Mechanism of hERG channel block by the psychoactive indole alkaloid ibogaine

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**ABSTRACT**

Ibogaine is a psychoactive indole alkaloid. Its use as anti-addictive agent was accompanied by QT prolongation and cardiac arrhythmias, which are most likely caused by hERG potassium channel inhibition. Therefore, the interaction of ibogaine with hERG channels heterologously expressed in tsA-201 cells was studied in detail. Currents through hERG channels were blocked regardless whether ibogaine was applied via the extra- or intracellular solution. The extent of inhibition was determined by the relative pH values. Block occurred during the activation of the channels and was not observed for resting channels. With increasing depolarizations ibogaine block grew and developed faster. Steady-state activation and inactivation of the channel were shifted to more negative potentials. Deactivation was slowed, while inactivation was accelerated. Mutations in the binding site reported for other hERG channel blockers (Y652A and F656A) reduced the potency of ibogaine, whereas an inactivation-deficient double mutant (G628C/S631C) was as sensitive as wild-type channels. Molecular drug docking indicated binding within the inner cavity of the channel independently of the protonation of ibogaine. Experimental current traces were fit to a kinetic model of hERG channel gating, revealing preferential binding of ibogaine to the open and inactivated state. Taken together, ibogaine blocks hERG channels from the cytosolic side either in its charged form alone or in company with its uncharged form and alters the currents by changing the relative contribution of channel states over time.
INTRODUCTION

In the human heart, delayed rectifying potassium currents are essential for the repolarisation of cardiac action potentials (Sanguinetti and Tristani-Firouzi, 2006). The rapid delayed rectifier current (I_{Kr}) provides the most important component of repolarizing currents and is carried by the human ether-a-go-go related (hERG) channel (Trudeau et al., 1995; Sanguinetti and Tristani-Firouzi, 2006). Mutations within the hERG gene may lead to reduced currents through these channels, to delayed cardiac repolarisation, and thereby to hereditary long-QT syndromes (Splawski et al., 2000). Carriers of these mutations are predisposed to an increased risk for arrhythmias and sudden cardiac death. Acquired long-QT syndromes occur by far more often than the inherited forms and are most commonly caused by a large variety of drugs that reduce currents through hERG channels. These drugs do not only comprise antiarrhythmics, but also antihistamines, antibiotics and psychoactive compounds, some of which have been withdrawn from the market because of their high risk of sudden cardiac death. As a consequence, chemicals are routinely tested for their effects on hERG channels early in the drug development process (Sanguinetti and Tristani-Firouzi, 2006).

Ibogaine is a psychoactive indole alkaloid that is frequently used as anti-addictive agent (Alper, 2001; Maciulaitis, Kontrimaviciute, Brefssolle, & Briedis, 2008), even though it has never been licenced as a therapeutic drug (Vastag, 2005; Alper et al., 2012). Therefore, ibogaine has never been subjected to thorough preclinical testing. Several cases of sudden death after ibogaine intake have been reported and were most likely caused by cardiac arrhythmias (Maas and Strubelt, 2006; Hoelen et al., 2009; Alper et al., 2012). Additional evidence for ibogaine’s cardiotoxicity is provided by case reports on acquired long-QT intervals and resulting cardiac tachyarrhythmias subsequent to ingestion of this alkaloid (Hoelen et al., 2009; Paling et al., 2012; Pleskovic et al., 2012). Most recently, we could provide direct experimental evidence for a reduction of currents through hERG channels by ibogaine: the alkaloid concentration that reduced the currents by 50% (IC_{50}) was shown to be
4 μM (Koenig et al., 2012, 2013). Low micromolar concentrations of ibogaine were also detected in human blood plasma after ingestion of typical doses (0.5 to 1 g, (Mash et al., 1998)) and plasma protein binding was found to be approximately 65% (Koenig et al. 2013). Drugs with IC50 values for hERG current inhibition within or close to their range of therapeutic plasma concentrations have a high propensity to cause QT prolongation and to trigger torsade de pointes arrhythmias (Redfern et al., 2003).

However, blockage of hERG currents by drug concentrations within the therapeutic range does not necessarily correlate with a high risk of long-QT intervals. For instance, the widely used antipsychotics chlorpromazine and thioridazine have been shown to block hERG channels equipotently and in the range of therapeutic plasma concentrations (Thomas et al., 2003; Milnes, Witchel, Leaney, Leishman, & Hancox, 2006), but only thioridazine and not chlorpromazine is likely to cause QT prolongation (Reilly et al., 2000). Thus, factors other than hERG channel affinities must also play a role, and differences in the underlying blocking mechanisms have been invoked as reasons for differences in cardiotoxicity (Vandenberg et al., 2001; Redfern et al., 2003). Along these lines, chlorpromazine has been suggested to bind to closed hERG channels (Thomas et al., 2003), while the inhibition by thioridazine was reported to be contingent on channel opening (Milnes et al., 2006). Moreover, changes in the gating properties of hERG - as caused by mutations - strongly influence cardiac repolarization (Chen, Zou, Splawski, Keating, & Sanguinetti, 1999; Nakajima et al., 1998). Therefore, it is essential to not only determine a drug’s affinity for hERG channels, but to also elucidate the mechanisms that lead to channel block, and to characterize resulting alterations in channel gating. Here, this has been done for the psychoactive alkaloid ibogaine.
MATERIAL & METHODS

Cell Culture and transfection
tsA-201 cells (American Type Culture Collection (ATCC, Manassas, VA, USA) were propagated in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, Vienna, Austria) containing 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 U/ml streptomycin, and were incubated at 37°C in a humidified incubator with 5% CO₂. Cells were transfected with pCEP4-plasmid containing the canonical coding sequence for the human cardiac ERG potassium channel (Kcnh2, Kv11.1, UniProt# Q12809) or with pcDNA3-plasmid containing the channel mutants Y652A, F656A or G628C/S631C (0.7 μg/3.5 cm dish). Cotransfection with pEGFP-C1-plasmid (0.02 μg) encoding green fluorescent protein (GFP) allowed the identification of successfully transfected cells. ExGen 500 (Fermentas, St. Leon-Rot, Germany) and FuGENE (Promega, Mannheim, Germany) were used according to the manufacturer’s protocols.

