Ibogaine, a Noncompetitive Inhibitor of Serotonin Transport, Acts by Stabilizing the Cytoplasm-facing State of the Transporter*

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Ibogaine, a hallucinogenic alkaloid with purported anti-addiction properties, inhibited serotonin transporter (SERT) noncompetitively by decreasing $V_{\text{max}}$ with little change in the $K_m$ for serotonin (5-HT). Ibogaine also inhibited binding to SERT of the cocaine analog 2β-2-carbomethoxy-3-(4-[125I]iodophenyl)tropane. However, inhibition of binding was competitive, increasing the apparent $K_D$ without much change in $B_{\text{max}}$. Ibogaine increased the reactivity of cysteine residues positioned in the proposed cytoplasmic permeation pathway of SERT but not at nearby positions out of that pathway. In contrast, cysteines placed at positions in the extracellular permeation pathway reacted at slower rates in the presence of ibogaine. These results are consistent with the proposal that ibogaine binds to and stabilizes the state of SERT from which 5-HT dissociates to the cytoplasm, in contrast with cocaine, which stabilizes the state that binds extracellular 5-HT.

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*Ibogaine is a hallucinogenic alkaloid found in the roots of the West African shrub Tabernanthe iboga. In Europe and North America, ibogaine has been promoted as a treatment for addiction, although clinical evidence has been difficult to obtain, partly due to its listing as a Schedule I controlled substance (1, 2) and partly due to concerns about toxicity (3). Ibogaine has affinity for α2 receptors, N-methyl-D-aspartate receptors, κ-opioid receptors, and serotonin and dopamine transporters (4–7). Ibogaine is demethylated to 12-hydroxyibogaine (noribogaine), which has been reported to persist in the blood for over 24 h and to have even higher affinity for 5-HT transporters than ibogaine (8).

The 5-hydroxytryptamine (5-HT)3 transporter (SERT) is responsible for reuptake of 5-HT released from serotonergic neurons (9). This protein is the target for antidepressant drugs, such as imipramine and fluoxetine, and psychostimulants, such as cocaine and 3,4-methylenedioxyamphetamine. SERT couples the entry of 5-HT into the cell to the entry of Na+ and Cl− and the exit of K+ (10). The transport of 5-HT has been envisaged as a two-step process, where 5-HT, Na+, and Cl− are transported in a single step and K+ is transported in a second step (11). These transport steps are proposed to interconvert two states of the transporter: an extracellular state that binds extracellular substrates and a cytoplasmic state that releases those substrates to the cytoplasm (9).

SERT belongs to a large family of Na+-dependent transporters in prokaryotes and animals designated the SLC6 or NSS family. The high resolution x-ray structure of the prokaryotic leucine transporter LeuT (12) appears to be a good model for other members of the family, and homology models for SERT and other neurotransmitter transporters have been generated using structure-based alignments (13). The utility of LeuT as a model for SERT has recently been validated by the identification of the Cl− binding site in SERT through analysis of the LeuT structure (14). This structure shows an aqueous pathway leading from the extracellular face of the protein almost to the leucine binding site. However, almost 20 Å of ordered protein structure separates that site from the cytoplasm, leaving unanswered the question of how bound substrates pass through to the cell interior.

In SERT, the cysteine reagent 2-(aminoethyl) methanethiosulfonate hydrobromide (MTSEA) reacted with cysteine residues placed in the intracellular half of transmembrane domain 5 (TM5) even at positions predicted by the LeuT structure to be inaccessible (15). The accessibility of these positions, as judged by the rate of the MTSEA reaction, varied in the presence of 5-HT and cocaine in a manner consistent with the proposal that TM5 contributes to the substrate permeation pathway between the cytoplasm and the binding site for 5-HT (16). According to this proposal, SERT undergoes conformational changes between two states. The extracellular state, stabilized by cocaine, is similar to the conformation of LeuT in the crystal structure, in which the TM5 residues would be buried and therefore less reactive with MTSEA. In the cytoplasmic state, favored in the presence of 5-HT, a cytoplasmic permeation pathway, formed partly by one face of TM5, connects the binding site with the cytoplasm, allowing dissociation of bound substrate and modification of cysteines placed in TM5.

