred krutimi moralističnimi napadi okolice, prinese pa preveč resigniran in substitucijsko naravnan pogled na uporabnika, kateremu je dodeljena preveč pasivna vloga v procesu »zdravljenja«, zdravniku oz. medicini pa preveč ekskluziven položaj v primerjavi z drugimi vrstami pomoči.

[4] »Videl sem najboljše ume svoje generacije, uničene od norosti, sestradane, histerične, gole, kako se ob zori vlačijo skozi črnske ulice, iščoč jezni fiks … A. Ginsberg: Tuljenje in druge pesmi, 1956.

[5] Pogosto se vsiljuje misel, da tu nekaj manjka. Človek iz urejenega okolja in z dobrim pedigrejem očitno štrli iz družinskega povprečja. Morda pa posameznik le ni samo produkt genov in okolja. Malček, v očeh in karakterju tako izoblikovan, iz primarne družine še pokukal ni, pa ni prav nič podoben staršem. »Od kod se je ta vzel?!« Morda pa le ni nag prijokal na ta svet ...

[6] Predvsem gra za prevelike zahteve do širšega okolja, saj izostanek cepljenja s stresom v zgodnji mladosti pusti mladostnika popolnoma nebogljenega ob stiku s svetom. Tako so negacije grandioznosti, ki je je otrok vajen v svojem primarnem okolju, v stiku z vrstniki vir občutka nezadostnosti in tesnobe in kar je še hujše; vir ogorčenja. Ker ni dobil, kar mu gre, ima vso pravico, da protestira. Zaupa samo sebi in svoji izkušnji in ko ob stiku z drogo začasno najde izgubljeno ravnovesje ... (Tomori, 2000)

Treba je poudariti, da ima substitucija v nekaterih primerih svoj prostor kot trajna terapija v smislu zazdravitve kronične motnje. Pri dolgoletnem uživalcu drog se lahko spremeni biokemično ravnovesje, ki se ga ne da popolnoma povrniti v izhodiščno stanje in s tem zagotoviti zadovoljive psiho-fizične konstitucije za kakovostno bivanje, delovanje in počutje.

V tem in le v tem primeru je smiselna stabilizacija z nadomestkom kot prvo in tudi zadnje dejanje v procesu zdravljenja, vsekakor pa ne pri ekperimentatorju, instrumentalni ali začetni habitualni rabi mladega, metabolno voljnega organizma. Obnova fizioloških ravnovesij je tu lahka in o kroničnosti motnje ne sme biti govora.[7]

Mit o dokončnosti možganskih sprememb je že leta 1986 razbila dr. Levi-Montalcinijeva z odkritjem živčnih rastnih dejavnikov – nevrotrofinov, ki spodbujajo obnovo in razrast živčnih celic – in za svoje delo prejela Nobelovo nagrado ...

To dokazujejo tudi okrevanja po poškodbah možganov, ki so sicer počasna, a pogosto privedejo do skoraj popolnega okrevanja in so pogojena s starostjo; mlajši ko je človek, hitrejše in temeljitejše so.

V tej zvezi je zanimiv ibogain. Če izvzamemo tradicionalno uporabo iboge v Afriki, jo Zahod najbolj pozna kot zdravilo za prekinjanje odvisnosti. Zadnjih trideset let so čedalje glasnejši namigi o uspešnosti iboge pri zmanjševanju abstinenčnih sindromov po odtegnitvi alkohola, nikotina, poživil in opiatov in trajno zmanjšanje želje po substanci.[8]

Po aplikaciji ibogaina je v možganih poskusnih živali dokazano povečano izločanje nevrotrofina GDNF (He in sod., 2005). Opazili so, da se pri podganah, vajenih pitja alkohola, po aplikaciji ibogaina zmanjša obseg popivanja. Po izkušnjah biomedicine je smiselno pričakovati podoben učinek pri človeku.

Slovenske študije so pokazale dodaten učinek; pospešeno izgorevanje sladkorjev in s tem večjo energetsko razpoložljivost pri vseh vrstah celic, kar omogoča hitrejši metabolizem, obnovo in aktivnost, ne glede na specializiranost in diferenciacijo (Paškulin in sod., 2006). Kaj pomeni povečanje števila živčnih celic, njihova razvejitev in premreženost možganov, skupaj z večjo živahnostjo in proženjem akcijskih potencialov? Asociacijsko pestrost, ostrejše zaznavanje in povečanje doživljajskega sveta lahko opišemo tudi z duhovnim besednjakom – razsvetljenje, kot psihološki »uvid« ali preprosto kot povečano kondicijo in odpornost na stres. Ne nazadnje, pospešen metabolni obrat olajša detoksikacijo in pospeši povrnitev zdravja, ne glede na osnovno nokso.

Tu se kaže prepletenost različnih nivojev bivanja in njihova neločljivost; delovanje na posamezen delček vpliva na vse druge aspekte celote. Res je, da se posamezna področja močno prekrivajo in prek presečišč prehajajo druga v drugo, pa vendar lahko pristop k zdravju oz. vstop v proces zdravljenja prvotno omejimo na eno področje in tako dobimo prijemališče, preko katerega vplivamo na vsa druga.

Vseeno pa se zdi, da je proces lažji v smeri od ideje k materiji. Vzrok težav večinoma tiči v napačnem dojemanju oz. nepoznavanju samega sebe, sledijo neprimerni odnosi do okolja, kar se konča v patološki manifestaciji na telesni ravni. To lepo dokazujejo t. i. civilizacijske bolezni. Zdravljenje se tako začne pri razumevanju vzrokov in pri sprejemanju odločitev v zvezi z odnosom do njih.

Simptomatsko zdravljenje tu vseeno ima svoj prostor in je na trenutke nenadomestljivo, včasih življenje rešujoče, a učinkuje kratkotrajno in težava ne preneha, dokler ni odstranjen vzrok. Tako je tudi substitucijska terapija kot nizkopražni ukrep za zmanjševanje škode zaradi

[7] Odvisnost je motnja; morda celo to ne. Enkrat preseženo odvisnost lahko vzamemo za poučen kos poti v osebnostnem razvoju. Vsak osebek se rodi popolnoma odvisen od okolice in si samostojnost šele izbori. Odvisnost katerekoli vrste bi tako najprimerneje pojmovali kot zastoj.

[8] www.ibogaine.mindvox.com

uporabe drog v tem kontekstu popolnoma na mestu. Namen nadomeščanja droge je pridobiti čas za osnovno socialno in psihološko obravnavo. Predvideti pa je treba njeno začasnost, saj najsubtilnejših sfer človekove samopodobe ni mogoče dojeti ali razumeti, niti spreminjati, ko je človek pod vplivom narkotika.

Iboga se na afriški celini uporablja od pamtiveka. Prek Pigmejcev je bila predstavljena ljudstvom Kongoške nižine, kjer ima svojo zdravilsko, socialno in duhovno vlogo.

Iboga, posušena in zmleta skorja korenine grma Tabernanthe iboga, vsebuje več sorodnih alkaloidov, predvsem ibogain. Je krepčilo in zdravilo za širok nabor bolezenskih stanj. Ta lahko izvirajo na telesni ravni, kot je rekonvalescenca po infekcijskih boleznih, ali pa na psihološko-socialni noti izgubljenosti posameznika v družbi, ki v svojem bivanju ne najde smisla in harmonije z okolico.[9]

Duhovne iniciacija se še danes množično uporablja v kultu Bwiti, ki s svojima dvema milijonoma privržencev pravzaprav predstavlja trdno in uveljavljeno verovanje, ki pa ni ekskluzivno do krščanstva ali islama. Predsednik Gabona Omar Bongo je po veroizpovedi musliman, hkrati pa nganga – svečenik v Bwiti.

Kot je bilo že rečeno, iboga oz. alkaloid ibogain velja za uradno nepreverjeno, a domnevno učinkovito sredstvo za prekinjanje odvisnosti. Vztrajno narašča tudi število znanstvenih člankov, ki na živalskih modelih odvisnosti potrjujejo to hipotezo. Kontrolirane, dvojno slepe klinične študije, še ni; ameriški National Institute on Drug Abuse (NIDA) je leta 1995, potem ko je leta 1993 sprejel protokole in odobril I. fazo kliničnih študij na Univerzi v Miamiju, projekt na podlagi mnenj predstavnikov farmacevtske industrije ustavil (Alper, 2001).

Tako je težišče dogajanja v povezavi z ibogo v zahodnem svetu preneseno v sfero nevladnosti. Bolj ali manj izkušeni laiki, pa tudi nekateri strokovnjaki namreč organizirajo tretmaje za odvisnike, pogosto pa tudi za duhovne iskalce samorealizacije, ki pri sebi ne prepoznajo težav z odvisnostjo. Mar odvisnik, ki išče svobodo, ni duhovni iskalec samorealizacije? Tu se tudi zastavi vprašanje izrazoslovja; kaj ta tretma sploh je? Tretma, seansa, zdravljenje, iniciacija, verski obred ...?

Če bi pri ibogainski obravnavi govorili o zdravljenju, bi moral biti ibogain registriran kot zdravilo in izvajalec zdravnik. Kot nakazuje razumevanje in interpretiranje zdravja v uradnem zdravstvenem sistemu, registracije tudi v bližnji prihodnosti ni na obzorju.

Bitka z odvisnostjo pa je bitka s časom. Kaj naj torej odgovori zdravnik, če ga mlad odvisnik, ki izgublja letnike v šoli, izgublja prijatelje in pridobiva spremljajoče bolezni, vpraša, kaj meni o ibogainu!?

Gre za vprašanje odločitve in vprašanje odgovornosti.

Po načelu relativnosti ni končnega, pravega pristopa k zdravljenju, pravilen je tisti, ki je prirejen potrebam in pričakovanjem uporabnika. To velja bolj ali manj pri vseh specialnostih, še kako pa na kompleksnem področju (medicine) odvisnosti. Pravi je torej tisti pristop, ki vključuje bolnika kot sotvorca zdravstvene obravnave in ki ga ne obravnava kot neposvečeno telo, ki le natančno sledi navodilom.

Pravzaprav je odvisnost sama infantilen odnos do okolja, kjer odvisnik pričakuje, da bo nekaj zunanjega rešilo njegovo težavo; od dojke k drogi. Tako je že sama odločitev za ibogainski tretma, če je seveda svobodna, sama po sebi terapevtska. Spominja na primarni krik: Dovolj!!! in s tem preobrat iz trpne v tvorno pozicijo.

[9] Nekateri učinek iboge povezujejo s pospešenim odstranjevanjem tujih snovi oz. razstrupljevanjem telesa, drugi prisegajo na psihoterapevtski uvid, ki je posledica kontemplativnega stanja pod vplivom iboge. Navidezno razhajanje je semantične narave, ko se pogovarjamo o istih stvareh z različnim izrazoslovjem.

[10] »... kaj bi rad, da rečem? Te stvari so prikrite, midva pa sva na isti strani tančice. Ko ta pade, naju ne bo več tu« O. Hajam

Vseeno pa ima obravnava z ibogainom poleg elementa dejanja in odločitve tudi materialni element vnosa substance v telo. Tu bi bila negacija določenih tveganj neodgovorna in zavajajoča. Tako kot za vsako zdravilo tudi tu velja, da mora biti pričakovano tveganje preseženo s koristjo, da je uporaba upravičena. Korist vsakršnega zmanjšanja škode pri uporabi drog je neoporečna, pa najsi gre za abstinenco ali vsaj za prehod na manj škodljive oblike uporabe. Vsekakor pa je odločitev na strani uporabnika. Odgovornost na strani uradnih struktur je v tem primeru zagotoviti primerno obveščenost javnosti o možnih tveganjih in koristnih, kar je temeljni pogoj za objektivno odločitev.

Tako imenovana ibogainska medicinska subkultura po zadnjih podatkih močno narašča (Alper, 2008). Mimo tradicionalne in turistične uporabe ibogaina v Afriki je bilo na Zahodu v letih 2001–2006 dokumentiranih 3414 tretmajev. To je štirikratna porast v primerjavi s predhodno petletko; dodati je treba še številne nedokumentirane tretmaje. Skupina, ki je izvedla poizvedbo, je naravo tretmajev uvrstila v štiri skupine:

- medicinski model,
- tretma z laičnim vodstvom,
- samopomoč,
- religiozno-duhovni obred.

Motiv uporabnika je bil pri dveh tretjinah težava z odvisnostjo in pri eni tretjini duhovna rast.

Omenjena tretjina uporabnikov iboge ali ibogaina v kontekstu »medicinskosti« ni sporna. To so vsekakor fizično, psihično in socialno zdravi posamezniki, ki se za tretma odločijo iz duhovnih nagibov z namenom duhovne rasti.

Duhovnost je (tudi) iskanje svoje kontinuitete v večnost. Iboga, v tem primeru kot sveti zakrament, na dozdaj še nepojasnjen način omogoča jasnejši vpogled v človekovo preteklost, kot nam to omogoča spomin, objektivnejši pogled z distance na sedanjost in odpira vizionarsko tehtanje možnosti za prihodnost. Psihologi bi temu rekli uvid, a je termin preveč razumske narave, da bi lahko opisal to vidno, tipno in slišno izkušnjo večnosti časa, sočasnosti trenutkov in občutenja prisotnosti višjih načel iz zakulisja (Naranjo, 1973).

Izkušnja prinese vizijo, (ponovno) osvetli cilj in osmisli posameznikovo bivanje. Sporočilo je v obliki simbolov, ki so včasih transparentni, drugič pa prikriti in se razkrijejo šele čez čas. In človek se spomni sebe. V izvirnem besednjaku je izkušnja z ibogo potovanje v Bwiti, v deželo prednikov oz. v deželo mrtvih, kjer se človek spomni svojega poslanstva.[10]

Je lahko prebujeno (za)upanje kot posledica onkraj videnega tako močno, da zmanjša, skoraj izniči sindrom odtegnitve in utiša smrtni gon po drogi?

Glede pravnega statusa je večina držav sveta ibogainu naklonjena; v izvornih deželah je iboga celo čislana kot sveta rastlina in ekonomski vir dohodka.

Izjeme so ZDA, Belgija, Danska, Švedska, Švica, Avstralija, kot zadnja je ibogain na seznam kontroliranih substanc uvrstila Francija, in sicer po nesreči, ko je pod vplivom ibogaina utonil človek. Znanih je tudi nekaj primerov, ko so pri toksikoloških analizah obdukcij v bioloških tekočinah našli sled ibogaina, a je vloga le-tega pri vzroku smrti neznana.

Vprašanje varnosti vsekakor je na mestu in dejstvo, da ni bilo kontroliranih kliničnih testiranj ibogaina kot zdravila, mora biti evidentno vsakomur, ki se odloči za tretma.

Zainteresirani pa ima po drugi strani pravico tudi do naslednjega podatka: iboga se v Centralni Afriki stoletja uporablja v družinskem krogu in v ožji skupnosti, kjer obred zaužitja simbolizira sprejetje mladostnika v svet odraslih. Posredujejo in organizirajo ga starši, kar

nakazuje na empirično dokazano zadovoljivo stopnjo varnosti. Farmakovigolanca – preživetje zdravila na trgu – je v tem primeru zelo visoka in kljub nekaterim rasnim razlikam dopušča prenosljivost izkušenj na Zahod.

V Sloveniji je tako registrirana verska skupnost Sakrament Prehoda (sacrament.kibla.si), ki je odprta tako za ateiste kot za ljudi vseh veroizpovedi in se ne oznanja kot ... edina prava vera ... Prav tako ni ekskluzivna do odvisnikov, se pa distancira od definiranja iniciacije kot zdravljenja odvisnosti (Knut, 1994).

Slovenska javnost ima priložnost spoznati pojave tovrstnih subkultur predvsem po zaslugi socialno-antropološke raziskave Anžeta Tavčarja s Filozofske fakultete Univerze v Ljubljani, ki je z etnografskimi tehnikami opazoval iniciacijske obrede Sakramenta Prehoda in predstavil lokalno podkulturo na podlagi globalnosti »ibogainskega gibanja« (Tavčar, 2006).

Tudi domača znanstvena in strokovna prizadevanja so bila uspešna pri evalvaciji ibogaina kot zdravila. Center za mentalno zdravje, Ministrstvo za zdravstvo in Urad za zdravila so bili pred desetimi leti resen sogovornik za izpeljavo kliničnih testiranj, a je bil projekt, kot že rečeno, ustavljen.