Electrophysiological studies using the whole-cell patch-clamp technique
Ionic currents were recorded from tsA-201 cells 24-48 h after transfection at room temperature (22 ± 2°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 1 and 2 MΩ when filled with the respective pipette solutions. 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 analyzed with Clampfit 10.2 (Axon Instruments) and Prism 5.01 (GraphPad Software, San Diego, CA) software. Rapid solution exchange was performed by a DAD-8-VC superfusion system (ALA Scientific Instruments, Westbury, NY). Normal pipette solution contained 130 mM KCl, 5 mM MgCl₂,
5 mM K$_2$-ATP, 5 mM EGTA, 10 mM HEPES (pH=7.2, KOH). pH was titrated to 6.8 and 8.2 with HCl and KOH and to 5.5 with citric acid, respectively. Normal bath solution 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, NaOH). A pH of 6.8 and 8.2 was adjusted with HCl and NaOH, respectively.

Ibogaine stock solutions were prepared in 0.1% HCl and stored at -20°C. On the day of the experiment, stock aliquots were freshly diluted 1:1000 with the respective bath solution. For intracellular application of 100 µM ibogaine a 30 mM stock was diluted 1:300 in the respective pipette solution.

**Data analysis and curve fitting**

Curve fitting was performed with nonlinear least square regression in GraphPad Prism 5.01. Normalized hERG activation data of individual experiments were fit to a Boltzmann function: $I_{\text{norm}} = 1/(1+\exp((V/V_{0.5}-V)/K))$ where $I_{\text{norm}}$ is the normalized hERG tail current, $V$ is the membrane potential, $V_{0.5}$ is the voltage at which half maximum activation occurred, and $K$ is the slope factor. Individual steady-state inactivation data were fit to a Boltzmann function: $I = I_{\text{max}}/(1+\exp((V-V_{0.5})/K))$ and normalized to the maximal amplitude, $I_{\text{max}}$. Steady-state inactivation data were not corrected for channel deactivation during the second voltage step; a significant amount of deactivation was only present at -100 mV, which however did not exceed 20%. Concentration-response data were fit with a Hill equation: $I_{\text{norm}} = 100/(1+(IC_{50}/C)^h)$ where the minimal and maximal values were constrained to 0% and 100%, respectively. $I_{\text{norm}}$ is the current during drug exposure in relation to the current during drug-free conditions, $IC_{50}$ is the concentration at 50% current inhibition, $C$ is the drug concentration, and $h$ is the Hill slope.

**Molecular drug docking**
The recently published hERG homology model (‘model 6’, (Stary et al., 2010)) after 50 ns molecular dynamics simulations was used as a starting point for docking investigations. Coordinates of ibogaine were obtained from the Pubchem structure database (http://pubchem.ncbi.nlm.nih.gov/) and the geometry optimized with Hartree–Fock, 6-31G* basis set, implemented in Gaussian09 (Frisch et al., 2009). Docking was performed with the program Gold 4.0.1 (Cambridge Crystallographic Data Centre, UK) using the Gold scoring function. The coordinates of the geometric center calculated among the Y652 and F656 residues were taken as binding site origin. The binding site radius was set equal to 10 Å. 100,000 operations of the GOLD genetic algorithm were used to dock the selected compounds into the open state hERG model.

**Modelling**

A Markov Model formalism was used to simulate the kinetics of the ibogaine induced HERG channel block. The linear gating model proposed by (Wang et al., 1997a), containing three closed, one open and one inactive state, was chosen as the starting configuration, for its fully identifiable parameters (Fink and Noble, 2009). For a review of available HERG models consult (Bett et al., 2011). To test the action of ibogaine, we expanded the original model topology by introducing up to three interacting drug-bound states (Fig. 8A). The most complex model (COIB) containing all three bound states was similar to that proposed by (Ganapathi et al., 2009) for R-roscovitine. It served as a reference, as it contained all rates of the three simpler nested models (OIB, OB, IB; Fig. 8A). The models were implemented, analytically solved and fit to the recorded data in MathWorks Matlab 2011b 64 bit, on a desktop computer with an Intel i7 quad-core CPU and 16GB ram. To reduce data size and computing time, traces were sub-sampled. The fitting procedure employed a sequential algorithm, consisting of an initial global genetic algorithm followed by constrained nonlinear optimization of a weighted least square error function:
\[ RSSE = \frac{1}{N} \sum \frac{(Y_{\text{data}} - Y_{\text{model}})^2}{Y_{\text{data}}}. \]

Starting values for the model parameters were manually introduced into the initial population and contained published model rates (Wang et al., 1997a), as well as cold (lower bounds) and hot (upper bounds) start configurations. Model fitness was assessed by applying a form of likelihood ratio test (Horn, 1987) for nested models:

\[ T = \frac{(SSE_s - SSE_f)}{SSE_f} \times \frac{(n - k_f)}{k_s}. \]

Where SSE_f and SSE_s are the error functions and k_f and k_s are the number of parameters of the full (f) and the nested (s) models. The number of data points is n and T is the F(k_f,n-k_s)–distributed statistic.

**Statistics**

All values are given as arithmetic mean ± sem. The statistical significance of differences between two groups was tested with a Student’s t-test. Experiments with three groups were tested by analysis of variance (ANOVA) with Tukey’s post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant.
RESULTS

All results were obtained by conventional whole-cell patch-clamp recordings in tsA-201 cells transiently expressing hERG (Kcnh2, Kv11.1) channels. Previously, ibogaine had been shown to reduce currents through recombinant hERG channels with half maximal inhibition at about 3 µM (Koenig et al., 2012, 2013). In all subsequent experiments that do not include complete concentration dependences, 3 µM ibogaine was used as representative concentration.

Kinetics and voltage dependence of hERG channel block by ibogaine

hERG currents were activated and deactivated from a holding potential of -80 mV once every 15 s by a voltage step to +10 mV with subsequent repolarization to -50 mV (Fig. 1A). Application of 3 µM ibogaine reduced current amplitudes by more than 50%; this inhibition reached its maximum within 30 s and was entirely reversible within 45 s (Fig. 1A). During the first 4 s depolarization in the presence of ibogaine, the current appeared to progressively decline. After the removal of ibogaine the current appeared to progressively increase. These observations suggest that the development of block as well as unblock is related to channel gating.