As a tool for identifying other parts of the cytoplasmic pathway, it would be advantageous to use an inhibitor that stabilized..."
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In contrast, most SERT inhibitors, including cocaine, are competitive inhibitors. This behavior is expected for a compound that binds to the same state of SERT that binds extracellular 5-HT. However, an inhibitor that bound to the cytoplasmic state might not act as a competitive inhibitor, since it would stabilize a conformation of SERT to which extracellular 5-HT could not bind. To our knowledge, there are only two examples of transporters with inhibitors binding specifically to each conformation. These are the adenine nucleotide exchanger of mitochondria, inhibited by atractylolyside from the intramembrane space and bongkrekic acid from the matrix (18), and the red cell glucose transporter, inhibited by cytochalasin B from the cytoplasm and ethylideneglucose from the cell surface (18). Neither of these transporters is in the NSS family, and in both cases, evidence for the sidedness of inhibitor binding was based primarily on kinetic measurements. In the present work, we used structural inferences from the LeuT structure and site-directed chemical modification to understand the mechanism by which ibogaine inhibits transport and binding by SERT.

EXPERIMENTAL PROCEDURES

Expression of Rat SERTs—Rat SERT cysteine mutants used here were described previously (15). Briefly, each mutant was subcloned into the X5C background construct, which is lacking all endogenous cysteine residues known to react with MTS reagents (19). The expression system used has been described in detail elsewhere (20, 21). Briefly, HeLa cells were cultured in 96-well culture plates and allowed to grow overnight. The confluent cells were infected with recombinant VTF7–3 virus and transfected with a plasmid containing rat SERT cDNA under control of the T7 promoter. Transfected cells were incubated for 20–22 h at 37 °C with 5% CO2 and then assayed for transport. Alternatively, LLC-PK1 cells stably expressing rat SERT were grown and assayed in 24-well plates as described (22). Protein concentration was determined with the Micro BCA protein assay reagent kit (Pierce).

Transport Assay—[3H]5-HT influx was assayed in monolayer cultures at room temperature. Transfected HeLa cells in 96-well plates were washed once with 100 μl of phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, and 1.4 mM KH2PO4, pH 7.3) containing 0.1 mM CaCl2 and 1 mM MgCl2 (PBS/CM). Transport of 5-HT was measured by adding 100 μl of PBS/CM containing 20 nM [3H]5-HT (PerkinElmer) to each well and incubating for 10 min at room temperature. The assays were terminated by three rapid washes with ice-cold phosphate-buffered saline. The cells were then solubilized in 30 μl of 0.1 M NaOH for 30 min. The extent of [3H]5-HT accumulation was determined by a Wallac Microbeta plate counter.

To measure the effect of ibogaine, cocaine, or 5-HT on inactivation by MTSEA, HeLa cells expressing the appropriate rat SERT mutant were incubated with 10 μM ibogaine, cocaine, or 5-HT for 10 min in 50 μl of PBS/CM. This incubation was followed by the addition of MTSEA at the indicated concentrations in 50 μl of PBS/CM. After 10 min, the cells were washed five times with 100 μl of PBS/CM (Cells were incubated with PBS/CM between washes and gently rocked to ensure that all residual MTSEA, ibogaine, cocaine, or 5-HT was removed). Control wells that did not receive ibogaine, cocaine, or 5-HT were subjected to the same washes. Transport activity was then measured by the addition of 20 nM [3H]5-HT in 100 μl of PBS/CM.