Vseeno pa nadaljujemo predklinične raziskave na modelih odvisnosti, kjer OMI Inštitut (www.omi.si), zavod za antropološko medicino, združuje laboratorije Medicinske fakultete, Fakultete za farmacijo in Biotehniške fakultete Univerze v Ljubljani. V desetih letih je s podporo Urada za droge, Inštituta za varovanje zdravja, Ministrstva za zdravje, Mestne občine Ljubljana, Fakultete za socialno delo, Centra za mentalno zdravje, Fakultete za računalništvo in informatiko in Open Society Institute uspel osvetliti nekatere vidike delovanja ibogaina in se lahko pohvali z dobro uveljavljenostjo publikacij in predavanj v svetu tako v strokovni kot v laični javnosti.

Kot je bralcu sedaj že jasno, je cela »reč« okoli ibogaina zelo kompleksna in pogled nanjo precej zaznamovan z vnaprejšnjimi stališči in predsodki. Za nekatere je neodtuljiva pravica do verovanja, za druge z ustavo zajamčena pravica do gibanja – pač navzgor in navzdol in ne samo horizontalno iz službe domov in v gostilno, za tretje nepovabljeno obiskovanje Olimpa, ki si zasluži kazen ...

Iskalci sebe in svoje poti so stari kot človeštvo.[11] Za petami so jim vedno tudi ponudniki dobrin in storitev. Ibogainska scena je postala tržna niša, tako v smislu duhovnega turizma v Afriko, kot tudi v obliki številnih ponudnikov na Zahodu. Pojavi se neobhodno in potrebno vprašanje ohranjanja kakovosti ponudbe.

Ta pa je včasih zelo nizka. Prodaja ibogaina po internetu s skopimi navodili za domačo uporabo je daleč od standardov in protokolov, ki jih neformalno občestvo iboga scene ima in spoštuje. Pričakovati medicinsko izobrazbo vseh ponudnikov bi bilo v tem trenutku iluzorno in tudi nepotrebno, v primeru duhovnih iniciacij tudi popolnoma irelevantno. Pri religioznem obredu gre namreč za odmerek, manjši od detoksikacijskega, in so pri izpolnjevanju osnovnih meril zdravja tveganja neznatna, tj. primerljiva športnim tveganjem. Minimum za vodstvo katerekoli vrste tretmaja pa naj bi bila vsaj osebna izkušnja z ibogo in s tem razumevanje procesov pred tretmajem, med njim in po njem.

Potrebna povratna informacija javnosti kot varnostna varovalka glede kakovosti je lahko zagotovljena tudi sama po sebi s pluralizmom ponudbe, kjer gre za preživetje učinkovitih in za odpad neprimernih. S sodobno informacijsko tehnologijo je posredovanje informacij in ozaveščanje zelo olajšano in to ne bi smel biti problem. Vsebinsko strokovne in preverjene

[11] γνῶθι σεαυτόν (gnothi seauton) - spozn(av)aj se. Napis na Apolonovem preročišču v Delfih, kjer je prerokovala Pitija.

informacijske spletne strani lahko za minimalne stroške posredujejo verodostojne podatke in so stičišča za izmenjavo mnenj in izkušenj. Za kaj takega pa zadeva ne sme biti ilegalna in stigmatizirana kot nekaj sramotnega in napačnega.

Bližnjica do navidezne varnosti, ki se je že nič kolikokrat izkazala za slepo ulico, je prohibicija. Sto let izkušenj je dokazalo poslabšanje kakovosti in kontrole ponudbe ter neuspeh pri zmanjševanju uživanja substanc.

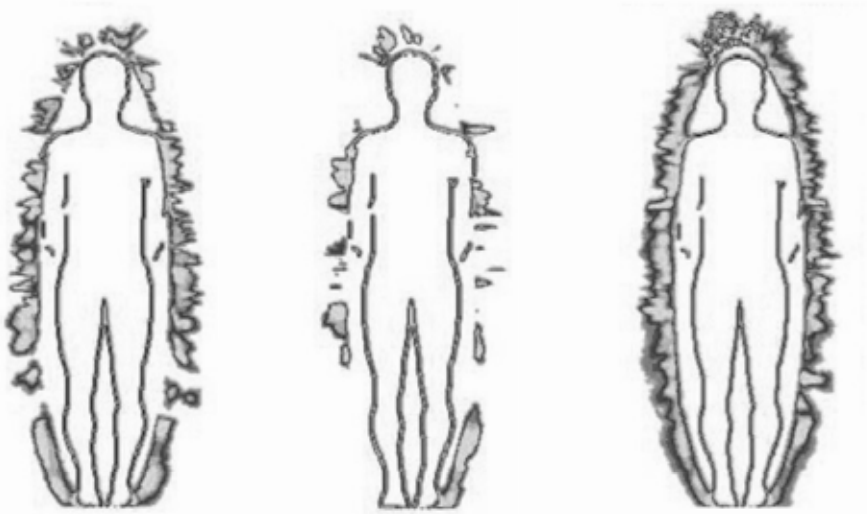
Trend izogibanja tveganju in neupravičenega precenjevanja varnosti na račun osebne svobode ogroža napredek; pa naj gre za osebnostni razvoj, ali pa za razvoj novih, učinkovitejših in cenejših metod zdravljenja oz. bolj zdravih in svobodnejših družbenih odnosov.

Vsekakor pa je treba nadaljevati znanstveno in strokovno evalvacijo ibogaina kot zdravila, ga primerno umestiti v javnozdravstveni okvir in s tem zadevo pravno urediti. Vsaj kar zadeva status in legitimizacijo zdravilnega potenciala substance; duhovni vidik tozadevno močno prednjači in je v Sloveniji formalnopravno urejen.

Pojav ibogainske subkulture je spontani odgovor na potrebe sodobnega človeka, na nezadovoljstvo nad pretirano materialistično naravnano medicino ne eni strani in na pretirano indoktrinirano duhovno ponudbo na drugi. Je eksistenčno vprašanje brez odgovora in je odgovor brez predhodnega vprašanja.

Bo Slovenija zbrala pogum in dovolj samozavesti, da povede posameznika sto let po dr. Klementu Jugu na vrh te gore? Tako kot se s tveganjem življenje lahko konča, se brez tveganja življenje niti začeti ne more ...

Slika: Indukcija energije na ravni celic se odraža na bioenergiji celega telesa – avri.
Primerjava avre človeka pred iniciacijo, med njo in po njej. (www.gape.org)



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2.4 INDUKCIJA ENCIMOV ENERGETSKEGA METABOLIZMA KVASOVKE *SACCHAROMYCES CEREVISIAE* IZPOSTAVLJENE IBOGAINU JE ADAPTACIJA NA AKUTNI PADEC ATP ENERGETSKE RAVNI

INDUCTION OF ENERGY METABOLISM RELATED ENZYMES IN YEAST *SACCHAROMYCES CEREVISIAE* EXPOSED TO IBOGAINE IS ADAPTATION TO ACUTE DECREASE IN ATP ENERGY POOL

Roman Paškulin, Polona Jamnik, Nataša Obermajer, Marija Slavić, Borut Štrukelj

European Journal of Pharmacology, 2010, 627: 131-135

POVZETEK

Ibogain je bil v zadnjih desetletjih predmet izčrpnih raziskav v povezavi z lastnostjo prekinjanja odvisnosti in zmanjševanja sle po drogi. V naši predhodnji študiji smo predstavili indukcijo encimov energetskega metabolizma podganjih možgan po aplikaciji ibogaina 20 mg/kg i.p. 24 in 72 ur pred proteomsko analizo. V tej študiji smo kultivirali modelni organizem kvasovko *Saccharomyces cerevisiae* ob prisotnosti ibogaina v koncentraciji 1 mg/l. Grozd encimov energetskega metabolizma t.j. gliceraldehid-3-fosfat dehidrogenaza, fosfoglicerat kinaza, enolaza in alkoholna dehidrogenaza so bili inducirani po petih urah izpostavitve. To je kompenzacija demonstriranega padca ATP ravni ob ibogainu. Kvas v stacionarni fazi rasti je uporaben model za študij osnovne presnove evkariontov, vključno človeka. Študija dokazuje, da učinek ibogaina na metabolizem ni ne vrstno, ne tkivo specifičen. Ta učinek ni posredovan preko v literaturi opisanih vplivov na receptorje, saj jih ta model nima.

European Journal of Pharmacology 627 (2010) 131–135

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Induction of energy metabolism related enzymes in yeast *Saccharomyces cerevisiae* exposed to ibogaine is adaptation to acute decrease in ATP energy pool

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ARTICLE INFO

Article history:
Received 1 June 2009
Received in revised form 23 September 2009
Accepted 13 October 2009
Available online 22 October 2009

Keywords:
Ibogaine
Tabernanthe iboga
Addiction therapy
Energy metabolism
Yeast
Proteomics

ABSTRACT

Ibogaine has been extensively studied in the last decades in relation to its anti-addictive properties that have been repeatedly reported as being addiction interruptive and craving eliminative. In our previous study we have already demonstrated induction of energy related enzymes in rat brains treated with ibogaine at a dose of 20 mg/kg i.p. 24 and 72 h prior to proteomic analysis. In this study a model organism yeast *Saccharomyces cerevisiae* was cultivated with ibogaine in a concentration of 1 mg/l. Energy metabolism cluster enzymes glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase and alcohol dehydrogenase were induced after 5 h of exposure. This is a compensation of demonstrated ATP pool decrease after ibogaine. Yeast in a stationary growth phase is an accepted model for studies of housekeeping metabolism of eukaryotes, including humans. Study showed that ibogaine's influence on metabolism is neither species nor tissue specific. Effect is not mediated by binding of ibogaine to receptors, as previously described in literature since they are lacking in this model.

1. Introduction

Ibogaine, an indole alkaloid present in the root bark of African plant *Tabernanthe iboga* has been extensively studied in the last decades in relation to its anti-addictive properties that have been repeatedly reported as being addiction interruptive and craving eliminative for opiates, stimulants, alcohol and nicotine (Alper et al., 1999; Maciulaitis et al., 2008). Although controlled clinical trials haven't yet been done, both lay and scientific literature suggest a certain level of ibogaine's efficacy (Lotsof, 2007; Alper et al., 2008). Claims are supported with results from *in vitro* studies and proven in animal models of drug addictions (Alper, 2001).

Multiple ligand binding and activity modulation actions of ibogaine on receptors, transporters and enzymes have been described in the scientific literature, in particular: 5-Hydroxytryptamine (5-HT), opioid, nicotinic and N-methyl-D-aspartate (NMDA) receptors, dopaminergic and 5-HT transporters and monoamine oxidase enzyme (MAO) (Alper, 2001; Glick et al., 2002; Leal et al., 2003).

Besides the effects on receptors, transporters and enzymes, the molecular aspects of ibogaine's influence on drug addictions concerning signal transduction and modulation of gene expression are becoming increasingly recognized (Ali et al., 1999; Onaivi et al., 2002). Consequent biochemical, neuroendocrine, structural and functional changes in terms of brain plasticity have been suggested (Ali et al., 1996; He et al., 2005; Carnicella et al., 2008).

Our recent work (Paškulin et al., 2006) showed the stimulating influence of ibogaine at a dose of 20 mg/kg i.p. on rat brain energy metabolism. We have observed changes in proteome at 24 and 72 h after i.p. application with induction of glycolysis and TCA cycle enzymes (glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase).

In the present study analysis of changes in proteome using 2-D electrophoresis was done again, this time on yeast *Saccharomyces cerevisiae* in stationary growth phase, at a concentration of 1 mg/l ibogaine in the media, which represents local bioavailability in brain tissue in previous experiment (Mash et al., 2000; Kontrimaviciute et al., 2006).

This cell suspension model doesn't show any cell differentiation, nor organization in tissue, and it lacks the influence on metabolism due to synaptic intercellular communication, as is the case in *in vivo* experiments. In spite of that, yeast is an accepted model for studies of basic metabolic pathways of higher eukaryotes, including mammalian cells (Ma, 2001; Menacho-Marquez and Murguia, 2007).

The aims of this study were to investigate if the effect of ibogaine is species and/or tissue specific and to find the cause for the induction of energy related enzymes.

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doi:10.1016/j.ejphar.2009.10.032

2. Materials and methods

2.1. Yeast strain and cultivation

Yeast strain S288C (*MATα mal gal2*) was used in this study. Cells were cultivated in YEPD medium with the following composition: 10 g/l glucose (Kemika), 5 g/l yeast extract (Biolife), and 5 g/l pepton (Oxoid), at 28 °C and 220 rpm to the stationary growth phase. Then cells were centrifuged for 5 min at 4000 rpm, washed once with 50 mM potassium phosphate buffer, pH 7.0 and suspended in the same buffer at a concentration of $1 \cdot 10^8$ cells/ml.

A fresh ibogaine stock solution (10 mg/ml) was added to the cell suspension to reach different concentrations: 0, 1, 4, and 20 mg/l. After 0.25, 0.5, 1, 2 and 5-h incubation at 28 °C and 220 rpm, samples were taken to measure cell energy metabolic status and intracellular oxidation of treated and non-treated cells. Protein profile of yeast cell extract was analyzed only at 5 h of exposure to the lowest concentration of 1 mg/l.

2.2. Two-dimensional electrophoresis

Cells were sedimented by centrifugation from 20 ml samples of the cultures and washed twice with 50 mM potassium phosphate buffer, pH 7.0. 0.1 g of cells (wet weight) was suspended in 0.5 ml extraction buffer (40 mM Tris–HCl, pH 8.0; 2% (w/v) 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 65 mM dithiothreitol (DTT)) containing protease inhibitor cocktail (Complete, Mini; Roche) — 1 tablet per 10 ml of buffer. The cells were disrupted by vortexing with glass beads five times, 1 min each with 1-min intervals for cooling the mixture on ice. The cell homogenate was centrifuged at 20000*g* for 20 min at 4 °C.

The protein content in the cell extracts was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Two-dimensional (2-D) 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 gradient (IPG) strips (GE Healthcare). After rehydration (13 h) isoelectric focusing (IEF) as first dimension was carried out at 20 °C on a Multiphor II (GE Healthcare). The following voltage program was applied: 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), the 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-PAGE as the second dimension was carried out with 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 stained with Sypro Ruby (Invitrogen). For each sample two 2-D gels were run at the same conditions.

2.3. Protein visualization and image analysis

2-D gels were recorded using the CCD camera G.BOX_HR (Syngene). Gel image analysis was done with the 2-D Dymension software version 2.02 (Syngene) and included spot detection, spot quantification, pattern aligning and matching. For all spot intensity calculations, normalized volume values were used. The results are expressed as a ratio of the normalized volume of protein spot in ibogaine-treated cells divided by normalized volume of matched protein spot in untreated control cells at the same time of exposure. Differences by a fold change >2 between treated and untreated cells were considered as significant.

2.4. Protein identification

The protein spots of interest were excised from the gels and analyzed by LC-MS/MS using an ESI-TRAP instrument. The Mascot software was used to search SwisProt 54.7 database. The following search parameters were applied: *S. cerevisiae* as species; tryptic digest with a maximum number of one missed cleavage. The peptide mass tolerance was set to ± 1.5 Da and fragment mass tolerance to ± 0.5 Da. Additionally, carbamidomethylation and oxidation of methionine were considered as possible modifications. Mascot protein scores greater than 29 were considered statistically significant ($P<0.05$).

2.5. Determination of cell energy metabolic status

Cell energy metabolic status was determined *via* the ATP pool by measuring luminescence with the commercially available kit BacTiter-Glo™ Microbial Cell Viability Assay (Promega) according to the manufacturer's instructions. Briefly, 100 µl of BacTiter-Glo reagent was added to 100 µl sample of cell culture and after 5 min luminescence was recorded using the microplate reader Safire II (Tecan). The results were expressed as a difference in ATP pool regarding the control.

2.6. Estimation of intracellular oxidation

Intracellular oxidation was estimated by using 2′,7′-dichlorofluorescein (H_2DCF), which is able to react with oxidant-reactive oxygen species. It is given as 2′,7′-dichlorofluorescein diacetate (H_2DCFDA), which easily penetrates the plasma membrane and is hydrolysed inside the cells by non-specific esterases. Non-fluorescent H_2DCF is oxidized to fluorescent 2′,7′-dichlorofluorescin (DCF), which is determined fluorimetrically (Jakubowski and Bartosz, 1997).

Cells were sedimented by centrifugation from 2 ml samples of the cultures, washed twice with 50 mM potassium phosphate buffer, pH 7.8, resuspended in the same buffer at a concentration of 1% (v/v) and preincubated at 28 °C for 5 min. H_2DCFDA was added as stock of 1 mM ethanol solution to a final concentration of 10 µM. After incubation (28 °C, 220 rpm, 20 min) 200 µl of cell suspension was transferred to the microplate and fluorescence was measured using the Tecan microplate reader Safire II (excitation and emission wavelengths of 488 and 520 nm, respectively). The results were expressed as a difference in reactive oxygen species level regarding the control.

