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Anti-addiction drug ibogaine inhibits voltage-gated ionic currents: A study to assess the drug's cardiac ion channel profile

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ABSTRACT

The plant alkaloid ibogaine has promising anti-addictive properties. Albeit not licenced as a therapeutic drug, and despite hints that ibogaine may perturb the heart rhythm, this alkaloid is used to treat drug addicts. We have recently reported that ibogaine inhibits human ERG (hERG) potassium channels at concentrations similar to the drugs affinity for several of its known brain targets. Thereby the drug may disturb the heart's electrophysiology.

Here, to assess the drug's cardiac ion channel profile in more detail, we studied the effects of ibogaine and its congener 18-Methoxycoronaridine (18-MC) on various cardiac voltage-gated ion channels. We confirmed that heterologously expressed hERG currents are reduced by ibogaine in low micromolar concentrations. Moreover, at higher concentrations, the drug also reduced human $Na_v1.5$ sodium and $Ca_v1.2$ calcium currents. Ion currents were as well reduced by 18-MC, yet with diminished potency. Unexpectedly, although blocking hERG channels, ibogaine did not prolong the action potential (AP) in guinea pig cardiomyocytes at low micromolar concentrations. Higher concentrations (≥ 10 μM) even shortened the AP. These findings can be explained by the drug's calcium channel inhibition, which counteracts the AP-prolonging effect generated by hERG blockade. Implementation of ibogaine's inhibitory effects on human ion channels in a computer model of a ventricular cardiomyocyte, on the other hand, suggested that ibogaine does prolong the AP in the human heart. We conclude that therapeutic concentrations of ibogaine have the propensity to prolong the QT interval of the electrocardiogram in humans. In some cases this may lead to cardiac arrhythmias.

Introduction

Ibogaine, an indole alkaloid derived from the root bark of the African shrub *Tabernanthe iboga*, has received much attention because of its promising "anti-addictive actions" (for reviews see Alper, 2001; Maciulaitis et al., 2008). Thus, in animals, ibogaine attenuates opioid withdrawal signs and reduces the self-administration of a variety of drugs including opioids, cocaine, nicotine, and alcohol (Alper, 2001; Glick and Maisonneuve, 1998). In addition, ibogaine hampers responses that are associated with addiction, such as dopamine release in the *nucleus accumbens* of the brain (Benwell et al., 1996; Maisonneuve et al., 1991). Ibogaine's precise mechanisms of action remain unclear, but its effects may emerge from complex interactions with multiple neurotransmitter systems. Accordingly, ibogaine interacts with numerous different cellular and molecular targets, e.g. neurotransmitter transporters, opioid receptors, sigma receptors, glutamate receptors, and nicotinic receptors (Alper, 2001; Glick and Maisonneuve, 1998; Glick et al., 2000; Maciulaitis et al., 2008).

Ibogaine has a long history of use as a medicinal and ceremonial agent in West Central Africa. Besides its own psychoactive properties, anecdotal evidence suggests that ibogaine also acts as an anti-addictive in humans. Thus, intake of this alkaloid alleviates drug craving and impedes relapse of drug use (Alper, 2001; Maciulaitis et al., 2008; Mash et al., 1998). In spite of its status as a banned substance in the U.S. and some European countries, ibogaine is legal in most of the world, and, although not licenced as a therapeutic drug, is currently used as an anti-addiction drug in alternative medicine (Alper et al., 2008; Vastag, 2005).

Because ibogaine has a complex pharmacology and is known to interact with numerous different targets (see above), its potential to generate adverse effects is significant. Besides the expected neurotoxic actions (e.g. (Alper, 2001; Maciulaitis et al., 2008; Molinari et al., 1996; O'Hearn and Molliver, 1993; Xu et al., 2000)), ibogaine also

Abbreviations: hERG, human Ether-à-go-go-Related Gene; 18-MC, 18-Methoxy-coronaridine; AP, action potential.

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affects the cardiovascular system. In rats, high doses of ibogaine decreased the heart rate without altering blood pressure (Binienda et al., 1998; Glick et al., 2000). This finding is consistent with anecdotal reports in humans that ibogaine slows the heart rate (Alper, 2001; Glick et al., 2000; Maciulaitis et al., 2008). Alarming are several cases of sudden deaths after ibogaine use with unclear cause (Alper et al., 2012; Donnelly, 2011), which have been hypothesised to be related to cardiac arrhythmias (Alper et al., 2012; Hoelen et al., 2009; Maas and Strubelt, 2006). Due to concomitant medications used and comorbidities present in the patients described in these cases, however, it is unclear whether ibogaine alone or in combination with other factors may contribute to the clinical adverse findings. Recently several cases of ibogaine-associated QT interval prolongation and arrhythmias were reported (Hoelen et al., 2009; Paling et al., 2012; Pleskovic et al., 2012). In a first attempt to elucidate the mechanism(s) by which ibogaine may account for the described clinical observations, we recently (Koenig et al., 2012) tested the drug's propensity to inhibit human ERG (hERG, IK_r) potassium currents. hERG channels are crucial for the repolarisation phase of the cardiac action potential (AP), and hERG channel blockade by drugs is considered the most common reason for drug-induced QT interval prolongation, which can be associated with an increased cardiac arrhythmia risk (Redfern et al., 2003; Sanguinetti and Tristani-Firouzi, 2006). Indeed, we found that ibogaine reduces hERG currents (IC_{50}, 4 μM) at concentrations similar to the drug's affinities for several of its known targets in the brain (Koenig et al., 2012). Thus our finding matches with the described reports of QT interval prolongation after ibogaine intake (Hoelen et al., 2009; Paling et al., 2012; Pleskovic et al., 2012).