To study the action of ibogaine on open and inactivated channels, in a second set of experiments cells were depolarized for 80 s to voltages between -20 and +40 mV (Fig. 1B). During each period of depolarization, 3 µM ibogaine was applied and subsequently removed. Under these conditions, the reduction of steady current levels by ibogaine was maximal within up to 5 s and entirely reversible within about 10 s (Fig. 1B). The kinetics of this decrease in current amplitude as well as its recovery after removal of ibogaine were well described by monoexponential functions with time constants of 0.9 s for the onset of block and of 5 s for the recovery. Within the voltage range tested (-20 to +40 mV), these time constants did not show significant changes (Fig. 1B).
The observation that onset and cessation of block was voltage-independent raised the possibility that diffusion across the membrane might be the rate-limiting step. To address this in a third series of experiments, cells were first exposed to 3 µM ibogaine for more than a minute, which allowed for equilibration of external and internal drug concentrations. Channels were then activated by increasing voltage steps to between -20 and +40 mV and the ratio of current amplitude in the presence and absence of ibogaine ($I_{\text{ibogaine}}/I_{\text{control}}$) was calculated (Fig. 1C). Under these conditions the onset of block as well as its extent was in fact voltage-dependent (Fig. 1C). Fitting the onset of block by monoexponential functions revealed that the time constants decreased from 900 ms at -20 mV to <100 ms at +40 mV. The extent of channel block, on the other hand, increased from 30 % at -20 mV to >70 % at +40 mV (Fig. 1C). The current amplitude ratio ($I_{\text{ibogaine}}/I_{\text{control}}$) is a measure for the fraction of unblocked channels. The fact that it had an initial value of one implied that resting channels were not blocked by ibogaine.

Together, these results suggest that (i) ibogaine binds to the open and/or inactivated state but not to the closed state of the channel, (ii) channel inhibition by the alkaloid is voltage-dependent, and (iii) the onset of channel block is rate limited by diffusion of the drug through the membrane.

**Ibogaine acts on hERG via the cytosolic side**

The above mentioned experiments pointed to an action of ibogaine on hERG channels from the cytosolic side, as previously reported for several other hERG channel blockers (Kiehn et al., 1999; Zhang et al., 1999). Most of these blockers contain tertiary amines within their structures. They can thus occur in a neutral or a charged form depending on the ambient pH value. Obviously, only the neutral form can readily diffuse across cell membranes to reach the cytosol.
Ibogaine also contains a tertiary amine (Fig. 2A) and has a $pK_a$ value of approximately 8.1 (see supplementary and (Alper, 2001)). Therefore, at physiological pH (7.4) 17% of ibogaine exists in the neutral form ([Ibo]$_n$), and 83% in the charged form ([Ibo]$^+$); acidification of the extracellular milieu will increase the non-permeant charged form, whereas higher pH values will favor the membrane permeant form. Thus, raising the extracellular pH ($pH_e$) can be expected to enhance the block of hERG channels by the alkaloid. In accordance with this prediction, increases in $pH_e$ from 6.8 to 7.4 or 8.2, respectively, enhanced the reduction of current amplitudes by 3 µM ibogaine (Fig. 2D; n=4, 6, 4; p<0.001).

Conceivably, the levels of intracellular ibogaine are determined by the diffusion of the neutral form into the cell and by diffusion out of the cell (Fig. 2B). Acidification of the intracellular milieu will decrease the neutral form and thus trap ibogaine in the cytosol. As expected, the block of hERG channels as assessed by tail current amplitudes was increased when the intracellular pH ($pH_i$) was lowered from 7.2 to 5.5 (Fig. 2E; n=6, 3; p<0.001). The above results indicate that ibogaine had to penetrate the membrane in order to be able to block the channels from the cytosol.

To further confirm this notion, the alkaloid was applied from the intracellular side. An ibogaine concentration of 100 µM added to the recording patch-pipette was necessary to reduced hERG currents to an extent similar to that achieved by extracellular application of 3 µM (Fig. 2F). Moreover, the reduction of hERG currents by intracellularly applied ibogaine was more pronounced at pH$_i$ of 5.5 than at pH$_i$ of 7.2 (Fig. 2F; n=6, 5; p<0.01). This confirms that ibogaine acted on the channel from the cytosolic side and that acidification of the cytosol enhanced the action. It should be noted that lowering the external pH itself caused profound changes of hERG current properties. In agreement with previous studies (Bérubé et al., 1999; Zhang et al., 1999), extracellular acidification decreased current amplitudes and accelerated deactivation (Fig. 1C). In contrast, lowering the internal pH from 7.2 to 5.5 did not result in any kinetic changes (not shown).
So far, either pH$_o$ has been elevated to promote diffusion of ibogaine through the membrane into the cytosol, or pH$_i$ has been lowered to trap ibogaine within the cell. In a third set of experiments, pH$_o$ and pH$_i$ were adjusted simultaneously to values of 6.8, 7.4, and 8.2, and ibogaine was applied extracellularly. With equal H$^+$ concentrations on both sides of the membrane, ibogaine cannot have a tendency to accumulate here or there and will reach equal concentrations extra- and intracellularly. Nevertheless, given the pK$_a$ value for ibogaine of 8.1 (supplementary and (Alper, 2001)), the relative fractions of the neutral and charged forms vary between 5 : 95 at pH 6.8 and 56 : 44 at pH 8.2. Thus, within this pH range the concentration of the neutral form increases by a factor of 10 while the concentration of the charged form is reduced by only 50%. As shown in Fig. 2G, the extent of current inhibition by ibogaine was not significantly different at these three different pH values. Thus, the neutral form of ibogaine can be excluded as unique mediator of channel inhibition. Hence, hERG channel block is rather mediated by the charged form of ibogaine, either alone or together with the uncharged form.

Effects of ibogaine on hERG channel activation and deactivation

To investigate ibogaine’s action on hERG channel activation, currents were elicited from a holding potential of -80 mV by a two pulse protocol. Step depolarizations to potentials between -70 and +50 mV were applied for 4 s to activate and inactivate hERG channels. A subsequent 6 s repolarizing step to -50 mV allowed for rapid recovery of the channels from inactivation and revealed tail currents that reflect deactivation of the channels (Perry et al., 2010). Ibogaine reduced current amplitudes during depolarizations as well as repolarizations (Fig. 3A). To evaluate its effects on the voltage-dependence of activation, tail current amplitudes were determined and plotted against the voltage of the preceding depolarization. After normalization to maximal current amplitudes, a hyperpolarizing shift in the voltage
dependence of activation became obvious (Fig. 3B): fitting these data to a Boltzmann function produced the following voltage values of half maximal activation (mV): -10 ± 1 (control), -19 ± 2 (3 µM ibogaine), and -10 ± 3 (washout; n=8; p<0.001); the slopes of these three curves were not different from each other (p>0.05). This hyperpolarising shift in the activation increased in a concentration-dependent manner at 0.03 to 30 µM ibogaine (n=3-8; Fig. 3C). Hence, ibogaine affected the voltage-dependence of gating and rendered the channels more sensitive towards depolarizations.