3. Results

We have repeated our previous *in vivo* experiment with rats, this time on yeast *S. cerevisiae* cell culture in stationary growth phase with 1 mg/l of ibogaine in media, which mirrors peak mammalian brain tissue concentration after 20 mg/kg i.p.

Proteins from 5 h ibogaine-treated and control yeast cells were separated with 2-D electrophoresis and analyzed using the 2-D Dymension software. Of all protein spots that showed a significant change in intensity compared to control samples 12 spots that fall in the category of interest were excised and analyzed by LC-MS/MS, which gave sufficient confirmation of protein identity for five spots.

Proteins that were induced in yeast cells treated with ibogaine relative to control samples were identified as metabolic enzymes involved in glycolysis: glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase and alcohol dehydrogenase (6.3-, 4.6-, 3.8-, and 3.2-fold, respectively); and one as a member of antioxidant defence: superoxide dismutase (2.2-fold) (Fig. 1, Table 1).

To find the background of enzyme induction, ATP pool was measured in dose and time escalation manner. Results showed that immediately after exposure of yeast cells to ibogaine, the ATP pool measured by luciferine/luciferase test significantly falls in a dose dependent manner (Fig. 2A). It reaches the minimum at 30 min and then gradually returns towards control.

Fig. 1. Representative partial 2-D gel of proteins extracted from *Saccharomyces cerevisiae* cells: left – control cells, right – cells treated with 1 mg/l ibogaine after 5-h exposure. Labeled proteins were found to be upregulated and were identified by LC-MS/MS. Details are listed in Table 1.

Samples grown for 5 h in the presence of ibogaine at 1 mg/l concentration, also used for 2-D proteomic analysis, were washed with phosphate buffer. Eliminating the ibogaine from culture gradually brings ATP levels above the control ones (Fig. 2B).

Reactive oxygen species levels were simultaneously measured and we have observed immediate elevation of reactive oxygen species production after the ibogaine lasting up to 1 h, then inverted to decrease reaching the minimum at 2 h and this was followed by gradual return towards control (Fig. 3).

Additionally, enhanced synthesis of numerous unidentified types of low abundance proteins with relative shift of protein quantity towards low abundance fraction was observed and processed by computer analysis of existing 2-D gels, where a normalized volume value of 0.5% was a distinguishing criterion for a spot to enter low vs. high abundance group. Quantity of low abundance group was relatively enriched by a factor of 1.27 in treated sample compared to control while high abundance group being relatively impoverished. While high abundance group can be considered as a representative of structural proteins being constant at non-growing yeast cells in stationary growth phase, elimination of its relative diminution gives an increase factor of 1.76 for low abundance group (Table 2).

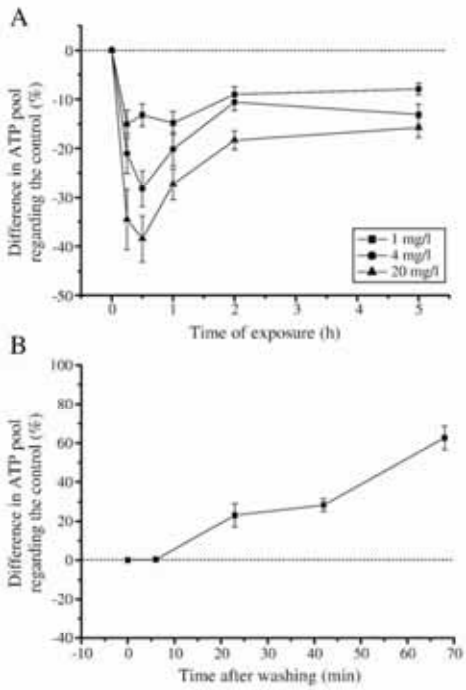

Fig. 2. A. Measuring of ATP pool during exposure of cells to different concentrations of ibogaine. Results are expressed as difference in ATP pool regarding the control and were measured in duplicate from two independent cultures for each concentration. The bars represent the averages ± S.E. B. Measuring of ATP pool after elimination of ibogaine from culture treated for 5 h at a concentration of 1 mg/l. Results are expressed as difference in ATP pool regarding the control and were measured in duplicate from two independent cultures. The bars represent the averages ± S.E.

4. Discussion

Molecular aspects of drug addictions are becoming increasingly recognized and findings suggest involvement of adaptation changes in gene expression patterns with the influence on cellular metabolism; to the very fundamental and ubiquitous housekeeping metabolism (Li et al., 2008). Reversibility of such changes is the platform for future anti-addiction treatments.

The induction of energy metabolism related enzymes due to ibogaine, previously triggered *in vivo* on rat model, was repeated on yeast *S. cerevisiae*, which is an accepted model for studies of primary metabolic pathways of higher eukaryotes (Ma, 2001; Menacho-Marquez and Murguia, 2007). A group of catabolism related enzymes was found to be induced after 5 h of exposure to ibogaine in a concentration of 1 mg/l (Fig. 1, Table 1); in higher proportion of induction and after shorter exposure compared to our previous study, assuming presently used concentration to be representative of brain tissue concentration in our previous experiment (Paškulin et al., 2006; Mash et al., 2000; Kontrimaviciute et al., 2006).

Ibogaine is known to affect numerous receptors and enzymes that are lacking in yeast cell. Therefore it is proved that enhanced expression of energy metabolism related enzymes is not mediated through receptor bindings, as previously described in the literature and it is not linked to cell differentiation or organization in tissue.

In search for a cause of enzyme induction, ATP pool and reactive oxygen species levels were investigated. ATP level falls to the minimum at 30 min of ibogaine's presence and then gradually returns towards control.

Table 1
List of *Saccharomyces cerevisiae* identified proteins whose expressions were stimulated by 1 mg/l ibogaine.

Spot enzyme	Swiss-Prot accession number	Fold ibogaine/control	Theor. M_r(Da)/pI	Matched peptides	Mascot score
1 Glyceraldehyde-3-phosphate dehydrogenase 3	P00359	6.3	35747/6.46	16	381
2 Phosphoglycerate kinase	P00540	4.6	44738/7.11	19	492
3 Enolase 2 (2-phosphopyruvate dehydratase 2)	P00925	3.8	46914/5.67	22	933
4 Alcohol dehydeogenase 1	P00330	3.2	36823/6.26	29	694
5 Superoxide dismutase (Cu–Zn)	P00445	2.2	15855/5.62	7	125

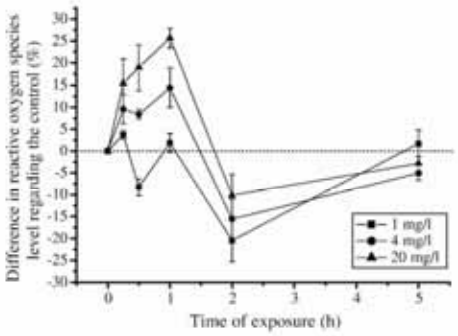

Fig. 3. Measuring of reactive oxygen species level during exposure of cells to different concentrations of ibogaine. Results are expressed as difference in reactive oxygen species level regarding the control and were measured in duplicate from two independent cultures for each concentration. The bars represent the averages ± S.E.

Table 2
Comparison of relative contents of low and high abundant protein fractions.

	Treated Σ normalized volumes (%)	Control Σ normalized volumes (%)	Ratio (treated/control)	Ratio (corrected)
High abundance proteins (normalized volume ≥ 0.5%)	36.398	50.103	0.726	1
Low abundance proteins (normalized volume < 0.5%)	63.602	49.897	1.275	1.756

Restoration of energy status is due to induced energy metabolism related enzymes being translated. Besides, some immediate alosteric feed-back stimulation of regulatory glycolytic enzyme activity due to low ADP/ATP ratio could be recognized, indicated by immediate elevation of reactive oxygen species as byproducts of energy metabolism. Latter fall of reactive oxygen species level compared to control is due to efficient endogenous antioxidative systems with quick onset, as shown by the induction of Cu–Zn SOD (Fig. 1). This shows a typical exercise rebound effect — reactive oxygen species load provocation exerts protective role by "alarming and awakening" of antioxidative defence (Jamnik et al., 2006; Hakkiwell and Gutteridge, 2007).

It was shown that the induction of energy metabolism related enzymes is not an event per se, but compensation to a transient dose dependent fall in ATP level in the first hours of exposure to ibogaine (Fig. 2A). Whether this fall is a consequence of lower ATP production or results from higher ATP consumption is answered by immediate increase in intracellular reactive oxygen species levels after the ibogaine (Fig. 3). Since reactive oxygen species are mainly products of ATP synthesis in mitochondria, results negate decreased production. Rather, they suggest enhanced consumption of ATP pool, which is insufficiently buffered by an immediate increase of production due to alosteric feed-back modulation of glycolytic enzymes. Only after sufficient translation of additional quantity of enzymes, levels of ATP approach control values.

Induction i.e. bigger amount of enzymes being the cause of elevated specific activity is additionally confirmed by rebound effect, when eliminating the ibogaine from milieu brings ATP levels of samples, previously grown in the presence of ibogaine, above the control ones (Fig. 2B).

In which processes the consumption of ATP is increased remains unclear. We have excluded ibogaine's toxicity to cells and consequent energy cost of repair (data not shown). Neither, energy is consumed for metabolism of ibogaine itself, since yeast does not have cytochromes P450 that are known to be responsible for degradation of ibogaine in human (Walker, 1998; Maciulaitis et al., 2008).

Enhanced synthesis of numerous low abundance proteins with relative shift to low abundance fraction (Table 2) was observed. This is by itself an anabolic process that requires energy, which additionally suggests that enlarged energy demands are the primary trigger for induction of enzymes.

What is the exact mechanism and purpose of this wide non-specific activation of transcription, translation and consequent metabolic changes remain unclear, but metabolic turnover acceleration with even further energy demands is suspected. Shifts in quantities of energy related enzymes with subsequent elevated energy availability affect all metabolic processes inside and outside of the cells of any type and functional state; directly by fuelling ATP dependent reactions and indirectly by facilitating the synthesis of functional units. This could facilitate different healing processes, including restoration of physiological homeostasis in functionally remodeled cells after the development of tolerance to drugs of abuse, termed detoxification.

It should be pointed out here that substance-related disorders are not just a matter of neuronal circuits, being tuned on a drug seeking, craving-reward cycles, but are also a matter of a single cell, being habituated to the presence of drug of abuse and missing it, when it is gone. Interference with energy supply might be the crucial meeting point of these diverse adaptations to different types of drugs. Briefly, ibogaine has the opposite effect on energy metabolism than most of the drugs of abuse, which after chronic use downcast cellular energy status (Ryman and Walsh, 1952; Sadava et al., 1997; Sharma et al., 2003). On the contrary, after acute deprivation the ibogaine's induction of enzymes supplies additional energy; the effect once triggered, not being dependent to the presence of the drug and thereafter lasting for a prolonged period of time (Fig. 2B).

The proposed mechanism of action extends indications of ibogaine for medical use beyond syndrome of addiction, since induced catabolism enzymes with accelerated metabolism turnover facilitates detoxification and renewal of tissues after numerous pathological conditions like reconvalescence after infectious diseases, recovery after trauma, general exhaustion of chronic systemic diseases, cancer cachexia, depression etc. Ibogaine could be an adjuvant, non-specific therapy in synergism with disease targeting drug.

Besides, rebound effect of elevated energy availability after washing ibogaine from culture represents human individual after treatment, elevated in mood, strength and will, being capable of exerting resistance to their addiction for a prolonged period of time. These life changing, mind opening properties are exactly what the ibogaine medical subculture votes for.

Acknowledgements

Ibogaine was donated by Sacrament of Transition, Maribor, Slovenia.

Special thanks to Prof. Dr. Francoise Bressolle, University of Montpellier for her help in the research.

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2.5 UČINEK TIANEPTINA NA SPONTANO IN S CA^{2+} POVZROČENO KRČENJE MATERNIČNE GLADKE MIŠIČNINE

TIANEPTINE'S EFFECTS ON SPONTANEOUS AND CA^{2+}-INDUCED UTERINE SMOOTH MUSCLE CONTRACTION

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Acta Physiologica Hungarica, 2012, 99: 140-147

POVZETEK

Tianeptin je novejši antidepresiv s primerljivo učinkovitostjo klasičnim antidepresivom. Dodatni ugodni učinki, ki vključujejo nevroprotektivno in protistresno delovanje ter zašito pred želodčno razjedo, niso popolnoma pojasnjeni, verjetno pa vključujejo vpliv na antioksidativni sistem. Tu smo preučevali učinek tianeptina na krčljivost izoliranih komponent podganje maternice in sestavne dele endogenega antioksidativnega zaščitnega sistema. Tianeptin v odvisnosti od odmerka povzroča spontane in s Ca^{2+} povzročene kontrakcije matrnične gladke mišičnine. Učinek je izrazitejši pri slednji. Tianeptin poveča aktivnost glutation peroxidaze (GSH-Px) in katalaze (CAT) pri spontani in Ca^{2+} vzpodbujani maternici. Pomemben padec glutation reduktazne (GR) aktivnosti pri obeh spontani in Ca^{2+} vzpodbujani maternici po aplikaciji Tianeptina nakazuje zmanjšano količino reduciranega glutationa in posledični pomik proti okidativnemu stanju. Pri spontano krčljivi maternici tianeptin povzroča padec baker-cink superoksid dismutazne (CuZnSOD) aktivnosti. Antidepresivni učinki tianeptina so morda dopolnjeni s prožitvijo kaskade celičnih adaptacij vključujoč inhibicijo gladkomišične krčljivosti in ustreznega antioksidativnega odziva.

Acta Physiologica Hungarica, Volume 99 (2), pp. 140–147 (2012)
DOI: 10.1556/APhysiol.99.2012.2.7

Tianeptine's effects on spontaneous and Ca^{2+}-induced uterine smooth muscle contraction

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Received: January 10, 2012
Accepted after revision: February 22, 2012

Tianeptine is a novel anti-depressant with an efficacy equivalent to that of classical anti-depressants. Additional beneficial effects include neuroprotection, anti-stress and anti-ulcer properties whose molecular mechanisms are still not completely understood but may involve changes in the anti-oxidant defence system. Herein, we have studied the effects of tianeptine on both contractile activity of isolated rat uteri and components of the endogenous anti-oxidative defence system. Tianeptine-induced dose-dependent inhibition of both spontaneous and Ca^{2+}-induced contraction of uterine smooth muscle. The effect was more pronounced in the latter. Tianeptine treatment increased glutathione-peroxidase (GSH-Px) and catalase (CAT) activities in spontaneous and Ca^{2+}-stimulated uteri. A significant decrease in glutathione-reductase (GR) activity in both spontaneous and Ca^{2+}-induced uterine contractions after tianeptine treatment indicated a reduction in reduced glutathione and consequently a shift toward a more oxidised state in the treated uteri. In spontaneously contracting uteri, tianeptine caused a decrease in copper-zinc SOD (CuZnSOD) activity. Tianeptine's anti-depressant effects may be accomplished by triggering a cascade of cellular adaptations including inhibition of smooth muscle contractility and an adequate anti-oxidative protection response.

Keywords: tianeptine, uterus, smooth muscle, contractility, antioxidative enzymes

Tianeptine (7-[(3-chloro-6, 11, dihydro-6-methyl-dibenzo [c,f] [1,2] thiazepin-11-yl) amino] heptanoic acid S-S dioxide, sodium salt) is a novel anti-depressant agent with an efficacy equivalent to that of classical anti-depressants (17). Its mechanism of action is quite different from that of tricyclic and other anti-depressants in that tianeptine increases serotonin reuptake both in brain and in platelets (18, 21, 26). Other therapeutic effects are related to modulation of the glutamatergic system (25, 31). Tianeptine inhibits the acute stress-induced increase in extracellular glutamate in the basolateral amygdala (34). Neuroprotective effects of tianeptine have also been reported (38). The anti-depressant activity of tianeptine is partly dependent on its ability to block stress-induced neuronal atrophy. Tianeptine has been demonstrated to prevent chronic stress-induced reduction of overall hippocampal volume (8), CA3 dendrites (39), hippocampal-dependent learning and memory (7) and to block chronic stress-induced increase in apoptotic cell death in the temporal cortex (23).

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A variety of pathophysiological processes including impaired transmitter metabolism, oligodendrocyte and mitochondrial dysfunction and dysregulation of receptor-mediated transmission via disturbed anti-oxidant and redox equilibrium have common denominators (5, 32). Bearing in mind the well-established link between transmitters metabolism and generation of reactive oxidative species (20, 33), it is tempting to speculate that tianeptine could have some direct metabolic effects and a part of tianeptine's neuroprotective ability could be connected with the anti-oxidative defence system. Recently, it has been shown that tianeptine inhibited complex I of isolated mitochondria at concentrations in excess of 20 μM and, thus, changed redox state (1).