Membrane Preparation and Binding Assay—Binding assays with membranes from transfected HeLa cells and platelets were performed as described (16, 23). Binding of the 125I-labeled high affinity cocaine analogue, 2β-carbomethoxy-3β-(4-iodo-phenyl)-tropane (β-CIT), was measured in crude membrane preparations. HeLa cells grown in 75-cm2 cell culture flasks were transfected with rat SERT cDNA as described above. After overnight transfection, the cells were rinsed once with room temperature 10 mM lithium-HEPES buffer (10 mM HEPES free acid brought to pH 8.0 with LiOH) and scraped into 10 ml of homogenization buffer (10 mM HEPES, pH 8.0, containing 0.5% of a protease inhibitor mixture (Sigma) and 100 μM phenylmethylsulfonyl fluoride). The cells were lysed by two cycles of freeze-thawing and sonication, and the resulting crude membrane fraction was collected by centrifugation at 15,000 × g for 20 min at 4 °C. The membranes were resuspended in 1 ml of homogenization buffer and stored at −80 °C in 0.1-ml aliquots until used.

For membrane binding assays, aliquots of membranes from cells expressing rat SERT mutants were thawed on ice and diluted with 1 ml of binding buffer (10 mM HEPES, adjusted to pH 8.0 with NaOH, 150 mM NaCl, 0.1 mM CaCl2, and 1 mM MgCl2). Binding was measured in Multiscreen-FB 96-well filtration plates (Millipore, Bedford, MA), which were pretreated by the addition of 200 μl of 0.1% polyethyleneimine to each well and incubated overnight at 4 °C. The polyethyleneimine was rinsed away with 3 × 100 μl of room temperature binding buffer, and then 100 μl of the diluted membrane solution was added per well. The membranes were washed twice by filtration with 200 μl of binding buffer, and then binding was initiated by the addition of 100 μl of binding buffer containing 0.1 nM [125I]β-CIT (RTI-55; PerkinElmer). Binding was allowed to proceed for 1.5 h at room temperature with gentle rocking. The reaction was stopped by washing all wells three times with 100 μl of ice-cold binding buffer. The filters were removed from the plate and counted after soaking in 150 μl of Optifluor.

For determination of MTSEA sensitivity, membranes in Multiscreen-FB wells preincubated with 5-HT, cocaine, or ibogaine, as indicated, were treated by the addition of MTSEA at varying concentrations for 15 min at room temperature, and then the reagent was removed by washing the membranes three times with binding buffer. The effect of MTSEA treatment was subsequently determined using [125I]β-CIT as described above.

Data Analysis—Nonlinear regression fits of experimental and calculated data were performed with Origin (OriginLab, Northampton, MA), which uses the Marquardt-Levenberg nonlinear least squares curve-fitting algorithm. The statistical analysis given was from multiple experiments. Data with error bars in the figures represent the mean ± S.D. for triplicate measurements. Statistical analysis was performed using Student’spaired t tests.

Materials—Ibogaine was purchased from Sigma. [3H]5-HT and [125I]RTI-55 were from PerkinElmer Life Sciences. Unla-
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RESULTS

Ibogaine Inhibits Transport and Binding by SERT—Ibogaine inhibits 5-HT accumulation by HeLa cells expressing SERT and also inhibits binding of the cocaine analog β-CIT to membranes prepared from those cells. The data in Fig. 1 demonstrate that transport was inhibited by ibogaine with an IC_{50} of 6.3 ± 1.3 μM. Binding of the high affinity cocaine analog [\textsuperscript{125}I]β-CIT also was inhibited by ibogaine with an IC\textsubscript{50} value of 2.5 ± 0.2 μM.

Ibogaine behaved as a noncompetitive inhibitor of 5-HT transport. The data in Fig. 2A demonstrate that inhibition of 5-HT transport into LLC-SERT cells was not reversed at higher concentrations of 5-HT. As shown in the Eadie-Hofstee plot (Fig. 2B), inhibition of 5-HT transport was predominantly an effect on the V\textsubscript{max} (ordinate intercept), and there was little change in the K\textsubscript{m} for 5-HT (−slope). To ensure that [\textsuperscript{3}H]5-HT and ibogaine had equal access to their respective binding sites on SERT, they were added simultaneously in this experiment. In separate experiments, ibogaine preincubation with the cells for 15 min had no effect on the extent of inhibition (data not shown).