Changes in the deactivation of hERG channels may lead to prolongation or shortening of the QT interval, e.g. (Chen et al., 1999). When deactivation was assessed by measuring tail currents at different voltages, ibogaine turned out not only to reduce current amplitudes, but also to slow the kinetics (Fig. 3D). Monoexponential fits of the tail currents revealed that the time constants of deactivation increased in a voltage-dependent manner. At voltages of -50 mV and above, these time constants were significantly prolonged in the presence of 3 µM ibogaine (Fig. 3E). A similar prolongation of deactivation was observed at 30 µM ibogaine, while at lower concentrations of the drug the effect was less pronounced (n=4-7; Fig. 3F). This indicates that ibogaine delays hERG channel deactivation in a concentration-dependent manner.

Effects of ibogaine on hERG channel inactivation

Rapid inactivation contributes to the inward rectifying properties of hERG channels and thereby to its preferential opening in the repolarizing phase of cardiac action potentials (Sanguinetti and Tristani-Firouzi, 2006). To investigate effects of ibogaine on hERG inactivation, the channels were first activated and simultaneously inactivated by a 300 ms depolarization, then recovered from inactivation (without causing deactivation) by a short hyperpolarization (-110 mV), and finally exposed to re-depolarization to different voltages to let them undergo inactivation again (Fig. 4A). The decaying outward currents during the
second depolarization reflect the onset of inactivation. They can be described by monoexponential functions and their time constants are parameters for the entry into inactivation. 3 µM ibogaine reduced the amplitudes of these decaying currents and speeded the entry into inactivation; this was reflected by a decrease in the calculated time constants at all test potentials (Fig. 4B). The decline in these inactivation time constants was concentration dependent between 0.03 and 30 µM ibogaine (n=3-6; Fig. 4C).

The voltage-dependence of steady-state inactivation was assessed as in Walker et al. (2000). Briefly, channels were first driven into inactivation (1 s depolarization to +30 mV); a second voltage step of 20 ms to between -100 mV and +60 mV allowed for rapid recovery and subsequent inactivation of the channels (Fig. 4D). Tail currents elicited by a final step to +30 mV were taken as a measure for the non-inactivated channels. Again, these current amplitudes were much smaller in the presence of ibogaine than in its absence (Fig. 4D). Plotting of normalized current amplitudes versus the preceding 20 ms voltage revealed that ibogaine caused a hyperpolarizing shift in steady-state inactivation (Fig. 4E): the voltages of half maximal inactivation as obtained by fitting the data to Boltzmann functions were (mV): -45 ± 3 (control), -63 ± 2 (3 µM ibogaine), and -49 ± 3 (washout; n=5; p<0.001); the slopes of these three functions were not significantly different from each other. Hence, ibogaine renders channels more sensitive towards inactivation and accelerates its onset.

**Ibogaine block of mutant hERG channels**

For several hERG channel blockers, inactivation gating seems to be important for high affinity drug binding (Guo et al., 2006). As ibogaine was found to affect inactivation, we tested the action of the alkaloid in the double mutant G628C/S631C, which does not undergo inactivation (Smith et al., 1996). This double mutant activated at the same voltage as wild-type channels, but the resulting currents had lower amplitudes; the tail currents were small and displayed slow deactivation kinetics when compared to wild-type. Ibogaine reduced
currents through the G628C/S631C mutant at +10 mV in a concentration-dependent and entirely reversible manner (Fig. 5A). The concentration response curve for the inhibition of currents in the G628C/S631C mutant was superimposable with that obtained with wild-type hERG currents (n=4; p>0.05; Fig. 5B). Thus, hERG channels with impaired inactivation are equally sensitive to ibogaine as wild-type channels.

Two aromatic residues located on the pore-lining S6 domain of hERG, i.e. Y652 and F656, have been identified as key molecular determinants for high affinity binding of channel blockers (Perry et al, 2010). To find out whether these residues were also involved in the binding of ibogaine, the mutant channels Y652A and F656A were investigated and their inhibition by ibogaine was compared with that of the wild type channels. Expression of the F656A mutant channel led to reduced current amplitudes and to abnormal channel gating as described before, e.g. (Mitcheson et al., 2000). Current inhibition, as quantified by tail current amplitudes at -50 mV, was shifted to higher ibogaine concentrations for both mutant channels in comparison with wild-type hERG. As a consequence, the calculated IC_{50} values were 5- and 50-fold higher for Y652A and F656A, respectively, than for wild-type hERG (n=4; Fig. 5C). These data suggest that ibogaine binds within the channel’s inner cavity and that ibogaine’s binding pocket includes the canonical residues Y652 and F656.

**Drug docking**

To further support the notion that ibogaine binds the channel’s inner cavity, we applied molecular drug docking to investigate the structural interactions of ibogaine with a previously published hERG homology model (Stary et al., 2010). These simulations suggest that ibogaine binds to the central cavity of hERG and forms hydrophobic interactions with the aromatic rings of F656 and Y652 of the S6 helices (Fig. 6). Further, the nitrogen atom of the indol moiety of ibogaine likely interacts with the selectivity filter serines 624 from two
adjacent subunits via hydrogen bonds. Docking poses obtained with protonated and non-protonated ibogaine species are predicted to be essentially the same.