As mechanisms underlying the activity of tianeptine are still not well understood, we explored tianeptine's effects on physiological function and anti-oxidant defence using non-vascular smooth muscle. The isolated uterus is a very suitable experimental model for this kind of study because of the presence of complex signal transduction systems, a high level of receptors and the redox sensitivity of smooth muscle (3, 28, 36). Tianeptine's effects on uterine contractile activity were studied and in the same tissue measurements of anti-oxidant enzyme activities were performed.

Material and Methods

Experimental system

All protocols for handling rats were approved by the local ethics committee for animal experimentation that strictly follows international regulations. Animals were kept at 22 °C, housed 3 per cage and fed *ad libitum*. Isolated uteri from virgin Wistar rats (200–250 g) in estrus were used. The estrous phase was determined by examination of a daily vaginal lavage (24).

Reagents

Tianeptine (Coaxil) was supplied by Les Laboratories Servier Industrie (Gidy, France). Salts for De Jalon's solution were obtained from Zorka Pharma (Sabac, Serbia), Merck and Centrohem d.o.o. (Stara Pazova, Serbia). All drugs were dissolved in ultra-pure water.

Isolated organ bath studies

All rats were killed by cervical dislocation. The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue and mounted vertically in a 10 ml volume organ bath containing De Jalon's solution (NaCl 154 mM, KCl 5.6 mM, $CaCl_2 \times 2H_2O$ 0.41 mM, $NaHCO_3$ 5.9 mM and glucose 2.8 mM), aerated with 95% oxygen and 5% carbon dioxide at 37 °C. The uteri, spontaneously active or Ca^{2+}-induced (the addition of 20% $CaCl_2$ to 6 mM final concentration in De Jalon's solution induces rhythmic contractile activity), were allowed to equilibrate at 1 g tension before addition of the experimental drugs.

After an equilibration period (about 30 minutes) when uteri achieved stable contractions, cumulative doses of tianeptine (0.03, 0.17, 0.23, 0.28, 0.43 and 0.51 mM) were added. The different concentrations of tianeptine were applied after another, without washing out the previous concentration. Controls were isolated, two hours incubated, nontreated uteri.

Myometrial tension was recorded isometrically with a TSZ-04-E isolated organ bath transducer (Experimetria, Budapest, Hungary).

After treatment, samples were immediately frozen using liquid nitrogen and then transferred to –80 °C until further analysis.

Determination of anti-oxidant enzyme activities

Thawed uteri were homogenised and sonicated in 0.25 M sucrose, 1 mM EDTA and 0.05 M Tris-HCl buffer pH 7.4 and centrifugated at 105 000 × *g* for 90 min. The supernatant was used to determine enzyme activities spectrophotometrically using a Shimadzu UV-160 spectrophotometer (Shimadzu Scientific Instruments, Shimadzu Corporation, Kyoto, Japan). Total superoxide dismutase (SOD) activities were determined by the adrenaline method (27). One unit of activity is defined as the amount of enzyme necessary to decrease by 50% the rate of adrenalin auto-oxidation at pH 10.2. Manganese SOD (MnSOD) activity was determined by incubating the samples with 8 mM KCN. Copper-zinc SOD (CuZnSOD) activity was calculated as the difference between total SOD and MnSOD activities. Catalase (CAT) activity was determined by the rate of hydrogen peroxide (H_2O_2) disappearance measured at 230 nm (4). The activity of GSH-Px was determined by a modified assay described by Paglia and Valentine (29). The reduction of *t*-butyl hydroperoxide by glutathione peroxidase (GSH-Px) was coupled with concomitant oxidation/reduction recycling of glutathione (GSH) by glutathione reductase (GR) using NADPH as a reducing agent. One unit of GSH-Px activity is defined as the amount of oxidised 1 nmol NADPH per min at 25 °C and pH 7.0. GR activity was determined using the method of Glatzle et al. (13). This assay is based on NADPH oxidation concomitant with GSH reduction. One unit of GR activity is defined as the oxidation of 1 nmol NADPH per min at 25 °C and pH 7.4. All enzyme activities were expressed as units/mg protein. The amount of protein was determined by Lowry method (22).

Data analysis and statistical procedures

Statistical analyses (descriptive statistics, analysis of variance – ANOVA and *F*-test) were performed using Statistical analysis software, version 9.1.3 (SAS Institute Inc., NC, USA). Effects of treatments on uterine contractions were calculated as the percentage of control untreated contractions. All data are expressed as the mean ± SEM. Data were analysed by two-way ANOVA (type of contractions and dose as factors), Boltzmann fitted and compared by the *F*-test. The activities of antioxidant enzymes were compared using one way ANOVA followed by the Tukey's HSD *post hoc* test (minimum significance was when $p < 0.05$).

Results

Tianeptine induced dose-dependant inhibition of spontaneous (Fig. 1) and Ca^{2+}-induced (Fig. 2) uterine activity (two-way ANOVA, significant dose effect, $p < 0.001$, Fig. 3). The degree of tianeptine-induced inhibition of spontaneous uterine activity was less than that found in Ca^{2+}-induced rhythmic activity (significant type effect ANOVA, $p < 0.001$) (Fig. 3). Moreover, tianeptine at the first applied dose (0.03 mM) increased spontaneous uterine activity compared to the non-treated period (up to 140 %), but further addition of tianeptine induced an inhibition similar to that obtained in Ca^{2+}-induced active uteri (Figs 1 and 3; statistical significance between the EC_{50} values (*t*-test) and slopes (*F*-test) was just beyond the significance limit – $p < 0.06$).

Tianeptine slightly reduced CuZnSOD activity (one-way ANOVA $p < 0.05$, significant $p < 0.05$ post hoc Tukey HSD effect) in spontaneously contracting uteri when compared to control treatment (Fig. 4). Tianeptine did not affect CuZnSOD activitity in Ca^{2+}-induced contracting uteri (one-way ANOVA non-significant effect, Fig. 4). Tianeptine caused an increase in CAT and GSH-Px activities in both spontaneous and Ca^{2+}-induced contracting uteri (significant one-way ANOVA $p < 0.001$, significant $p < 0.01$ post hoc Tukey HSD

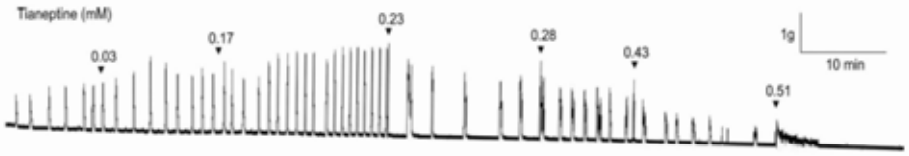

Fig. 1. The effect of increasing concentrations of tianeptine (0.03, 0.17, 0.23, 0.28, 0.43 and 0.51 mM) on spontaneous uterine contractions. An original trace is shown

Fig. 2. The effect of increasing concentrations of tianeptine (0.03, 0.17, 0.23, 0.28, 0.43 and 0.51 mM) on Ca^{2+}-induced contractions. An original trace is shown. Contractions were induced by addition of 20% (w/v) $CaCl_2$ (6 mM final concentration)

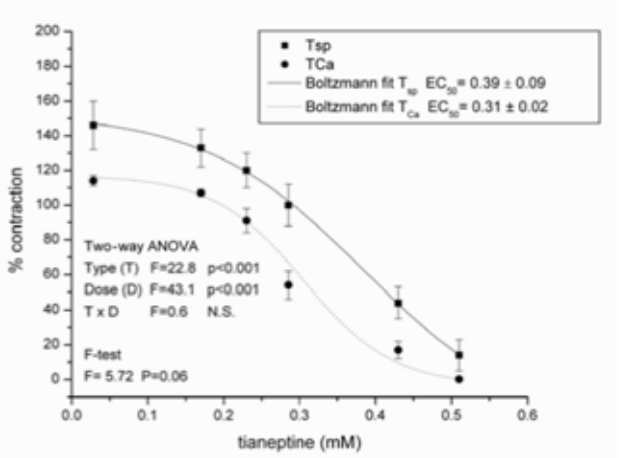

Fig. 3. Dose-response sigmoid fit curves for tianeptine-induced inhibition of spontaneous (Tsp) and Ca^{2+}-induced (TCa) rhythmic activity of the isolated rat uterus. Data are given as the percentage of control of untreated contractions and expressed as mean ± SE (n = 7). Effects were statisticaly compared by two-way ANOVA (treatments – treatment (t) and dose (d) as factors; F and p values are given. Both factors are significant at the level of $p < 0.001$). The sigmoid fit was performed according to the Boltzmann equation. The slopes of fitted curves have been testted by F-test ($p < 0.06$, non-significant effect). EC50 were calculated from Bolzmann fitted curves and compared by t-test ($p < 0.06$, non-significant effects)

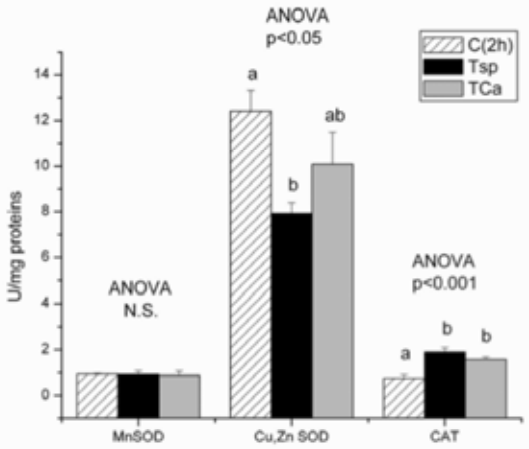

Fig. 4. The activities of MnSOD, CuZnSOD and CAT in two hours incubated controls [C(2h)] and tianeptine treated (0.03, 0.17, 0.23, 0.28, 0.43 and 0.51 mM) spontaneous (Tsp) and Ca^{2+}-induced (TCa) uteri. Data are expressed as mean ± SE (n = 7). Data were compared by one-way ANOVA and the post hoc Tukey's HSD test (different letters represent statistically significant differences $p < 0.01$)

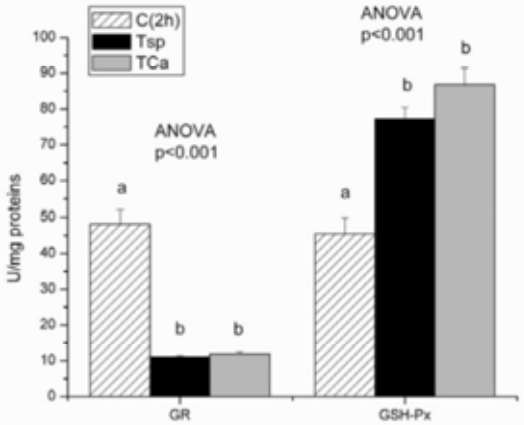

Fig. 5. The activities of GR and GSH-Px in two hours incubated controls [C(2h)] and tianeptine treated (0.03, 0.17, 0.23, 0.28, 0.43 and 0.51 mM) spontaneous (Tsp) and Ca^{2+}-induced (TCa) uteri. Data are expressed as mean ± SE (n = 7). Data were compared by one-way ANOVA and the post hoc Tukey's HSD test (different letters represent statistically significant differences $p < 0.01$)

effect) (Fig. 5). A significant decrease in GR activity after tianeptine treatment was also found in both spontaneous and Ca^{2+}-induced contracting uteri (significant one-way ANOVA $p < 0.001$, significant $p < 0.01$ post hoc Tukey HSD effect) (Fig. 5).

Discussion

Previous studies have suggested that the main mode of tianeptine action was related to serotonin- and glutamate-mediated neurotransmission (21, 25). Tianeptine has no effect on dopamine (D) or noradrenaline (NA) reuptake (26) and does not bind to 5-HT1A, 5-HT1B, 5-HT2 receptors, nor to pre-synaptic 5-HT receptors including α1, α2 and β adrenergic receptors; dopamine D2 receptors; GABA; glutamate receptors; histamine, muscarinic receptors and imipramine-binding sites or calcium channels (2, 15, 18, 26). Furthermore, it has been shown that tianeptine in acutely dissociated dorsal raphe neurons does not induce any current by itself suggesting no direct effect on ion channels (19). However, when the effects of tianeptine were studied in isolated rat stomach and colon preparations, in which neuronally mediated (predominantly cholinergic) contractions were evoked by electrical field stimulation, tianeptine concentration-dependent inhibition of these contractions in both stomach (0.3–10 μM) and colon (1–10 μM) was found (6). The inhibitory activity of tianeptine was unaffected by inhibitors of 5-HT and noradrenaline re-uptake, adenosine metabolism, nitric oxide synthesis and tryptophan dehydroxylase (6). Treatment of isolated uteri with increasing doses of tianeptine in our experiments also revealed dose-dependent inhibition of both spontaneous and Ca^{2+}-induced uterine smooth muscle contractions. Recent results have indicated tianeptine interaction with adenosine A1 receptors (37, 38). The expression of A1 adenosine receptors (AR) is cell specific, but results have shown that A1AR activation increases intracellular calcium in smooth muscle cells (9, 12). Increased spontaneous uterine activity after low tianeptine doses can be explained by possible stimulatory effects due to increased intracellular calcium concentrations. In Ca^{2+} activated uteri calcium concentrations are already elevated by exogenous added calcium and low doses of tianeptine might not additionally activate uterine contractions. However, it seems that after dose treshold other metabolic effects of tianeptine can be activated.

Tianeptine at concentrations above 20 μM inhibits mitochondrial beta-oxidation, complex I and the TCA cycle, changing redox equilibrium of cells and, thus, could promote oxidative stress (1, 10). Our results suggest that anti-oxidant enzymes responds to higher tianeptine doses by the elevation of its activity. Tianeptine caused a significant increase in CAT and GSH-Px activities. This may have been a consequence of elevated intracellular H_2O_2 levels. Our previous results demonstrated that isolated uteri exposed to H_2O_2 dose-dependently decreased contractions and was accompanied by an increase in GSH-Px activity (3). These results suggest that a part of tianeptine's inhibitory activity could be H_2O_2 mediated. Tianeptine is metabolized mainly by peroxisomal beta-oxidation of its heptanoic side chain where the high-potential electrons are transferred to O_2, which yields H_2O_2 (10). Intensive decomposition of H_2O_2 by increased GSH-Px activity is followed by extensive consumption of reduced glutathione suggesting state of oxidative stress. The significant decrease in GR activity after tianeptine treatment indicated a further decrease in reduced glutathione and conseqently a shift toward a more oxidised state in the treated uterus. Discrete differences at the level of anti-oxidant enzymes after tianeptine treatment depended on the type of uterine contractile activity. In spontaneously active uteri, higher H_2O_2 impact, in addition to a higher rate of H_2O_2 elimination, could impair CuZnSOD activity (16, 30). Therefore, decreased

CuZnSOD activity found in spontaneously active uteri after tianeptine treatment could be a consequence of H_2O_2's inhibitory activity.

Tianeptine possesses beneficial systemic effects. Clinical studies have shown that anxiolytic and anti-depressant drug therapy involving tianeptine is beneficial for patients with ulcers (depression involving psychotic and somatic symptoms is present in patients with gastrointestinal tract diseases) (11, 14, 35). However, our results of tianeptine's inhibitory effect on non-vascular smooth muscle suggested complex metabolic effects for *in vitro* doses above 20 μM. These implies that its effects could influence different cellular systems depending on the dose applied and on the multitargeted mechanisms of action.

Acknowledgements

This work was supported by a grant from the Ministry of Science and Technological Development of the Republic of Serbia, project No: 173014 "Molecular mechanisms of redox signalling in homeostasis: adaptation and pathology", University of Belgrade.

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Acta Physiologica Hungarica, Volume 99 (3), p. 364 (2012)
DOI: 10.1556/APhysiol.99.2012.3.13

ERRATUM

To the paper:

Tianeptine's effects on spontaneous and Ca^{2+}-induced uterine smooth muscle contraction

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published in Acta Physiologica Hungarica, Volume 99 (2), pp. 140–147 (2012) on page 140, the correct names and affiliations of the authors are:

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2.6 METABOLNA PLASTIČNOST IN UČINEK ENERERGETSKE VARČNOSTI IBOGAINA, GLAVNEGA ALKALOIDA RASTLINE *TABERNANTHE IBOGA*

METABOLIC PLASTICITY AND THE ENERGY ECONOMIZING EFFECT OF IBOGAINE, THE PRINCIPAL ALKALOID OF *TABERNANTHE IBOGA*

Roman Paškulin, Polona Jamnik, Tjaša Danevčič, Gordana Koželj, Rok Krašovec, Dijana Krstić-Milošević, Duško Blagojević, Borut Štrukelj

Journal of Ethnopharmacology, 2012, 143: 319-324

POVZETEK

Etnofarmakološki pomen
Skorja korenine rastline iboga - *Tabernanthe iboga* se v Centralni Afriki tradicionalno uporablja kot psihoaktivna substanca pri religioznih obradih, medtem ko je v manjših odmerki cenjena kot poživilo. Skorja korenine, izvleček ali čisti ibogain so na Zahodu poznani kot sredstvo proti odvisnosti in njihova uporaba narašča.