Here, to study in more detail the possibly harmful impacts of ibogaine on the heart's electrophysiology, we explored the drug's effects on the function of various cardiac voltage-gated ion channels. Therefore, human ion channels that contribute significantly to the action potential (hERG potassium channels, $hNa_v1.5$ sodium channels, and $hCa_v1.2$ calcium channels) were heterologously expressed in TSA-201 cells. In addition, ibogaine's effects on the cardiac AP were assessed in experiments on ventricular cardiomyocytes derived from adult guinea pig hearts, and in simulations using a computer model of a human ventricular cardiomyocyte. In an effort to estimate margins of safety for this drug between the ion channel potencies in vitro and human clinical exposure, we determined human plasma protein binding of the drug. Moreover, we also tested its synthetic congener 18-Methoxycoronaridine (18-MC), which exhibits anti-addictive effects but is believed to be less toxic (Glick et al., 1996, 2000, 2001, 2011).

Materials and methods

The study complies with the rules of the University Animal Welfare Committee. All of the procedures were conducted in accordance with the European Community Council Directive for Care and Use of Laboratory Animals.

Culture and transfection of TSA-201 cells

TSA-201 cells (American Type Culture Collection (ATCC, Manassas, VA, USA)) were propagated in a Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Vienna, Austria) containing 10% foetal bovine serum (FBS), 50 U/ml penicillin, and 50 U/ml streptomycin, and were incubated at 37 °C in a humidified incubator with 5% CO_2. Cells were transfected with pCEP4-plasmid (0.7 μg per 3.5 cm dish) containing the coding sequence for the human cardiac hERG potassium channel, or pGEM3-plasmid (1–2 μg per 3.5 cm dish) containing the coding sequence for the human cardiac $hNa_v1.5$ sodium channel. For $hCa_v1.2$ calcium current expression, an equimolar ratio of cDNA (1 μg per 3.5 cm dish) encoding for $hCa_v1.2$ α_1 subunit (77-pcDNA3) together with auxiliary $rCa_v\beta_3$ and $rCa_v\alpha_2\delta_1$ subunits was used. 77-pcDNA3 (Soldatov et al., 2000) was used in this work with the permission of Dr. Nikolai M. Soldatov (Humgenex, Inc., Maryland, USA). Cotransfection with pEGFP-C1-plasmid (0.02 μg) encoding green fluorescent protein (GFP) allowed the identification of successfully transfected cells. For transfection of hERG, $hNa_v1.5$ and $hCa_v1.2$, ExGen 500 (Fermentas, St. Leon-Rot, Germany) and FuGENE (Promega, Mannheim, Germany) were used according to the manufacturer's protocol.

Isolation of adult guinea pig ventricular cardiomyocytes

Ventricular cardiomyocytes from adult female Dunkin-Hartley guinea pigs (200–400 g) were isolated using a Langendorff setup following procedures previously described (Koenig et al., 2011).

Drug sources and preparation of stock solutions

Ibogaine hydrochloride originated from Sacrament of Transition (Maribor, Slovenia). 18-Methoxycoronaridine (18-MC) racemate was purchased from Obiter Research (Champaign, IL, USA). Both drugs were dissolved in 0.1% HCl, and stock solutions with various concentrations in the range of 1 μM to 30 mM (upper solubility limit) were prepared. These stocks were stored at −20 °C. On the day of the experiment, stock aliquots were freshly diluted 1:100 with the respective bath solutions to obtain final drug concentrations (range between 0.01 and 300 μM). The drug-free control bath solutions contained the same amount of HCl than the experimental solutions. Because of limitations in drug solubility in our bath solutions, we refrained from using higher drug end concentrations than 300 μM. E-4031 and isradipine were purchased from Sigma-Aldrich (Vienna, Austria).

Electrophysiological studies using the whole cell patch clamp technique

A detailed description of the electrophysiological recordings is given in our earlier work (Mille et al., 2009; Zebedin et al., 2008). Ionic currents were recorded from TSA-201 cells 24–48 h after transfection, and from adult cardiomyocytes up to 12 h after preparation at room temperature (22 ± 1.5 °C), using an Axoclamp 200B patch clamp amplifier (Axon Instruments, Union City, CA). Pipettes were formed from aluminosilicate glass (A120-77-10; Science Products, Hofheim, Germany) with a P-97 horizontal puller (Sutter Instruments, Novato, CA), and had resistances between 0.8 and 2 MΩ when filled with the respective pipette solutions (see below). Data acquisition was performed with pClamp 6.0 software (Axon Instruments) through a 12-bit A-D/D-A interface (Digidata 1200; Axon Instruments). Data were low-pass filtered with 1–10 kHz (−3 dB) and digitised at 10–100 kHz. Data analysis was performed using Clampfit 10.2 (Axon Instruments) and GraphPad Prism 5.01 (San Diego, USA) software. Rapid solution changes were performed by a DAD-8-VC superfusion system (ALA Scientific Instruments, Westbury, NY, USA).

hERG potassium currents. The pipette solution contained 130 mM KCl, 5 mM $MgCl_2$, 5 mM K_2-ATP, 5 mM EGTA, 10 mM HEPES (pH = 7.2, adjusted with KOH). Recordings were made in a bath solution that consisted of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, pH = 7.4 adjusted with NaOH.

Sodium currents. The pipette solution contained 105 mM CsF, 10 mM NaCl, 10 mM EGTA, 10 mM HEPES, pH = 7.3 adjusted with CsOH. Recordings of $hNa_v1.5$ sodium channels expressed in TSA-201 cells were made in a bath solution that consisted of 140 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH = 7.4 adjusted with NaOH.