**Frequency-dependence of hERG channel block**

The antiarrhythmic agent amiodarone, as one prominent example, blocks hERG channels in a frequency-dependent manner, and this kind of action may be one reason for its comparably low proarrhythmic potential (Kiehn et al., 1999). To examine whether the block by ibogaine was also frequency-dependent, trains of biphasic pulses of variable lengths (30, 100 and 300 ms) were applied at frequencies of 0.1, 1 and 3 Hz (Fig. 7A). These trains were interrupted for 1 min to expose the cells to 3 µM ibogaine and were then continued in the presence of the alkaloid. Channel block was analyzed by means of tail current amplitudes, and all values were normalized to the first amplitude within a train. At all frequencies, the extent of steady-state block increased with the length of the pulses (n=4-7; Fig. 7B-D). With 300 ms pulses, steady-state block was reached almost immediately after the 1 min incubation period and the extent of block was the same at both frequencies of 0.1 and 1 Hz (compare Fig. 7B and C). With shorter pulses, steady-state was not achieved immediately, but the block developed during continuous pulsing; this additional, slowly developing block was dependant on the number of pulses being applied. Importantly, for all the different pulse lengths tested, the levels of initial block and of steady-state block, respectively, were very similar at all three frequencies (Fig. 7B-D). Thus, hERG channel block does not depend on the frequencies but rather on the lengths of the pulses applied to elicit currents.

**A gating model explains the hERG channel block by ibogaine**

The data presented above suggest that ibogaine does not block closed, but rather open and inactivated channels, although inactivation did not appear to be a prerequisite for current inhibition. To comprehensively analyze the underlying mechanisms and functional
consequences of channel block by ibogaine, we used a gating model of hERG channels based on the work of (Wang et al., 1997a). This model foresees three closed states (C1, C2, and C3), one open (O), and one inactivated (I) state of the channel (Fig. 8A). Transitions between these states are voltage-dependent with one exception: C2 ↔ C3 (Wang et al., 1997a). This model was amended to allow for ibogaine being bound to the open (OB), the inactivated (IB), and the closed state (CB), respectively, or for a combination of ibogaine bound states (OIB and COIB; Fig. 8A). To identify the set of parameters, which best describe the observed hERG channel-ibogaine interaction, these models were globally fit to our experimental data. For that purpose we used original current traces obtained in the absence and presence of 3 µM ibogaine from recordings testing for activation (Fig. 3A), deactivation (Fig 3C), and inactivation (Fig. 4A). A likelihood ratio based test for nested models (Horn, 1987) was used to rank the fitness of the candidate models (Fig. 8A). When compared to models with only a single bound state (OB, IB), the more complex models containing two or three bound states (OIB, COIB), respectively, better described the measured data (p<0.01). Tested against each other, the introduction of an ibogaine-bound closed state (COIB) did not significantly enhance the results generated by the simpler OIB-model (p>0.05, Fig. 8B). Comparisons of simulated and recorded current traces are shown in Fig. 8C. Model parameters for the state transitions within the model OIB are displayed in Table 1. To validate our model and the calculated rate constants, we simulated the inhibition of currents by ibogaine during depolarizations to +10 mV (Fig. 8D). The concentration response curve generated by the model precisely replicated our experimental data (Fig. 5B). Furthermore, our kinetic model offers the additional benefit of revealing the state occupancies during 4 s depolarizations to +10 mV and 6 s repolarizations to -50 mV: in the presence of ibogaine, the relative contribution of the open state is promoted, whereas the contribution of the inactivated state is diminished (Fig. 8E, compare panels control with ibogaine combined). This is paralleled by preferential binding of ibogaine to the inactivated state early during depolarizations (Fig. 8E, panel ibogaine drug
bound). The longer the depolarization is maintained, the more the ibogaine-bound open state prevails, and this also holds true for the repolarizing phase (Fig. 8E, panel ibogaine drug bound). Thus, the model predicts that the majority of ibogaine is first bound to the inactivated state of the channel (IB), which then transits to the drug bound open state (OB). Nevertheless, removal of the inactivation state from the model hardly altered the calculated concentration response relation of current inhibition (Fig. 8D). Hence, although being dispensable at first sight, binding of ibogaine to the inactivated state is an essential component of hERG channel block by the alkaloid.

**DISCUSSION**

The present results elucidated the mechanism of channel block and revealed that ibogaine (i) passes the membrane to bind to the channel from the cytosolic side, (ii) blocks the channel either in its charged form alone or in company with the neutral form, (iii) is bound to the open and inactivated configuration of the channel, and (iv) alters the transition of the channel between the closed, open and inactivated states.

**Intracellular action of ibogaine**

Generally, there are innumerable K⁺ channel blockers that act either from the extracellular or the intracellular side (Leung, 2012). Most hERG channel blockers are believed to access the channel via the cytosol after passive diffusion through the membrane (e.g. Zhang et al., 1999). The respective drugs are weak bases that occur either charged or uncharged depending on the ambient pH. Consequently, evidence for membrane diffusion as prerequisite for hERG channel block is twofold: (i) Permanently charged analogues do not block the channels as they cannot pass the membrane, e.g. N-methyl verapamil (Zhang et al., 1999). This approach relies on the implicit assumption that the drug and its minimally modified analogue do not
differ in their receptor interaction. This, however, might not always hold true. A positively charged derivative of 4-aminopyridine, for instance, was found 100-fold less potent than its parent compound even though it shared the same binding site (Kirsch and Narahashi, 1983). In any case, a permanently charged analogue of ibogaine is not available. (ii) A pH-dependent action that is based on the fact that due to the Henderson-Hasselbalch equation the neutral, membrane permeable form of a drug can be enriched by an increase in pH (see supplementary). In line, extracellular acidification was shown to reduce current inhibition for clinically relevant hERG channel blockers, such as quinidine, azimilide, flecainide, and verapamil. Like ibogaine, all these agents have alkaline pKₐ values, and their loss of effect due to lowered pHᵢ is taken as evidence for membrane permeation prior to channel block (Zhang et al., 1999; Dong et al., 2004). Further evidence for membrane permeation was provided by the enhanced action of ibogaine under intracellular acidification. Under these conditions, the alkaloid is trapped in its charged form and thus accumulates in the cytosol. This conclusion is further supported by the finding that acidification of the cytosol enhanced the effect of ibogaine also when applied via the recording pipette. The block of K⁺ channels by intracellular verapamil is also enhanced the more acidic the cytosol is in relation to the extracellular solution (DeCoursey, 1995). Thus, intracellular channel blockers rapidly diffuse out of the cells, and this is counteracted by intracellular decreases and extracellular increases in pH. Therefore, comparably high concentrations of channel blockers are required to efficiently inhibit currents when applied via the recording pipette (present results and (DeCoursey, 1995)).