Cilj študije
Pretekle študije so pokazale prehodno zmanjšanje ATP ravni ob uporabi ibogaina, ki jo spremlja indukcija encimov energetskega metabolizma. Sedanja študija razkriva vzrok za to energetsko prikrajšanje in išče takojšnji in kasnejši vpliv na metabolizm. Celoten projekt poskuša razkriti skupen mehanizem delovanja pri vseh navidezno različnih namenih uporabe iboge, predvideva potencialne neželene učinke in ustvarja pogoje za njeno varno in koristno uporabo.

Materiali in metode
S plinsko kromatografijo smo merili hitrost podukcije ogljikovega dioksida (CO_2) kot markerja energetskega metabolizma kvasovke v aerobnih stacionarnih pogojih ob prisotnosti ibogaina v koncentracijah 1, 4 in 20 mg/l tekom petih ur. Celokupno oksidativno obremenitev smo florimetrično določili uporabaje 2,7-dikloroflorescein diacetat (H_2DCFA), *in vitro* antioksidanten potencial ibogaina pa smo določili s pomočjo 1,1-difenil-2-pikrilhidrazil (DPPH) testa.

Rezultat
Ibogain v odvisnosti od odmerka začasno poveča produkcijo CO_2. Povečana potrošnja energije kot zgoden učinek ibogaina je bil dokazan, saj se ATP raven kljub njegovi povečani proizvodnji sočasno zniža. Čeprav povečano celično dihanje proizvaja proste radikale, ibogain znižuje oksidativno obremenitev. Ker ibogain *in vitro* nima pomembnega

antioksidativnega delovanja, rezultat nakazuje njegov vzpodbujevalni učinek na fiziološki antioksidativni sistem.

Sklep

Uporaba iboge izzove preoblikovanje hišnega metabolizma. Ob uvodnih energetskih izdatkih to rezultira v povečani učinkovitosti fizioloških antioksidativnih sistemov, ki zmanjšujejo oksidativno obremenitev in energetske stroške bazalnega metabolizma. Ob sočasni indukciji katabolnih encimov se vzpostavi novo metabolno ravnovesje, ki varčuje z energijo, v primeru dodatnih potreb pa omogoča njeno povečano razpoložljivost. Zdrav organizem lahko tako vzdrži večje fizične in mentalne napore brez tveganja stresne preobremenitve. Po istem načelu iboga omogoča hitrejše okrevanje v primeru bolezni, vključno z motnjo odvisnosti.

Journal of Ethnopharmacology 143 (2012) 319–324

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

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

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ARTICLE INFO

Article history:
Received 18 December 2011
Received in revised form
14 April 2012
Accepted 22 June 2012
Available online 30 June 2012

Keywords:
Tabernanthe iboga
Ibogaine
Addiction
Energy metabolism
Antioxidative system
Adaptogen

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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http://dx.doi.org/10.1016/j.jep.2012.06.039

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 Odič 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

the dark for 20 min at room temperature. Thereafter, the absorbance was measured at 517 nm. All tests were performed in triplicate, with trolox and ascorbic acid as physiological controls. The percent inhibition of the DPPH radical by ibogaine was calculated according to the formula:

$$\%\text{Inhibition} = [(A_{\text{blank}} - A_{\text{test}})/A_{\text{blank}}] \times 100$$

where A_{blank} is the absorbance of the DPPH in solution without test sample (antioxidant), and A_{test} is the absorbance of DPPH in solution with ibogaine.

2.6. Yeast viability

Cell viability was measured as cell-membrane integrity using LIVE/DEAD Funga Light™ Yeast Viability Kits (Molecular Probes), according to the manufacturer instructions. Briefly, the cells from 1 ml cell cultures were centrifuged (14,000 × *g*, 5 min) and washed once with filtered PBS, and cell suspensions at 1×10^6 cells/ml were prepared in PBS. Then 1 μl SYTO® 9 and 1 μl propidium iodide were added in the dark and the samples incubated at 37 °C for 30 min. After the incubations, the fluorescence was measured using a microplate reader (Tecan). The excitation/emission wavelengths for these two dyes are 480/500 nm for SYTO® 9 and 490/635 nm for propidium iodide.

2.7. Ibogaine kinetics

Yeast cells at a concentration of 1×10^8 cells/ml were suspended in different ibogaine buffer solutions (0, 1, 4, 20 mg/l) and incubated at 28 °C. Samples were taken in 15 min intervals, prepared and adequately diluted for analysis according to a modified method which was initially developed for the determination of ibogaine and noribogaine (internal standard prazepam) in biological samples (Koželj, 2010). The compounds were separated on Zorbax XDB-CN (75 mm × 4.6 mm i.d, 3.5 μm) by using an Agilent 1100 HPLC system and detected in the tandem quadrupole mass spectrometer Quattro micro™ API from Waters, the software used was MassLynx 4.1. All samples and standards were treated adequately to prevent decomposition of ibogaine and noribogaine due to daylight exposure.

3. Results

We have proceeded with our previous *in vivo* experiments on Wistar rats and yeast *Saccharomyces cerevisiae* in stationary growth phase in relation to ibogaine's influence on energy metabolism. In the present experiment, yeast in the same metabolic state was treated with 0, 1, 4 and 20 mg/l of ibogaine and CO_2 production was measured at 0.25, 0.5, 1, 2, 3, 4 and 5 h time points. Immediately after addition of ibogaine there was a dose dependent raise in CO_2 production with peak values of 16, 67, 142% (1, 4 and 20 mg/l of ibogaine, respectively) relative to control, which ceased in an hour and raised again between 2 and 4 h with second peak of 15, 11, 27% (1, 4 and 20 mg/l of ibogaine, respectively) at 3 h time point. After 3 h there was a progressive decline of CO_2 production crossing the control values at 4 h and dropping further until the end of experiment at 5 h, when reduction of the energy metabolism of 10, 9 and 31% (1, 4 and 20 mg/l of ibogaine, respectively) relative to the control was observed and further diminution expected through extrapolation from late trends (Fig. 1).

The total oxidative load in the enhanced catabolism part of the experiment was decreased by 24, 23, 57% (1, 4 and 20 mg/l of ibogaine, respectively) relative to the control (Table 1). Influence of the ascorbic acid in *in vitro* equipotent concentrations

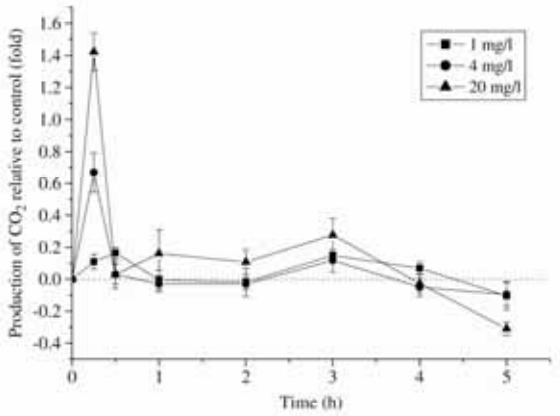

Fig. 1. Time dependent CO_2 production in yeast under 1, 4 and 20 mg/l of ibogaine in media. Results are expressed as the relative change in CO_2 production between exposed and control cells at each time point. They were collected from four independent cultures for each concentration. The data represent average values and standard errors.

Table 1
Concentrations of ibogaine in yeast cytosole after equilibrium, the influence of ibogaine at different concentrations on the total oxidative load and the influence of *in vitro* equipotent concentration of ascorbic acid are represented. The values represent averages and standard errors and are results of the experiment in triplicates.

Calculated ibogaine concentration [mg/l]	Ibogaine concentration in cytosol [mg/l]	Oxidative load ratio treated/control [%]	Ascorbic acid concentration [μM]	Oxidative load reduction by ascorbic acid
1	0.83 ± 0.03	76.26 ± 1.69	1	Non-significant
4	3.89 ± 0.06	76.67 ± 1.56	2	Non-significant
20	18.14 ± 0.34	43.45 ± 1.30	4	Non-significant

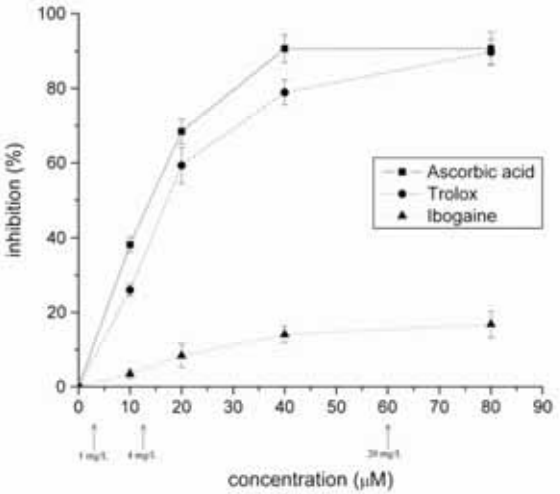

Fig. 2. *In vitro* anti-oxidant properties of ibogaine represented in molar concentrations. Results are expressed in percentage of DPPH reduction and were measured in triplicates. The bars represent averages and standard errors.

estimated in Fig. 2 to be 1, 2 and 4 μM was used as a positive control and no significant influence on oxidative load was found.

Ibogaine's intrinsic antioxidative potential was measured *in vitro* by DPPH test. Molar concentrations were used to compare with Trolox and ascorbic acid efficacy. No significant direct antioxidant properties of ibogaine were found in concentrations, relevant for experiment (Fig. 2).

The viability of yeast cells was examined by cell-membrane integrity test which excluded toxicity due to ibogaine presence at any concentration of concern (data not shown). Also, cell's morphology and growth after the treatment didn't show any deviations from control.

Ibogaine kinetics was examined in the yeast in phosphate buffer for different concentrations of ibogaine. Concentrations inside and outside the cells reached the balance in 2 h with a half of shift occurred in 15 min after beginning of the experiment. The results of measurements after equilibrium are shown in Table 1. Ibogaine as a highly lipid soluble molecule freely enters the cell and accumulates in the membranes causing a slight drop in concentration of water dissolved fraction. No noribogaine, a principal metabolite of ibogaine in humans was found neither in intracellular nor extracellular compartment at any time during the experiment.

4. Discussion

In our first work (Paškulin et al., 2006) induction of energy related enzymes in rat brain was demonstrated as a consequence of ibogaine administration. In our previous work (Paškulin et al., 2010) we have demonstrated similar results of ibogaine's influence on energy metabolism cluster in yeast *Saccharomyces cerevisiae*, while following the changes in ATP pool showed its transient reduction in a dose dependant manner. In the present experiment, the influence of ibogaine on metabolism was further studied on yeast in the stationary phase under aerobic conditions, this time by measuring the rate of CO_2 production and followed by search for energy consumers.

4.1. Energy metabolism acceleration

Transient oxidative energy metabolism acceleration was directly confirmed by increased CO_2 production after ibogaine exposure, in a dose dependant manner. Interestingly, this elevation is not permanent but shows rather interesting dynamics with biphasic elevation of CO_2 production, followed by the calming of catabolism at the end of the experiment.

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

4.2. Possible energy consumers

4.2.1. Oxidative stress

Parallel to observation of the catabolism the level of oxidative stress as the sum of produced ROS was tracked under the same conditions. Surprisingly, we have observed that apart from undisputable increase in ROS formation due to stimulated ATP production (Halliwell and Gutteridge, 2007) there was a significant drop in the total oxidative load on the cell (Table 1). Since ibogaine doesn't show any significant *in vitro* antioxidant activity at the concentrations of concern and due to the failure of *in vitro* equipotent concentration of ascorbic acid to exert such effect *in vivo*, the impact on the physiological antioxidative systems must be responsible for such improvement, in a pro-antioxidant manner. Unlike the antioxidants that directly scavenge free radicals, pro-antioxidants act indirectly either by modulation of direct agents or by regulation of the biosynthesis of antioxidant proteins (Dinkova-Kostova and Talalay, 2008; Stevenson, 2012; Vertuani et al., 2004). Indeed, in our previous work (Paškulin et al., 2010) we have observed the 2,2 fold induction of Cu–Zn SOD enzyme expression after ibogaine treatment. Therefore, the reduction of oxidative load lowers energy expenses for cellular maintenance and saves the energy. This correlates with the later reduction of metabolic turnover as seen in Fig. 1.

4.2.2. Ibogaine kinetics

Energy consumption due to the ibogaine uptake cannot be responsible for energy load since active transport by specialized ATP coupled transporters is highly improbable at simple yeast model (Walker, 1998). Besides, it would show constant inward pumping effort opposing the escape of highly lipid soluble molecule (Maciulaitis et al., 2008) that would be presented as a constant rise in CO_2 production, rather than being expressed as biphasic production acceleration with latter inversion.

Redox and energy linked metabolism of ibogaine was checked regarding transformation to noribogaine, in mammals being catalyzed by CYP 450 enzyme reduction. This reaction is not possible since yeast does not posses CYP 450 system (Walker, 1998). Also, we have not found any measurable levels of noribogaine in the yeast cells, treated with ibogaine. Other possible degradation products were not undoubtedly excluded but any kind of energy coupled degradation would again manifest itself by constant CO_2 production elevation, prolonged far beyond our experiment until all ibogaine being metabolized.

4.2.3. Ibogaine toxicity

The ibogaine's toxicity with potential energy cost of cellular repairs was excluded by the test of cellular membrane's integrity and even further excluded by observations of normal growth and morphology after ibogaine exposure (data not shown). Also, literature denies antimicrobial activity of indole alkaloids from Apocynaceae family against yeast (Verpoorte et al., 1983).

Since transient, intermittent effort of coupled oxidative energy catabolism to compensate ATP pool diminution is not a permanent effect of ibogaine's presence, its toxicity or kinetics are not expected to be the subject of energy expenditure. Rather, a trigger mechanism is suspected where ibogaine serves as an elicitor of some finite energy consuming process. In our recent work (Paškulin et al., 2010) we have found induction of low abundance, functional protein fraction in yeast (including energy metabolism and antioxidative system enzymes in question), whose synthesis seems to be responsible for these energy expenditures. Deeper insight into the systems biology of complete proteome changes needs to be done for profound understanding of the ibogaine effect.

4.3. Acute effect

The ibogaine initiates the energy consuming process and manifestation of the effects depends on capability of catabolism to compensate the energy outputs in this dynamic equilibrium. This might be the case with low doses quickly gaining moderate improvement of physical and mental performance in a stimulant manner, while higher doses initially overcome cellular catabolism buffering capacity with energy flux being exclusively occupied with metabolic plasticity, not leaving much of free energy for physical activities. This puts the user in a period of lethargy under high ritual doses while full invigorating effect appears later (Fernandez, 1982).

Mind altering property of iboga might also be mediated by this mechanism of reduced energy availability (Magistretti, 2006).

Brain activity modulation due to interference of ibogaine in neurotransmitter release, action and reuptake extensively described in literature is undoubtedly involved in perception and cognitive shifts, but also reduced disposable energy has its implication. Brain cortex as energetically most demanding tissue and a site of cognition as well as psychological inhibitions can in case of ATP pool reduction move into a different mode of action. Decreased effectiveness of K/Na-ATPase due to acute lack of ATP might cause changes in electroencephalogram (EEG) patterns. This includes observed theta and delta trance state that precipitate subconscious psychological material into the awareness (Binienda et al., 2011; Strubelt and Maas, 2008).

Many factors influence the efficacy and safety of ibogaine use and so the justification of its use. Detoxification from drugs of abuse or any other recovery after illness is a restitution of physiological balance that by itself represents considerable energy load. Although ibogaine quickens such adaptation, such aid and its justification have its limits in terms of energy overload. Accelerated detoxification of severe addiction with the use of high ibogaine dose can overcome the body's buffering capacities and result in complications, so medical surveillance during such treatments is highly recommended.

4.4. After-effect

Under the cost of transitional energy expenditures ibogaine enables changes in proteome with the shift to a more economical and cytoprotective metabolic equilibrium. Besides, induced energy metabolism related enzymes serve as an extended energy source for a prolonged period of time. Resulting metabolic state is of special value in state of elevated energy demands under different stress in an adaptogen manner.

4.5. Ibogaine and anti-addiction effect

Tolerance is adaptation of an organism to the presence of drugs and their withdrawal causes the abstinence syndrome. Physical weakness with lack of will is recognized as one of symptoms. Besides subjective descriptions as "run out of gas", literature describes choking influence of diverse drugs of abuse on the energy metabolism (Chen et al., 2007; Hargreaves et al., 2009; Ryman and Walsh, 1951, 1952; Sadava et al., 1997). Reversibility of such changes is the platform for future anti-addiction treatments.