Drugs that exert their action from the intracellular side are believed to bind to the inner cavity of hERG. The putative binding pocket comprises several amino acids within the pore helix of the S6 domain amongst which two aromatic residues, Y652 and F656, are of utmost importance (Perry et al, 2010). Mutation of these aromatic residues to e.g. alanine greatly reduces the potencies of several hERG channel blockers including cisapride, halofantrine,
quinidine, ketokonazole and Mk-499 (Mitcheson et al., 2000; Sánchez-Chapula et al., 2002, 2004; Ridley et al., 2006; Kamiya et al., 2008). Likewise, the IC$_{50}$ of ibogaine for current inhibition was significantly reduced in these mutants (Fig. 5C). This confirms an intracellular site of action of the alkaloid and hints at a common binding site shared by numerous drugs that are infamous hERG channel blockers.

**State-dependent block of ibogaine**

It is widely accepted for most of these drugs that hERG channels need to open before block can occur (Perrin and Kuchel, 2008), although there appear to be exceptions such as chlorpromazine (Thomas et al., 2003). Our results obtained with ibogaine can also be interpreted along these lines. In particular, the lack of current inhibition during initial phases of depolarization as determined by the fractional currents (Fig. 1C) strongly argued against a resting state block. Here, a drug targeting the closed state would be expected to produce a significant level of block as was observed for e.g. dofetilide (Tsujimae et al., 2004). Obviously, the binding site within the channel is exposed only upon depolarization and allows for access of the drug. *Vice versa* this implies that ibogaine must unbind upon repolarization when channels close despite the continuous presence of the drug. These unbinding events account for the observed slowing of deactivation, as the unbound channels have to transit the open state before deactivating. The same conclusion can be reached from the results of our gating models. The full model (COIB) shows a high K$_d$-value (~690 µM, data not shown) for the bound closed state, as well as slow transition rates. Accordingly, unbinding occurs predominantly in the OB and IB states. From there, channels then have to enter the open state before deactivating. Thus, C3 bound state occupancy is low during all phases, rendering this topology (COIB) obsolete. This is supported by the lack of a significant difference in model fitness among COIB and OIB (Fig. 8B; p>0.05), as described above.
hERG channel blockers may display preferential binding to the inactive over the open state. Mutant channels with impaired inactivation have been employed to test for this possibility (Tseng, 2001). For members of the methansulfonanilide family, e.g. dofetilide and E-4031, it has been shown that affinities were reduced by more than an order of magnitude when inactivation was removed (Wang et al., 1997b; Ficker et al., 1998). In contrast, ibogaine displayed no change in its IC$_{50}$ value when applied to the inactivation-deficient double mutant G628C/S631C (Fig. 5). This suggested no preferential binding to the inactive state, but argued for equal affinity to both states or preferential binding to the open state. Still, the significant acceleration of the entry into inactivation (Fig. 4B) as well as the left shift in steady-state inactivation (Fig. 4E) indicated a prominent role of the inactivated state in block development.

Additional insights were provided by our kinetic hERG models. The best model, determined by a likelihood ratio test, contained an open as well as an inactive bound state (OIB, Fig. 8A). In this model, ibogaine binds the open state slowly but with high affinity, whereas the inactivated state is bound rapidly but with reduced affinity. However, binding of ibogaine to the open state is so slow that most of the open channels undergo inactivation before substantial binding to the open state can occur. Despite the higher affinity for the open state, the major part of the initial drug binding thus results from the kinetically favoured association of ibogaine to the inactive state. Only then is the open bound state populated via the inactive bound state, which is reflected in the delayed rise in OB probability compared to IB probability (Fig. 8E). Nevertheless, removal of the inactivated states from the model hardly altered the concentration response relation for current inhibition (as did the use of the inactivation deficient double mutant; see above). This emphasises the importance of high affinity binding of ibogaine to the open state of hERG channels.

Relevance of hERG inhibition by ibogaine
As a consequence of ibogaine’s state-dependent binding, hERG channel activation, inactivation, and deactivation were significantly altered. Similar observations had been obtained with drugs such as cisapride (Walker et al., 1999), azimilide (Walker et al., 2000) and halofantrine (Tie et al., 2000). Like ibogaine, these substances target hERG with low µM potency and within or close to their therapeutic concentration range. They all share basic chemical properties including molecular weight, lipophilicity (logP) and pH-dependent protonation status (pKa), as well as a preference for the open and inactivated conformation of hERG channels. Moreover, they do not only reduce current amplitudes, but also cause changes in channel gating, such as a left shift in steady-state activation and inactivation, a slowed deactivation and an accelerated inactivation. In contrast, chlorpromazine and ketoconazole, suggested to bind hERG only in the closed conformation (Dumaine et al., 1998; Thomas et al., 2003), did not alter channel gating in the same manner. Hence, it is tempting to speculate that cisapride, azimilide, and halofantline share a common mechanism of hERG channel block with ibogaine. All of the former drugs carry an increased risk to develop cardiac arrhythmias (Redfern et al., 2003; Sánchez-Chapula et al., 2004). In particular, halofantrine has a measurable incidence of torsade de pointes (Tie et al., 2000; Sánchez-Chapula et al., 2004; and refs therein). Cisapride was withdrawn from the market due to the intolerable risk of its administration (Redfern et al., 2003). Azimilide is a class III antiarrhythmic specifically developed to target hERG (Walker et al., 2000). On the other hand, chlorpromazine and ketoconazole display little torsadogenic propensity (Reilly et al., 2000; Redfern et al., 2003). Thus, ibogaine queues in a prominent list of drugs known to have a high incidence of torsade de pointes arrhythmias.

For an overall arrhythmogenic risk assessment, hERG inhibition alone is clearly insufficient, and inhibition of other cardiac ion channels has to be taken into account (Redfern et al., 2003). Indeed, we could recently show that ibogaine also targets cardiac Na1.5 sodium and Ca1.2 calcium channels (IC50 values of 142 μM and 163 μM, respectively (Koenig et al.,
2013)) without affecting K\textsubscript{v}7.1 channels mediating I\textsubscript{Ks} (IC\textsubscript{50} >> 100 µM, unpublished data).

When tested on isolated guinea-pig cardiomyocytes, the AP prolonging effect of hERG channel inhibition by clinically relevant ibogaine concentrations was counteracted by the simultaneous block of calcium channels. However, if such a “compensatory” mechanism also matters in human is not known.

A final judgment and a quantitative assessment of human risk after ibogaine intake remain difficult as no randomised clinical trials have been carried out. It was estimated though that about 5000 people took ibogaine between 1990 and 2006 (Alper et al., 2008), while in the same period of time 11 cases of ibogaine related deaths were reported (an incidence of about 0.2%). However, the number of people taking ibogaine is increasing (Alper et al., 2008) and case reports of ibogaine triggered cardiac arrhythmias and related sudden cardiac deaths have accumulated recently, 8 in the past 2 years (Paling et al., 2012; Pleskovic et al., 2012; Jalal et al., 2013; Mazoyer et al., 2013; Papadodima et al., 2013; Vlaanderen and Martial, 2013). This asks for thorough investigations in predictive in vivo models and ultimately for standardized clinical trials in human.
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Authorship Contribution

Participated in research design: Thurner, Koenig, Hilber, Boehm, Sandtner

Conducted experiments: Thurner, Koenig, Stary-Weinzinger, Gafar, Kudlacek, Gawali

Contributed new reagents or analytic tools: Thurner, Kudlacek, Gawali

Performed data analysis: Thurner, Stary-Weinzinger, Koenig

Wrote or contributed to the writing of the manuscript: Thurner, Zezula, Koenig, Hilber, Boehm, Sandtner
References


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FIGURE LEGENDS

Fig. 1 Kinetics of hERG channel block. (A) Original hERG current traces elicited every 15 s by the indicated pulse protocol. Shown are the first traces after external application of 3 µM ibogaine (wash in) and after removal of the drug (recovery). Current amplitudes evaluated at the end of the first depolarizing voltage-step are plotted versus the pulse number (right). (B) Sustained hERG current elicited by a 80 s depolarizing voltage-step to -20 mV (left). External application of 3 µM ibogaine is indicated by the black bar. Ibogaine wash in and recovery time constants as obtained from single exponential fits are summarized for all tested voltages of -20 to +40 mV (right). (C) Superimposed traces of currents before administration and after equilibration with 3 µM ibogaine for depolarizing voltage-steps to -20 mV (left) and +40 mV (right). Pulse protocol shown on top. Beneath, plot of current ratios (I_{ibogaine}/I_{control}) during the initial 4 s of depolarization to -20 mV and +40 mV. At the bottom, voltage-dependence of time constants obtained from single exponential fits to the decay in I_{ibogaine}/I_{control} (left) and voltage-dependence of block determined as the unblocked channel fraction after 4 s depolarization (right).

Fig. 2 Intracellular action of ibogaine. (A) Chemical structure of ibogaine containing a tertiary amine with a pKₐ value of 8.1. (B) Fractional distribution of ibogaine into a neutral, membrane-permeable form ([Ibo]₀) and a charged, impermeable form ([Ibo]⁺) is dependent on intra- and extra-cellular pH, pHi and pHo, respectively. (C) Original hERG current traces as elicited by the indicated protocol recorded at a pHo of 6.8, 7.4 and 8.2 under control conditions and after equilibration with extracellularly applied 3 µM ibogaine. (D) Summary of hERG tail current block levels as shown in C, normalized to control at different pHo values. (E) Tail current block by extracellularly applied 3 µM ibogaine for an internal pH (pHi) of 7.2 and 5.5, respectively. (F) Current density values at a pHi of 7.2 and 5.5 for internal application of 100 µM ibogaine normalized to the respective drug free control (relative current density).
(G) Summary of hERG tail current block levels normalized to control by extracellular application of 3 µM ibogaine as observed for simultaneous change of pHᵢ and pHₒ to values of 6.8, 7.4 and 8.2. *** p<0.001; ** p<0.01; n.s., not significant.

**Fig. 3** hERG channel activation and deactivation. (A) Pulse protocol, to test for activation of hERG channels with the corresponding original current traces under control conditions, after equilibration with 3 µM ibogaine and after wash out of the drug. (B) Voltage-dependence of steady-state activation. Tail current peak amplitudes elicited by the 6 s repolarizing voltage-step to -50 mV were normalized to the maximal value and plotted as a function of the pre-pulse voltages. Data points were fit with a Boltzmann function. The dotted line represents rescaled ibogaine data for comparison. (C) Shift of half maximal activation relative to drug free control (ΔV₀.₅) in the presence of the indicated concentrations of ibogaine. (D) Pulse protocol to test for deactivation of hERG channels with corresponding original current traces under control conditions, after equilibration with 3 µM ibogaine and after wash out of the drug. (E) Time constants (τ) of deactivation were obtained from monoexponential fits to the decay of tail currents during the second 2.5 s voltage-step to various potentials. * p<0.05, *** p<0.001. (F) Changes in deactivation time constant relative to control (Δτ) at a potential of -40 mV in the presence of the indicated concentrations of ibogaine.

**Fig. 4** hERG channel inactivation. (A) Pulse protocol and representative original current traces to determine the time constants of inactivation. Currents traces before and after application of ibogaine are depicted on an expanded timescale (marked as grey shaded area in the pulse protocol). (B) Time constants (τ, ms) of inactivation were obtained from monoexponential fits to the decay of tail currents during the third 300 ms voltage-step. Values under control conditions, after equilibration with 3 µM ibogaine and after wash out of the drug are plotted as a function of the applied test potentials. (C) Changes in the inactivation
time constant relative to control (delta tau) at a potential of -20 mV in the presence of the indicated concentrations of ibogaine. (D) Pulse protocol and corresponding representative current traces on an expanded time scale to test for the voltage-dependence of hERG channel steady-state inactivation. (E) Tail current amplitudes determined during the third 100 ms step to +30 mV were fit with a Boltzmann function and normalized to maximal amplitude values. Data were plotted versus the applied voltages during the second pulse for control conditions, after equilibration with 3 µM ibogaine and after wash out of the drug. Lines represent best fits of the mean data with a Boltzmann function.

Fig. 5 Ibogaine block of mutant hERG channels. (A) Voltage-clamp protocol (same as in Fig. 1A) and representative hERG currents recorded from the inactivation-deficient double mutant G628C/S631C during depolarisations to +10 mV (grey shaded area) under control conditions, after equilibration of extracellularly applied ibogaine (0.3, 3 and 30 µM) and after wash out of the drug. The fraction of unblocked channels was determined at the end of the 4 s voltage-pulse to +10 mV. (B) Concentration-response relation from a series of such experiments for wild-type and G628C/S631C channels. Data were fit with the Hill equation to give IC$_{50}$ values of 1.2 µM and 1 µM for the wild-type and the mutant, respectively. (C) Concentration-response relation for the channel mutants Y652A and F656A evaluated at the tail current during repolarisation to -50 mV (see results). Calculated IC$_{50}$ values were 1, 5 and 50 µM for wild-type, Y652A and F656A, respectively.