The prolonged anti-addiction effect of ibogaine at least partially consists of improved energy accessibility that can be considered as stabile metabolic shift in the epigenetic landscape (Huang et al., 2009; Waddington, 1957). While escaping the genetic determinism (Noble, 2006) such recognition of ibogaine as a causal remedy puts a question mark upon the definition of addiction as a chronic and relapsing disorder.

Nevertheless, full benefit of iboga use arises only from conjunction of its invigorating quality with the spiritually initiated intent for a life change—either initiating adulthood or quitting addiction (as if there was any difference…).

5. Conclusions

The increased energy consumption is confirmed to be the cause of acute energy depletion due to ibogaine. The proteome changes including induction of energy metabolism and antioxidative enzymes are responsible for initial energy expenditures. After the shift is accomplished the new metabolic equilibrium results in improved fitness.

As the dose distinguishes remedy from poison, the same is true for beneficial eustress influence of the adaptogen that can be overcame by adverse distress of metabolic overload. Special attention must be paid to pace at which the adaptation from one metabolic equilibrium to another is conducted.

Acknowledgements

Special thanks Prof. Francoise Bressolle, University of Montpellier for the donation of noribogaine standard. Special thanks to Sacrament of Transition, Maribor, Slovenia for the donation of ibogaine HCl. Special thanks to Miss Irena Ocepek for designing the graphical abstract.

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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

Preučevali smo vpliv ibogaina, ključnega alkaloida iz skorje korenine grma iboga na izražanje genov v možganih Wistar podgan in kvasovke *Saccharomyces cerevisiae*, ki je modelni organizem za študij metabolizma evkariontskih celic, vključno človeka (Ma, 2001).

Tropska rastlina *Tabernanthe iboga* se v Centralni Afriki uporablja kot tradicionalno zdravilo in obredna droga. V nižjih odmerkih deluje kot poživilo, povečuje odpornost na stres in pospešuje okrevanje po bolezni. V višjih odmerkih sproži stanje transa, ki posreduje uvid v duhovnem smislu in odgovarja na eksistencialna vprašanja. Iniciacija z zaužitjem iboge je družbeno sprejet obred prehoda mladostnika v odraslost s polno močjo in odgovornostjo in je analogija zahodnjaški maturitetni simboliki. Zaužitje substance ima tako farmakološko, simbolno, sociološko, vzgojno in duhovno komponento. Zahod je ibogo spoznal kot sredstvo za prekinjanje zasvojenosti. Preseneča večplastno delovanje iboge t.j. olajšano razstrupljevanja telesa s ponovno vzpostavitvijo fiziološkega ravnovesja, odpravljanje prisilnih vedenjskih vzorcev in doseganje duhovnega blagostanja. Vstopno vprašanje teze se je glasilo: »Ali se duhovna izkušnja odraža na materiali ravni; kaj jo v biokemijskem smislu sproži in kakšne so njene presnovne posledice?«

Kljub številnim znanstvenim objavam, ki opisujejo vpliv ibogaina na različne receptorje, encime, transporterje in hormonske osi, mehanizem delovanja ni do kraja pojasnjen (Alper, 2001). Zanimiv je predvsem podaljšan učinek iboge, ki sega daleč onkraj same prisotnosti substance v organizmu. Kljub lipidotopnosti ibogaina s kinetiko dvorazdelnega sistema in posledično relativno dolgo razpolovno dobo ibogaina in njegovega aktivnega metabolita noribogaina, trajne spremembe bivanjskega modusa, katero opisujejo številni opisi primerov, ni mogoče razložiti z neposrednim farmakodinamskim učinkovanjem same substance (Mash in sod., 2000). To je mogoče le kot posledica temeljitih strukturnih in funkcijskih sprememb sproženih s strani iboge; torej novo metabolno ravnovesje, ki je sposobno samovzdrževanja. Gre za premik v epigenetski pokrajini (Waddington, 1957).

Metoda izbora je bila dvodimenzionalna elektroforeza proteinov, saj je ta metodologija primerna za sam vstop v raziskavo, kadar na samem začetku zaradi pomanjkanja ustreznih podatkov ni moč postaviti hipoteze (Gorg, 1991). Po identifikaciji sprememb proteoma pri podganah t.j. indukcija encimov energetskega metabolizma smo uspešno ponovili rezultate na modelu kvasovke, kjer smo dodatno odkrili še stimulacijo endogene antioksidativne obrambe in pa nespecifično aktivacijo frakcije nizko zastopanih proteinov. Nadalje smo iskali vzrok za aktivacijo energetskega metabolizma. Možnosti sta dve; ali je indukcija neposredna posledica delovanja ibogaina na genom, ali pa posredna reakcija celice na potencialno energetsko deprivacijo zaradi ibogaina. Zato smo preverili energetski status celic z metodo merjenja luminiscence luciferin/luciferaznega testa, ki pokaže količino ATP molekul, t.i. ATP raven. Zasledili smo prehodni padec ATP ravni po aplikaciji ibogaina. Postavilo se je novo vprašanje; ali je ta padec posledica povečane porabe ATP ali

zmanjšanje njegove proizvodnje? Odgovor smo najprej dobili posredno z merjenjem nastajanja prostih radikalov, ki so stranski produkt oksidativne fosforilacije oz. sinteze ATP, ki smo ga določili z uporabo 2,7-diklorofluorescein diacetata (H_2DCFDA) in merjenjem fluorescence njegovega oksidiranega metabolita. Prehodno povečanje nastajanja prostih radikalov je nakazovalo povečano proizvodnjo ATP, vendar pa je graf pokazal kasnejši padec oksidativne ravni v celicah, kar bi lahko bila posledica odbojnega učinka provokacije in aktivacije endogenih antioksidativnih sistemov v smislu hormeze, kar nakazuje tudi zaznana indukcija encima superoksidne dismutaze. Dokončno smo povečan katabolizem potrdili z merjenjem nastajanja oglikovega dioksida s plinsko kromatografijo, pro-antioksidativno delovanje ibogaina pa z določitvijo celokupne oksidativne obremenitve v času eksperimenta ob izključitvi intrinzične antioksidantne narave samega ibogaina z merjenjem stopnje reducije 1,1-difenil-2-pikrilhidrazila (DPPH).

Odprto je ostalo vprašanje porabnika energije. Energetska obremenitev po vnosu tuje substance najprej sugerira stresni učinek. Poškodbo celic smo izključili z metodo merjenja membranske integritete. Tudi obremenitve zaradi same kinetike prerazporejanja so izključene, saj ibogain neodvisno prehaja membrano. Energetsko vezana presnova samega ibogaina je bila izključena z odsotnostjo njegovih metabolitov. Najverjetneje gre za energetsko obremenitev zaradi aktivacije sinteze encimov, odprto pa ostaja vprašanje deleža te obremenitve zaradi sinteze encimov energetskega metabolizma. Padec energije namreč aktivira sintezo katabolnih encimov, kar pa je samo po sebi dodatna energetska obremenitev, ki se nadalje odraža z dodatno aktivacijo transkripcije in translacije… Še vedno je možno, da je prvi dogodek direktna elicitorska aktivacija grozda energetskega metabolizma ali pa gre prvenstveno za energetsko obremenitev drugega tipa in je posredna aktivacija le pridružena osnovnemu dogodku.

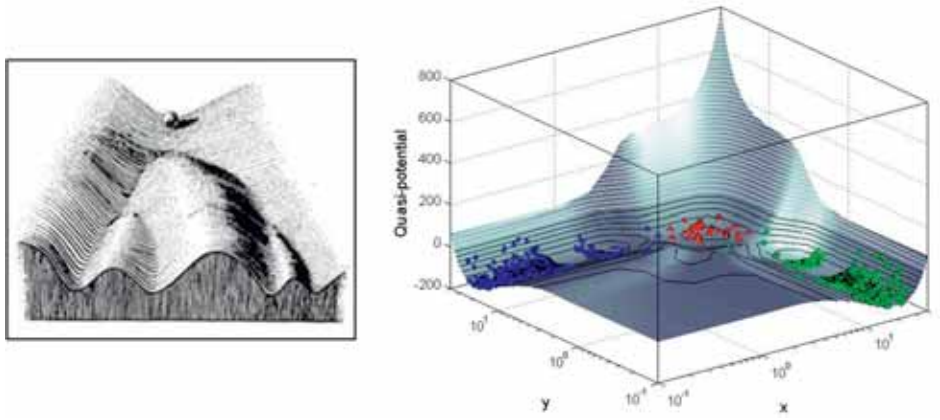
Potrditev *in vivo* rezultatov na modelu kvasovke je dokazala, da indukcija encimov in metabolni pomik ni posledica v literaturi opisane vezave ibogaina na receptorje sesalskih celic in da ni ne tkivno, ne vrstno specifična.

Dokazali smo, da ibogain prehodno poveča porabo energije za sintezo številnih encimov, med drugim tudi samih katabolnih in antioksidativnih encimov. Kljub povečanju metabolnega obratu in sočasni proizvodnji prostih radikalov ibogain znižuje oksidativno obremenitev. Ker ibogain *in vitro* nima pomembnega antioksidativnega delovanja, rezultat nakazuje njegov vzpodbujevalni učinek na fiziološki antioksidativni sistem kot pro-antioksidant, kar dokazuje tudi povečanje količine antioksidativnih encimov. Dolgoročno se to kaže v preoblikovanju hišne presnove. Ob uvodnih energetskih izdatkih to rezultira v povečani učinkovitosti fizioloških antioksidativnih sistemov, ki zmanjšujejo oksidativno obremenitev in s tem energetske stroške obnove oksidativno poškodovanih celičnih struktur. Ob sočasni indukciji katabolnih encimov se vzpostavi novo metabolno ravnovesje, ki varčuje z energijo, v primeru dodatnih potreb pa omogoča njeno povečano razpoložljivost. Zdrav organizem lahko tako vzdrži večje fizične in mentalne napore brez tveganja stresne preobremenitve. Po istem načelu iboga omogoča hitrejše okrevanje v primeru bolezni, vključno z motnjo odvisnosti.

3.2 SKLEPI

Študija je prikazala prehodno zmanjšanje ATP ravni ob uporabi ibogaina, ki jo spremlja indukcija encimov energetskega metabolizma in antioksidativnih sistemov, razkriva vzrok za to energetsko prikrajšanje in nakazuje takojšnji in kasnejši vpliv na metabolizem.
Uporaba iboge izzove preoblikovanje hišne presnove. Ob uvodnih energetskih izdatkih to rezultira v povečani učinkovitosti fizioloških antioksidativnih sistemov, ki zmanjšujejo oksidativno obremenitev in energetske stroške celične obnove. Ob sočasni indukciji katabolnih encimov se vzpostavi novo metabolno ravnovesje, ki varčuje z energijo, v primeru dodatnih potreb pa omogoča njeno povečano razpoložljivost (Slika 1). Zdrav organizem lahko tako vzdrži večje fizične in mentalne napore brez tveganja stresne preobremenitve. Po istem načelu iboga omogoča hitrejše okrevanje v primeru bolezni, vključno z motnjo odvisnosti. Gre za stabilni premik v epigenetski pokrajini (Slika 2).

Slika 1: Ilustrativni prikaz ATP energetske ravni ob in po ibogainu (Paškulin in sod., 2012)
Figure 1: Illustration of ATP energy pool under and after ibogaine (Paškulin et al., 2012)



Slika 2: Ilustrativni prikaz epigenetske pokrajine (Bhattacharya in sod., 2011)
Figure 2: Illustration of epigenetic landscape (Bhattacharya et al., 2011)

4 POVZETEK

Skorja korenine rastline iboga - *Tabernanthe iboga* se v Centralni Afriki tradicionalno uporablja kot psihoaktivna substanca pri religioznih obredih, medtem ko je v nižjih odmerkih cenjena kot zdravilo in poživilo. Skorja korenine, izvleček ali čisti ibogain so na Zahodu poznani kot sredstvo za odpravo odvisnosti in njihova uporaba narašča. Doktorska teza poskuša razkriti skupni mehanizem delovanja pri vseh navidezno različnih namenih uporabe iboge, predvideti potencialne neželene učinke in ustvariti pogoje za njeno varno in koristno uporabo.

Z metodo dvodimenzionalne elektroforeze in masne spektrometrije smo identificirali spremembe v proteomu podganjih možgan in kvasovke po aplikaciji ibogaina. Energetski status celic smo merili z luminiscenco luciferin/luciferaznega testa, ki pokaže količino ATP molekul, t.i. ATP raven. Nastajanje prostih radikalov in stopnjo znotrajcelične oksidacije smo določili z uporabo 2,7-diklorofloresceina diacetata (H_2DCFDA) in merjenjem fluorescence njegovega oksidiranega metabolita. *In vitro* antioksidativni potencial ibogaina smo določili z merjenjem količine reduciranega 1,1-difenil-2-pikrilhidrazila (DPPH). Stopnjo oksidativnega katabolizma smo določili z merjenjem nastajanja CO_2 s plinsko kromatografijo.

Rezultati so pokazali povečanje količine oz. indukcijo encimov energetskega metabolizma in endogene antioksidativne obrambe. Pri podganjih možganih so bili 72 ur po intraperitonealni aplikaciji 20 mg/kg t.t. ibogaina inducirani encimi gliceraldehid-3-fosfat dehidrogenaza, aldolaza A, piruvatna kinaza in malatna dehidrogenaza, pri kvasovki po 5 urah kultivacije v mediju z 1 mg/L ibogaina pa encimi gliceraldehid-3-fosfat dehidrogenaza, fosfoglicerat kinaza, enolaza in alkoholna dehidrogenaza ter superoksidna dismutaza. Računalniška analiza rezultatov je pri slednjem modelu pokazala tudi nespecifično aktivacijo sinteze frakcije nizko-zastopanih proteinov. Ti zaenkrat ostajajo še neidentificirani. Pri kvasovki smo zaznali tudi od odmerka odvisen prehodni upad ATP ravni ob sočasno povečani proizvodnji CO_2.

Dokazali smo, da ibogain prehodno poveča porabo energije za sintezo številnih encimov, med drugim tudi samih katabolnih in antioksidativnih encimov. Kljub povečanju metabolnega obrata in sočasni proizvodnji prostih radikalov ibogain znižuje oksidativno obremenitev. Ker ibogain *in vitro* nima pomembnega antioksidativnega delovanja, rezultat nakazuje njegov vzpodbujevalni učinek na fiziološki antioksidativni sistem kot pro-antioksidant, kar dokazuje tudi povečanje količine antioksidativnih encimov. Dolgoročno se to kaže v preoblikovanju hišne presnove. Ob uvodnih energetskih izdatkih to rezultira v povečani učinkovitosti fizioloških antioksidativnih sistemov, ki zmanjšujejo oksidativno obremenitev in s tem energetske stroške obnove oksidativno poškodovanih celičnih struktur. Ob sočasni indukciji katabolnih encimov se vzpostavi novo metabolno ravnovesje, ki varčuje z energijo, v primeru dodatnih potreb pa omogoča njeno povečano razpoložljivost. Zdrav organizem lahko tako vzdrži večje fizične in mentalne napore brez tveganja stresne preobremenitve. Po istem načelu iboga omogoča hitrejše okrevanje v primeru bolezni, vključno z motnjo odvisnosti.

5 SUMMARY

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 remedial and 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. Thesis aims 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.

With the method of two-dimensional electrophoresis and mass spectrometry we have identified proteome changes in rat brain and yeast cells after the application of ibogaine. Cellular energy status was defined by luminiscence of lucipherin/lucipherase test that shows the level of ATP pool. Free radicals production and the level of intracellular oxidation was defined with 2`,7`-dichlorofluorescein diacetate (H_2DCFDA) by measuring fluorescence of its oxidized metabolite. In vitro antioxidative potential of ibogaine was estimated by its ability to reduce 1,1-diphenyl-2-picrylhydrazyl (DPPH). The level of oxidative catabolism was defined by tracing of CO_2 production with gas chromatography.

The results have shown the induction of energy metabolism and antioxidative defence enzymes. In rat brain 72 hours after intraperitoneal application of 20 mg/kg per body weight of ibogaine the enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase had been induced. Yeast after 5 hours of cultivation in media with ibogaine 1 mg/L showed induction of glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase, alcohol dehydrogenase and superoxide dismutase. In silico analysis also showed nonspecific activation of synthesis of low abundance protein fraction that remains unidentified. In the yeast model we have also observed transitory fall in ATP pool accompanied by enhanced CO_2 production.