Fig. 6 Ibogaine docking pose. hERG inner cavity with flanking S6 helices and pore loops (brown and green). Only 3 side chains are shown for clarity: residues Y652 (yellow) and F656 (blue) of the S6 helices, and residues S624 (orange) on the pore loop as part of the selectivity filter. Ibogaine in its protonated form is shown in purple. Hydrogen bonds are shown as blue dotted lines.
Fig. 7 Frequency-dependence of hERG channel block. (A) Biphasic voltage-clamp protocols applied at variable frequencies (0.1, 1 and 3 Hz) and variable pulse duration (30, 100 and 300 ms). Beneath, typical hERG currents elicited with 300 ms pulse duration at 1 Hz under control conditions and the first and second trace after application of 3 μM ibogaine. Pulsing was suspended for 60 s with the wash in of ibogaine (3 μM, black bar). After equilibration, repetitive pulsing was resumed. hERG current amplitudes determined at the peak of the tail currents (-120 mV) are plotted versus time for the three tested frequencies 0.1 Hz (B), 1 Hz (C) and 3 Hz (D).

Fig. 8 Best model fit of the experimental data. (A) Different hERG channel model topologies with additional drug binding for the open (OB), inactivated (IB), open and inactivated (OIB) and closed, open and inactivated (COIB) state. (B) Weighted sum of squares error values for the tested models. *** p<0.001, F-test. (C) Recorded original traces of hERG activation, deactivation and inactivation elicited with the same protocols as employed above (Fig. 3A, Fig. 3D and Fig. 4A, respectively) under control conditions (left) and under 3 μM ibogaine (right). Shown beneath is the best global data fit generated by the OIB model. All six experimental data sets, i.e. activation, deactivation and inactivation for control and 3 μM ibogaine, respectively, were simultaneously fit with a genetic algorithm followed by constrained nonlinear optimization. (D) Simulated dose-response relations for wild-type and inactivation deficient channels as predicted by the OIB model. G628C/S631C was simulated by abrogation of the O ↔ I and OB ↔ IB transitions. (E) State probabilities during the activation protocol under control conditions (left) and under 3 μM ibogaine (right).
Table 1. Transition rates of the OIB model.

Voltage-dependent rate constants were calculated as \( k = A \exp(zF/RT*V) \); \( A \) is the rate constant at 0 mV, \( z \) is the charge moved; \( F \) is the Faraday constant, \( R \) is the gas constant and \( T \) is the temperature. Forward and backward rates of the OIB model are given in s\(^{-1}\), except for the transitions O ↔ OB and I ↔ IB which are given in \( \mu \text{M}^{-1}\text{s}^{-1} \). \([c]\) is the concentration of ibogaine in \( \mu \text{M} \). Values were rounded to four significant digits.

<table>
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<th>forward</th>
<th>A</th>
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<td>x</td>
<td>C3 → C2</td>
<td>( k_2 )</td>
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<td>205,9105</td>
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Figure 2

A. **Ibogaine**

B. Chemical structure of Ibogaine with pKa=8.1

C. Graph showing current-voltage relationship with pH changes:
   - pHo 6.8
   - pHo 7.4
   - pHo 8.2

D. Bar graph showing Ibogaine/Control ratio with pH changes:
   - pHo 6.8
   - pHo 7.4
   - pHo 8.2

E. Graph showing Ibogaine/Control ratio with pHi changes:
   - pHi 7.2
   - pHi 5.5

F. Graph showing relative current density with pH changes:
   - pHo 7.4

G. Graph showing Ibogaine/Control ratio with pHo/pHi changes:
   - pHo/pHi 6.8
   - pHo/pHi 7.4
   - pHo/pHi 8.2

Notes:
- **pH** indicates the external pH conditions.
- **pHi** indicates the internal pH conditions.
- **n.s.** indicates not significant.
Figure 4

A

B

C

D

E

- □ control
- ○ 3 μM ibogaine
- ⌂ wash

- □ control
- ○ 3 μM ibogaine
- ⌂ wash

- □ control
- ○ 3 μM ibogaine
- ⌂ wash

- □ control
- ○ 3 μM ibogaine
- ⌂ wash

- □ control
- ○ 3 μM ibogaine
- ⌂ wash
Mechanism of hERG channel block by the psychoactive indole alkaloid ibogaine

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Supplemental material

Estimation of ibogaine’s pKₐ value

The obtained data of hERG inhibition by extracellularly applied 3 µM ibogaine at various pHₒ values can be used to get an estimate for the drug’s dissociation constant (pKₐ). The fractional distribution of a weak base between the neutral ([Ibo]ⁿ⁻) and the charged form ([Ibo]⁺) is dependent on the ambient pH and governed by the Henderson-Hasselbalch equation: [Ibo]ⁿ⁻/[Ibo]⁺ = 10^(pH –pKₐ). In a simple two compartment model as displayed in Fig. 2A, total internal ibogaine concentration is given by the following equation: [Ibo]ᵢ = [Ibo]ₒ * (1+10^(pKₐ-pHₒ))/((1+10^(pKₐ-pHᵢ)))(1+10^(pKₐ-pHₒ))). It is derived by applying the Henderson-Hasselbalch equation to both compartments and assuming only the neutral form to permeate the membrane. [Ibo]ᵢ and [Ibo]ₒ are the total internal and external drug concentrations, pHᵢ and pHₒ the internal and external pH values, respectively. A variation of pHₒ results in a change of the total internal concentration of ibogaine given by the above equation, but leaves the internal distribution between neutral and charged form untouched. For fixed pHᵢ and pHₒ, internal ibogaine concentration is thus solely determined by the drug’s pKₐ value. Under the assumption that ibogaine acts solely from the intracellular side, the observed levels of hERG
channel inhibition at the three tested pH values must thus obey the same concentration-
response relation as determined by various externally applied ibogaine concentrations at fixed
pH. Systematically varying the $pK_a$ value and fitting the resulting data to a logistic
concentration-response relation with the previously determined Hill coefficient of 0.8 (Koenig
et al., 2012) returned a value of 8.1 as the best estimate. Our estimated $pK_a$ value for ibogaine
is in line with the work of (Alper, 2001).

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doi:10.1111/j.1369-1600.2012.00447.x