It has been proven that ibogaine transitory increases energy consumption due to synthesis of numerous enzymes including catabolism and antioxidative defence enzymes of concern. In spite of increased metabolic turnover and consequent free radical production the overall oxidative load was decreased. Since ibogaine doesn't show some significant *in vitro* antioxidative properties, the results suggest stimulating influence on intrinsic physiological antioxidative systems in a pro-antioxidant manner, which is in concordance with observed induction of antioxidative enzymes. In a long term ibogaine effect manifests itself as an adaptation of house keeping metabolism. Under the initial energy load it results in increased efficacy of physiological antioxidative systems, which reduce oxidative damage and costs of cellular repair. 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.

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ZAHVALA

Materi, očetu in ostalim sorodnikom na tej in na oni strani, ki so mi omogočili ravno pravi življenski tempo, da sem imel možnost odkriti te čudovite skrivnosti življenja

Poloni, da verjame v moje delo kljub temu, da ji življenski vidik mojih eksperimentov nikakor ni na kožo pisan

Prof. dr. Borutu Štruklju, mentorju in prijatelju, za vso pomoč pri raziskavah in doktoratu

Vsem ljubiteljem znanosti, ki so podprli projekt s svojim časom, sredstvi, mnenji in odločitvami

Srbski ekipi; они већ знају зашто…

Prof. Bressollovi in dr. Violeti za francoski prispevek; Merci!

Gregorju za njegovo vero v svobodo in enakost

Maši, Svenu in ostalim prijateljem za vso izkazano podporo in potuho

PRILOGE

Korespondenca in izvedensko mnenje dr. Gregorja Lesjaka

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IZVEDENSKO MNENJE

O NEKATERIH VIDIKIH RELIGIJSKE OZIROMA DUHOVNE RABE IBOGINA V SLOVENIJI

Za pripravo izvedenskega mnenja me je decembra leta 2011 prosil gospod Roman Paškulin. O tem sva si izmenjala nekaj elektronske pošte, se dvakrat osebno sestala in dogovorila o rokih za pripravo mnenja. Gospod Paškulin me je natančno seznanil s kontekstom svoje prošnje in mi omogočil vpogled v sodne spise, iz katerih po njegovem mnenju izhaja potreba po izvedenskem mnenju.

Postavil mi je naslednja vprašanja:

1. Kakšna je vloga in položaj iboge in ibogaina v luči verovanja v Republiki Sloveniji?
2. Kakšna je vloga in položaj iboge in ibogaina v duhovno - religioznem smislu v svetu?
3. Ali je to edini primer uporabe psihoaktivne substance kot zakramenta in posledične spremembe pojmovanja sveta in smisla obstoja?
4. Kako so te prakse urejene?
5. Ali lahko prejem zakramenta posredno vpliva na motiviranost za svoboden načina življenja, med drugim na osvobajanje od drog?
6. Ali menite, da je zaradi tega učinka prejem zakramenta avtomatično pojmovan kot zdravljenje?

Ker menim, da so pojasnila mnogo bolj učinkovita v kontekstu, spodaj odgovarjam v vezanem besedilu. Ob vsakem poglavju so navedena vprašanja, na katera odgovrja besedilo. Na posamezno vprašanje praviloma odgovarjam v več kontekstih.

Z odgovorm na vprašanje pod zaporedno številko 1 v prvem poglavju ne presegam pristojnosti sodnega izvedenca tako, da bi razpravljal o konkretnem primeru in pravnih sredstvih, ki so stvar sodišča. V mnenju pojasnjujem zgolj splošni ustavno-pravni položaj obravnavane materije, po katerem sem bil vprašan, in ki sodi v področje večdisciplinarne ekspertize sociologa novih religijskih gibanj.

Odgovor na vprašanje pod zaporedno številko 6 je samo nakazan, ker bi razprava zahtevala soočenje z izvedencem medicinske stroke. Pojma duhovnega in konvencionalnega zdravljenja se lahko v večjem ali manjšem delu stikata ali prekrivata, v vsakem primeru pa drži, da sta obe področji tudi avtonomni, in da prevedba pomena v celoti ni mogoča ne v eno in ne v drugo smer.

Delo sem opravil iz akademskega interesa in pro bono.

dr. Gregor Lesjak

Log Čezsoški, 3. 3. 2012

VLOGA IN POLOŽAJ IBOGE IN IBOGAINA V LUČI VEROVANJA V REPUBLIKI SLOVENIJI

KAZALO

1. USTAVNOPRAVNI OKVIR

Vprašanji, na kateri se nanaša odgovor

Kakšna je vloga in položaj iboge in ibogaina v luči verovanja v Republiki Sloveniji?

Kako so te prakse urejene?

Povzetek

Človekovo pravico do svobode vesti in s tem do verske svobode v Republiki Sloveniji v enaki meri uživajo vsi posamezniki ter vse registrirane in vse neregistrirane verske skupnosti[1]. Slovenski pravni red ne prepoveduje iboge, ampak jo uvršča med zdravilne rastline, katerih uporaba za zdravljenje zahteva zdravniški nadzor. Zaradi javnega interesa oziroma vrednote (zdravja) je tako omejena splošna svoboda ravnanja, ki lahko posega tudi v versko svobodo.

Definicije in podrobnejša pojasnila

(*Tabernanthe iboga*) iboga je afriška tropska rastlina, skorja korenine te rastline pa ima v določenih količinah halucinogen učinek.

Pravilnik o razvrstitvi zdravilnih rastlin s seznamom zdravilnih rastlin (Ur. l. RS, št. 103/08) v 5. členu določa, da se v "kategorijo ZR razvrstijo zdravilne rastline, katerih uporaba zahteva zdravniški nadzor. Zanje je značilna strupenost pri prekoračenih odmerkih, v priporočenih odmerkih pa so možni neželeni škodljivi učinki, tako da varnost ni zagotovljena brez zdravniškega nadzora zdravljenja. V tej kategoriji so tudi rastline s toksikomanogenim učinkom. Tako rastline kot izdelki za peroralno uporabo, ki vsebujejo zdravilne rastline iz kategorije ZR v naravni ali predelani obliki, se praviloma razvrstijo med zdravila, za katera je potreben zdravniški recept". Zdravilna rastlina Tabernanthe iboga se razvršča v kategorijo ZR.

Versko svobodo v Republiki Sloveniji urejata ustava in zakon. Verska svoboda sodi med človekove pravice. V sklopu širše pravice do svobode vesti jo določa 41. člen ustave (Ur. l. RS, št. 33/91 in dalje)[2]. Verske skupnosti so v Republiki Sloveniji enakopravne, svobodne v svojem delovanju in ločene od države (tudi država je ločena od verskih skupnosti); tako določa 7. člen ustave. Položaj

[1] Pridobitev primerne pravne osebnosti je v Sloveniji všteta celo v obseg človekove pravice do verske svobode, zato je ustavno sodišče leta 2010 razveljavilo stroge kriterije za registracijo verske skupnosti. Pravica do vere je enaka pravici do svetovnega nazora in nevere (prim. OdlUS št. U-I-92/07, Ur. l. RS. št. 46/2010).

[2] 41. člen Ustave RS pravi: »Izpovedovanje vere in drugih opredelitev v zasebnem in javnem življenju je svobodno. Nihče se ni dolžan opredeliti glede svojega verskega ali drugega prepričanja. Starši imajo pravico, da v skladu s svojim prepričanjem zagotavljajo svojim otrokom versko in moralno vzgojo. Usmerjanje otrok glede verske in moralne vzgoje mora biti v skladu z otrokovo starostjo in zrelostjo ter z njegovo svobodo vesti, verske in druge opredelitve ali prepričanja«.

verskih skupnosti podrobneje opredeljuje Zakon o verski svobodi (Ur.l. RS, št. 14/07 in 46/10 – odločba US).

Človekovo pravico do svobode vesti uživajo tudi neverske svetovnonazorske skupnosti. Pravice, ki izhajajo iz človekove pravice do svobode vesti in jih v sklopu verske svobode uživajo verske skupnosti, se ne razlikujejo od pravic, ki izhajajo iz človekove pravice do svobode vesti in jih uresničujejo neverske svetovnonazorske skupnosti. Tudi neverske svetovnonazorske skupnosti so enakopravne, v svojem delovanju svobodne in ločene od države, tako kot so enakopravne, svobodne in od države ločene verske skupnosti (glej OdlUS, št. U-I-92/07, Ur. l. RS. št. 46/10)[3].

Človekova pravica do svobode vesti pa ne ščiti vsakega verskega in neverskega prepričanja. Ustavno sodišče pravi: »Pojem vesti (...) sodi na področje etike. Vest človeku pove, kaj je 'prav'. Predmet varstva v okviru 41. člena Ustave so zato samo *[1] opredelitve in prepričanja s področja etike oziroma morale*, zlasti vsa teistična, ateistična in neteistična prepričanja. V tem smislu lahko taka prepričanja opredelimo tudi kot svetovnonazorske opredelitve, torej kot filozofske ali ideološke teorije oziroma kot miselne sisteme, ki razlagajo človeka, njegovo bistvo in svet, v katerem prebiva, lahko tudi, čeprav ne nujno, z neke višje, metafizične ravni... Šele če notranje in zunanje lastnosti [tega] prepričanja kažejo na njegovo *[2] konsistentnost, tehtnost, resnost, kohezivnost in pomembnost*[4], je utemeljen sklep, da gre za vero oziroma drugo prepričanje v smislu 41. člena Ustave« (OdlUS št. U-I-92/07, Ur. l. RS. št. 46/2010, točka 75).

Človekova pravica do svobode vesti (v kateri je utemeljena verska svoboda) je torej v slovenskem pravnem redu v enaki meri zagotovljena vsem posameznikom in skupinam, ne glede na to, ali je njihovo prepričanje versko ali neversko; to prepričanje mora izpolnjevati le oba pogoja iz zgornjega odstavka. V takšnem primeru bi bilo uživanje iboge, če ga seveda upravičeno utemeljimo v svobodi vesti, povsem enako zaščiteno v primeru religijskega (duhovnega) ali nereligijskega svetovnonazorskega konteksta. Omejitev splošne svobode ravnanja iz Pravilnika o razvrstitvi zdravilnih rastlin, ki bi morda posegala v pravico do svobode vesti, bi bila ustavno dopustna, če bi prestala strogi test sorazmernosti[5].

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[3] Pravica neverskih svetovnonazorskih skupnosti do posebne pravno-organizacijske oblike v zakonodaji še ni urejena (tako kot je za verske skupnosti urejena pravno-organizacijska oblika verske skupnosti), zato imamo na tem mestu protiustavno pravno praznino.

[4] Ustavno sodišče ni pojasnilo, kako naj razlagamo navedene lastnosti, sklicevalo pa se je na prakso Evropskega sodišča za človekove pravice oziroma zadevi Campbell in Cosans proti Združenemu kraljestvu (25. februar 1982) ter Leela Förderkreis E. V. in drugi proti Nemčiji (6. november 2008).

[5] V okviru tega testa sodišče odgovarja na naslednja vprašanja: (1) ali je poseg v človekovo pravico sploh nujen (potreben), (2) ali je ta poseg primeren za dosego zasledovanega cilja in (3) ali je teža posledic tega posega proporcionalna vrednosti zasledovanega cilja oziroma koristim, ki bodo zaradi posega nastale.

2. RAZLIČNE OBLIKE RELIGIOZNOSTI V SLOVENIJI

Vprašanji, na kateri se nanaša odgovor

Kakšna je vloga in položaj iboge in ibogaina v luči verovanja v Republiki Sloveniji?
Kakšna je vloga in položaj iboge in ibogaina v duhovno - religioznem smislu v svetu?

Povzetek

Uživanje ibogaina zasledimo med praktikami slovenskih novoreligijskih organizacij in posledično v segmentu individualizirane slovenske novodobniške duhovnosti. Nova religijska gibanja (New Religious Movements) in novodobniška duhovnost (New Age) nedvomno sodijo v sklop relevantnih religijskih pojavov.

Podrobnejša pojasnila

Sociologiija pozna številne (še aktualne) tipologije religijskih organizacij, ki se med seboj razlikujejo predvsem po namenu razvrščanja in okolju, v katerem to razvrščanje poteka. Za Slovenijo se zdi, da njene posebnosti najbolje odrazi delitev religijskih organizacij med tradicionalne religije okolja, krščanske denominacije, imigrantske religije ter novoreligijske in novodobniške skupine. V takšni tipologiji je upoštevana razlika med religisjkimi (in novoreligijskimi) ter duhovnimi oziroma novodobniškimi skupinami in/ali gibanji, a hkrati tudi jasno demonstrirana njihova enost. Vse je namreč mogoče razumeti v sklopu najširšega pojmovanja religije in religioznosti[6].

Pojav novih religij in novodobniške duhovnosti v Sloveniji spremljamo od osemdesetih let prejšnjega stoletja, čeprav se nekatere skupine (na primer Teozofsko združenje) že ponašajo tudi s častitljivo tradicijo. Slovenske nove religije so k nam sprva prenašale zelo raznolike nauke in organizacijske strukture tujih skupin, od leta 2000 naprej pa med njimi opažamo tudi samonikle religijske inovacije. Po razpoložljivih podatkih v tem trenutku v Sloveniji deluje več kot 100 novoreligijskih in novodobniških organizacij oziroma skupin (prim. Goljevšček, 1992; Pribac, 1992 in Lesjak, 2007).

Novodobniška duhovnost se od (stare in nove) religije pomembno razlikuje. Vnanjo razliko najprej prepoznamo v tem, da novodobniki praviloma ne tvorijo socialnih ali večjih skupin, ampak ohlapne socialne mreže, zato tudi ne poznajo klasičnih religijskih organizacij. Če skušamo le-te vseeno opredeliti, so najpogosteje sestavljene zgolj iz enega ali več specialistov, ki svoje storitve ponujajo na izrazito individualiziranem novodobniškem trgu. Instutucionalno nevezani posamezniki na tem trgu po lastnih potrebah in preferencah izbirajo tisto, kar v danem trenutku najbolj potrebujejo in tako oblikujejo specifično ekonomijo vzajemnega obdarovanja, ki praviloma vključuje tudi gmotno

[6] Takšno sodbo brez vsakega zadržka tudi v svoji enciklopedijski produkciji podpira globalna strokovna skupnost sociologov novih religijskih gibanj (npr. Partridge, 2004; Introvigne in Zoccatelli, 2006 ter Melton, 2009).

povračilo za prejeto uslugo[7]. Novodobniki ne poznajo religijskih avtoritet, dogem in svetih besedil. Njihovo skupnost opredeljuje njihova praksa, v katero vključujemo prepoznavne težnje ter značilne, a načeloma odprte nabore idej in praktik[8]. Novodobnik (v tradicionalnem pomenu) veruje samo tisto, kar je izkusil sam (prim. Ban, 2008).

Zaužitje ibogaina je pomemben del prakse ene izmed samoniklih slovenskih novodobniških organizacij, registrirane verske skupnosti Sakrament prehoda. Ta organizacija pretaka svojo prakso v segment individualizirane novodobniške duhovnosti. Informacije o tej praksi so zaradi njenih specifičnih lastnosti (ibogain naj bi občutno zmanjševal in/ali prekinjal različne zasvojenosti) zanesljivo razširjene v subkulturi zasvojencev.

3. (SLOVENSKE NOVE) RELIGIJE IN DROGA

Vprašanji, na kateri se nanaša odgovor

Kakšna je vloga in položaj iboge in ibogaina v luči verovanja v Republiki Sloveniji?

Ali je to edini primer uporabe psihoaktivne substance kot zakramenta in posledične spremembe pojmovanja sveta in smisla obstoja?

Povzetek

Verska skupnost Sakrament prehoda in njegova socialna mreža sta edini znani primer povezave med drogami in religijo oziroma duhovnostjo v Sloveniji. Učinke halucinogenih drog na človekovo doživljanje je mogoče primerjati z opisi pristnih mističnih in meditativnih izkušenj.

Podrobnejša pojasnila

Povezav med (novo)religijskimi in/ali duhovnimi praktikami in drogami v Sloveniji pred Sakramentom prehoda nismo zaznali. Nobeden izmed delujočih ali nedelujočih skupin ali posameznikov, o katerih imamo podatke, v svoji verski/duhovni dejavnosti v Sloveniji doslej ni uporabljal (nesocializiranih) drog.

Literatura pove, da sta odkrivanje in raba drog stara prav toliko, kot so stare človekove civilizacije; da se raba drog v nekaterih tradicijah z religijo povezuje prav toliko časa, kolikor poznamo religijo (prim.

[7] »Obstaja prepričanje, da zaračunavanje storitev s področja duhovnosti ni 'duhovno'. Kako to? Mar ni zdravilec prav tako vložil veliko časa ter energije v to, da se je naučil alternativno zdraviti? Ali ni predavatelj vložil precej časa, da je dosegel stanje, ko lahko predava, pripravi delavnico, seminar ali predavanje? Da napiše knjigo? Torej je za vloženi čas ravno tako upravičen zaračunati storitev. Pa ne samo tisti konkretni čas, čas tistega predavanja ali terapije, temveč sorazmerno še vse tiste ure, ki jih je porabil za to, da sedaj lahko predava ali kakorkoli deluje. Zakaj bi bilo torej 'neduhovno' zaračunavati storitve s področja duhovnosti?« (Pajnkiher Prem, 2004: 182).

[8] Na primer težnje k samorealizaciji ali razvoju človekovih potencialov ter različne tehnike meditacij, joge, holističnega zdravilstva, hipersenzornih zaznav, itn.

npr. Swatos, 1998); ter da se *učinki halucinogenih drog oziroma psihotropnih snovi, predvsem v občutkih depersonalizacije, derealizacije in blaženosti, ki včasih ta doživetja spremlja, približujejo pristnim mističnim doživetjem* (prim. Milčinski, 1978 ter James, 1902; Huxley, 1954; Phanke, 1964; Leary, 1964, Clark 1969 in Nelson 2009).

4. SAKRAMENT PREHODA[9] IN IBOGAIN

Vprašanja, na katera se nanaša odgovor

Kakšna je vloga in položaj iboge in ibogaina v luči verovanja v Republiki Sloveniji?
Kakšna je vloga in položaj iboge in ibogaina v duhovno - religioznem smislu v svetu?
Ali lahko prejem zakramenta posredno vpliva na motiviranost za svoboden načina življenja, med drugim na osvobajanje od drog?

Povzetek

Izkušnja zaužitja ibogaina posameznika pogosto nagovarja na eshatološki ravni. V novodobniškm kontekstu, ki ga gradi Sakrament prehoda, iniciant tako vzpostavlja stik s svojim pravim/resničnim sebstvom, prek katerega si odgovarja na vprašanja: kaj je dobro (zame), kdo sem, od kod prihajam in kam grem. Praksa te verske skupnosti je samonikla; na afriške bwitije se sklicuje zgolj v izvoru in učinku svete rastline, iboge.

Podrobnejša pojasnila

Predpostavlja se, da je Sakrament prehoda, leta 1999 registrirana verska skupnost, nosilec ali vsaj primarni prenašalec idej in prakse duhovne rabe ibogaina v Sloveniji. Ta verska skupnost se je razvila iz Ustanove iboga, ki je delovala med letoma 1995 in 2005. Namen te ustanove je bilo iskanje alternativnih možnosti in sredstev za prekinjanje različnih zasvojenosti in iskanje podpore strokovnih krogov in laikov[10].

Sakrament prehoda nima svojih temeljnih besedil. Najstarejši med tistimi, ki jih vseeno navaja na svojih spletnih straneh (Knut, 1994)[11], neposredno povezuje slovensko prakso z »zadnjim valom uživanja drog (zlasti halucinogenov), ki je zajel mlado generacijo Zahoda« (Milčinski, 1978: 59), čeprav z njegovim stranskim ali zapoznelim krakom. To je najprej čas hipijevske subkulture in pojava prvih novih religijskih gibanj v Združenih državah Amerike v šestdesetih letih minulega stoletja ter

[9] Namen tega zapisa ni v profiliranju verske skupnosti Sakrament prehoda. Na tem mestu niso zbrani niti vsi razpoložljivi podatki o tej skupnosti niti obravnavana vsa vprašanja, ki jih delovanje te skupnosti postavlja raziskovalcu.
[10] Prim. http://www.archive.org/web/web.php (http://www.ustanova-iboga.si)
[11] Glej: http://sacrament.kibla.si, 24. 2. 2012

kasnejše delo Howarda Lotsofa (1943 – 2010) – zasvojenca, ki je heroin opustil po zaužitju ibogaina ter življenje posvetil spodbujanju raziskav in promociji ibogaina kot sredstva za prekinjanje zasvojenosti[12].

Ibogain je opisan kot droga, ki je povsem drugačna od vseh ostalih. Njeno delovanje je dolgotrajno, trajalo naj bi tudi 36 ur, vendar ni euforičen halucinogen[13], potenciala za zlorabljanje naj ne bi vsebovala[14] in prekinjala naj bi, kot rečeno, različne odvisnosti[15]. Duhovni interes pritegnejo skoraj identični opisi halucinogene faze, na primer: »Razlog, zakaj se ne premikaš, je v tem, da si popolnoma okupiran z gledanjem, kaj vse se nahaja v tvojem duhu. Spomini so kot film. Kaže ti, kje v življenju si delal napake, pokaže ti tudi, kaj moraš storiti, da jih popraviš. Dobesedno to ti dela. Hočem reči, da vidiš čisto vse« (Amon, 1994: 65). Jakost takšne izkušnje, ki posameznika pogosto nagovarja ne le na osebni in bivanjski, ampak tudi na eshatološki ravni (prim. Humski, 2009: 199) v svoji shemi delovanja ibogaina ponazarja Lotsof: »Najprej je halucinogena faza, v kateri se sprostijo subjektovi potlačeni spomini; v drugi fazi možgani intelektualno predelajo vizije iz prve faze; in tretja, ko se vsi vtisi integrirajo v pacientovo osebnost« (prav tam).

Če pritrdimo podobnosti učinkov halucinogenih drog in mistične izkušnje na človekovo doživljanje, kot to preizkušajo, dopuščajo in/ali zatrjujejo nekatere zgoraj navedene raziskave, ter zatrjevano izkušnjo zaužitja ibogaina primerjamo z opisi (kemično nespodbujenih) mističnih oziroma meditativnih doživetij, se občasna, začasna ali trajna integracija izkušnje zaužitja ibogaina v posameznikovo osebnost ne zdi nemogoča. Milčinski (1990: 384) med posledicami mistične izkušnje na primer navaja tudi trajne pozitivne spremembe v naravnanosti in vedenju posameznika, ki se izražajo kot: (1) krepkejša integracija osebnosti, s čimer se sprostita kreativnost in storilnost pa srečno, optimistično razpoloženje; (2) večja senzibilnost, tolerantnost, pristnost in naklonjenost do drugih ljudi; (3) korekcije v svetovnem nazoru, vrednotenju služenja drugim ljudem in stvarstvu nasploh ter (4) pozitivna naravnanost do mističnega doživetja nasploh – lastnega ali tujega.

Ibogain prihaja iz religijske prakse bwiti nekaterih ljudstev v Gabonu in Kongu v Srednji Afriki (prim. Fernandez, 1982). Osrednja poteza tradicionalnega bwitija je čaščenje prednikov. Njihov močno struktutriran obred iniciacije temelji na zaužitju svete rastline, iboge, in posledičnih spremenjenih stanjih zavesti. V njih inicianti ne srečujejo zgolj duhov svojih preminulih prednikov, ampak navežejo tudi stik s svojim stvarnikom (prim. Pratt, 2007). Bwiti je tudi afriško sinkretistično novo religijsko gibanje, ki približno od leta 1910 združuje tradicionalne religijske prvine s krščanstvom in v svojem obredju prav tako uporablja ibogo (prim. Woodhead in drugi, 2002). V praksi Sakramenta prehoda in

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[12] Glej: http://www.nytimes.com/2010/02/17/us/17lotsof.html, 24. 2. 2012

[13] »Kdo bi si želel vzeti trip, ki traja 36 ur,« se sprašuje Lotsof (Knut, 1994: 16). Ibogain je »zanimiv na svoj način, vendar ni takšne vrste droga, da bi se zadel in se šel na plažo zabavat s prijatelji... Res je, da te resnično vrže prve tri do štiri ure, potem hodiš okoli v tisti visoko energetski fazi in po štiriindvajsetih urah si že pošteno izčrpan. Potem zaspiš in šele, ko se zbudiš, ugotoviš, da se ti je zgodilo nekaj res enkratnega« (prav tam: 27).

[14] »V večini primerov je faza haluciniranja izostala po tretji zaporedni dozi. Ni bilo ničesar več, kar bi vznemirjalo podzavest« (prav tam: 27).

[15] »Od 20 oseb, ki smo jim dali ibogain, je bilo sedem zasvojenih s heroinom. Dva dni kasneje pet od teh sedmih oseb ni več občutilo želje po drogi. In niso imeli nobenih abstinenčnih težav. Pri tistih, ki so vzeli ibogain le enkrat, je ta abstinenca trajala kakih šest mesecev, eni od teh oseb smo dali ibogain petkrat in ni občutila želje po heroinu leto in pol. Če veste vsaj nekaj malega o zasvojenosti s heroinom, vam bo jasno, da je to nekaj neverjetnega« (prav tam: 20-1).

najverjetneje tudi v vseh drugih izpeljankah uživanja ibogaina v Sloveniji, ne najdemo verodostojnega prenosa ontologije, kozmologije in/ali obredja bwitijev (tako kot lahko, na primer, med slovenskimi novimi religijami najdemo prenos nekaterih drugih naukov in praks afriškega šamanizma), pač pa zgolj pritrjevanje (razmeroma pogosti) eshatološki izkušnji zaužitja iboge. Morebitnega obstoja drugih religijskih skupin, ki bi svoj nauk in/ali prakso utemeljevale v zaužitju ibogaina in/ali tradicionalnih ali novih bwitijih, ne morem potrditi niti v Sloveniji niti v svetu[16].

Sakrament prehoda ibogain imenuje Sveti Sakrament[17]. Zaužitje Svetega Sakramenta se imenuje inicijacija[18] ali duhovno zdravljenje. Količino in obliko zaužitega Svetega Sakramenta[19] ter podrobnosti protokola[20] določi duhovnik (prim. Resnovič, 2009). Zaužitje Svetega Sakramenta je deklarirano kot eden izmed dveh načinov samorealizacije oziroma neposrednega stika inicianta z njegovo pravo naravo in/ali najvišjo realnostjo oziroma bogom. Drugi način opredeljuje BIOfizika 7., 13., 33. in 53. dimenzionalnega prostora oziroma BIOkibernetika, kot jo je razvil Slavko Anton Gorenc iz Ljubljane[21]. BIOkibernetika boga imenuje S-Alfa. Šele obe praktiki skupaj tvorita celoto: Sveti Sakrament (dovolj pogosto) zagotavlja osnovno religiozno izkušnjo, biokibernetika pa kakovosten način življenja in nadaljnjo kultivacijo osnovne religiozne izkušnje. Zanimiva je visoka individualiziranost obeh sestavin prakse. Sakrament prehoda prve izkušnje ne kontekstualizira, ampak jo v celoti prepušča iniciantu; taka je tudi BIOkibernetika, ki posameznikove izkušnje opremlja zgolj in samo s tehniko (prim. Resinovič, 2002).

Sakrament prehoda je postopoma razvil neposredno spodbudo za preučevanje učinkov ibogaina, ki jo je prejel preko dela Howarda Lotsofa[22]. Nevladna organizacija Ustanova iboga je izkazovala neipridobiten raziskovalni interes in voljo do pustolovščine v poslovanju z državnimi organi[23]. Iniciant danes prek zaužitja Svetega Sakramenta na tipično novodobniški način vzpostavlja ali preverja stik s svojim pravim/resničnim sebstvom in si tako odgovarja na vprašanja: kaj je dobro (zame), kdo sem, od kod prihajam in kam grem. Od prakse bwitijev, na katero se sklicuje zgolj v izvoru in učinku Svetega Sakramenta, se razlikuje tudi v tem, da v nadaljnjih duhovnih iskanjih ne uporablja Svetega Sakramenta (čeprav je mogočih več iniciacij, ne samo ena), ampak druge novodobniške tehnike.

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[16] To pomeni, da takšne skupine lahko obstajajo, vendar jih raziskovalci v svojem delu nismo niti opazili niti o njih poročali svoji strokovni skupnosti. To je zaradi narave predmeta preučevanja tudi razumljivo. Med religijskimi organizacijami, ki v svoji praksi uporabljajo halucinogene (splošno prepovedane, a ponekod zaradi uresničevanja verske svobode dopuščene) snovi, danes v javnosti izstopata Ameriška domorodna cerkev (Native American Church) in izvorno brazilska skupnost Santo Daime.

[17] Imenovanje v nauku ni tematizirano, vsekakor pa izraža vsaj zelo spoštljiv odnos do te substance.

[18] Iniciacija v tradicionalnem pomenu sodi med obrede prehoda in označuje vstop ali sprejetje posameznika v skupino ali družbo; v bwitiju na primer mladostnik z zaužitjem iboge postane mož in Afričan. Pri novodobniški iniciaciji se izgublja skupnostni in poudarja individualni vidik prehoda. Oseba, čeprav s pomočjo druge ali v našem primeru kemične substance, z iniciacijo v sebi doživi prehod iz ene ravni zavedanja v drugo, doživi preobrazbo, transformacijo, postane drugačna (prim. Pajnkiher Prem, 2004: 91).

[19] »Pri religioznem obredu gre... za odmerek, manjši od detoksikacijskega in so pri izpolnjevanju osnovnih meril zdravja tveganja neznatna, t.j primerljiva s športnim tveganjem« (Paškulin, 2009: 258).

[20] Prim. http://www.ibogaine.desk.nl/SofTInitiationProtocol.html, 24. 2. 2012.

[21] Prim. Resinovič, 2002 in http://www.ati-e-import.si, 24. 2. 2012.

[22] »Ibogain smo vzeli zato, da bi se zadrogirali, vendar smo naenkrat ostali brez potrebe po drogah... Moral sem se odločiti, kaj bom s svojim življenjem. Zavedal sem se, da lahko z ibogainom napravim ogromno dobrega, ob tem pa tudi dobro zaslužim« (Amon, 1994: 64).

[23] Prim. http://www.archive.org/web/web.php (http://www.ustanova-iboga.si)

5. IBOGAIN, ZASVOJENOST IN ZDRAVLJENJE

Vprašanji, na kateri se nanaša odgovor

Ali lahko prejem zakramenta posredno vpliva na motiviranost za svoboden načina življenja, med drugim na osvobajanje od drog?
Ali menite, da je zaradi tega učinka prejem zakramenta avtomatično pojmovan kot zdravljenje?

Pojasnila

Celostna skrb religijskih organizacij za človekovo duhovno in socialno dobrobit, v katero lahko brez dvoma umestimo tudi pogosto skrb za zasvojence, tvori jedro njihove dejavnosti. Ta zveza je v kontekstu duhovne rabe ibogaina še močnejša in praktično primarna: substanca, ki zagotavlja temeljni duhovni uvid (in lahko s tem, kot rečeno, krepi tudi senzibilnost, tolerantnost, pristnost in naklonjenost do drugih ljudi), hkrati vsaj domnevno lajša slabo, napačno, odtujeno in življenje ogrožajoče stanje – zasvojenost človeka z drogami.

Religijske organizacije po definiciji izvajajo družbeno povezovalno (lat. re-ligiare)[24] in z njo tudi korektivno funkcijo ter takšno skrb spodbujajo, zapovedujejo in institucionalizirajo, bodisi v izrecno religijskem bodisi pretežno socialno-skrbstvenem kontekstu. V sklopu slovenske Katoliške Cerkve tako, na primer, danes deluje 138 organizacij, ki se primarno ukvarjajo s humanitarno dejavnostjo (Karitas); v njeni strukturi najdemo redove in druge skupnosti, ki svojo primarno dejavnost posvečajo skrbi za dobrobit sočloveka ali celo za katero izmed obrobnih, posebej ranljivih ali depriviligiranih družbenih skupin, na primer zasvojencev z drogami.

Skrb za zasvojence je med slovenskimi religijskimi organizacijami pogosta. Med njimi najdemo registrirane (povečini evangelijske) verske skupnosti, ki aktivno ali celo primarno pomagajo zasvojencem. Taka je, na primer, prekmurska Cerkev Novo Življenje[25]. Skrb verskih skupnosti za zasvojence je celostna in najpogosteje izhaja iz prepričanje, da bo posamezniku pomagalo oziroma ga duhovno ozdravilo predvsem spoznanje Boga. Literatura pove, da lahko religijsko doživljanje, in v sklopu tega posebej vzbujanje spremenjenih stanj zavesti (ki so pogosto socialno inducirana), odpravlja oziroma prekinja ali premešča različne zasvojenosti, med njimi tudi zasvojenost z drogami (prim. Galanter, 1999).

[24] Na tem mestu se ne sklicujemo na sodobnejše spoznanje, da je mogoče povezovanje verujočih opazovati tudi kot izločanje drugače verujočih in neverujočih.
[25] Prim. npr. http://www.rtvslo.si/odprtikop/duhovni_utrip/cerkev-novo-zivljenje-in-odvisniki, 24. 2. 2012.

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