In contrast to the noncompetitive inhibition of transport, ibogaine was a competitive inhibitor of [\textsuperscript{125}I]β-CIT binding to human platelet plasma membranes. Fig. 3 is a Scatchard plot showing that the maximum amount of β-CIT binding in the presence of 5 μM ibogaine (abscissa intercept) is similar to the control. Ibogaine decreased the slope of the Scatchard plot, which is equivalent to −1/K_{D} (app). From the increase in the apparent K_{D} for β-CIT, we calculated a K_{D} of 2.26 ± 0.05 μM for ibogaine.

Ibogaine Influences the Reactivity of SERT S277C—To determine whether ibogaine had an influence on the equilibrium of SERT between cytoplasmic and extracellular states, we deter-
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Activity was determined by incubating with [125I]β-CIT. After washing membranes free of MTSEA and ibogaine, residual binding to membranes prepared from cells expressing SERT mutants X5C, K279C, and S277C were treated with MTSEA at 2 mM (for X5C and K279C) or 50 μM (for S277C) in the presence of the indicated concentrations of ibogaine. After washing membranes free of MTSEA and ibogaine, residual binding activity was determined by incubating with [125I]β-CIT as described under “Experimental Procedures.” B, membranes from cells expressing S277C were treated as in A with 50 μM MTSEA in the presence of the indicated concentrations of cocaine, ibogaine, and 5-HT. Maximal protection from MTSEA inactivation required 0.58 ± 0.11 μM cocaine, and half-maximal potentiation of inactivation required 0.98 ± 0.18 μM ibogaine or 0.68 ± 0.11 μM 5-HT.

Mined its ability to influence the reactivity of cysteine residues positioned in the cytoplasmic and extracellular permeation pathways. We previously demonstrated that in TM5 of SERT, cysteine residues placed on one face of the cytoplasmic half of that helix reacted faster in the presence of 5-HT and slower in the presence of cocaine, consistent with the ability of cocaine to favor the extracellular state of SERT and 5-HT to trigger the conversion to the cytoplasmic state (16).

These measurements of reactivity depend on the ability of reagents such as MTSEA to inactivate binding of the cocaine analog β-CIT. The SERT mutants studied contained cysteine residues accessible only from the cytoplasmic face of the plasma membrane. In intact cells expressing these mutants, β-CIT binding was not sensitive to extracellular MTSEA, but binding to membranes prepared from these cells was sensitive (15). Because β-CIT, like cocaine, is thought to bind to an extracellular site, it is unlikely that MTSEA inactivated binding by directly modifying the binding site, but rather by an allosteric mechanism. We proposed that by modifying cysteines in or near the cytoplasmic permeation pathway, MTSEA prevented the conformational change required to close that pathway and open the extracellular cocaine binding site (16).

Fig. 4A shows the effect of ibogaine on inactivation of three SERT mutants. X5C is the background construct in which the five reactive endogenous cysteine residues have been mutated (19); S277C and K279C (in the X5C background) have cysteine residues positioned on the accessible face of TM5 and on the opposite face, respectively.

As previously shown, [125I]β-CIT binding to X5C was insensitive to MTSEA concentrations up to 2 mM. K279C and S277C were both sensitive, and in this experiment 30% inactivation of binding activity required MTSEA concentrations of 0.05 and 2 mM in S277C and K279C, respectively, due to their different reactivities. When the inactivation was carried out in the presence of ibogaine, the reactivity of S277C increased, leading to more inactivation, but ibogaine did not increase inactivation of X5C or K279C. Fig. 4B shows that the effect of ibogaine on S277C was similar to that of 5-HT but opposite the effect of cocaine, indicating that ibogaine and 5-HT both increase the reactivity of Cys-277.

By measuring the amount of inactivation as a function of MTSEA, we can estimate the rate constant for inactivation. Fig. 5 shows that the concentration of half-maximal inactivation varied over 14-fold when the reaction was performed in the presence of 10 μM ibogaine, 5-HT, or cocaine. The MTSEA concentration giving half-maximal inactivation of β-CIT binding (after washing to remove MTSEA and drugs) was used to calculate inactivation rate constants as plotted in Fig. 6. In this experiment, half-maximal MTSEA concentrations and corresponding rate constants were as follows: MTSEA alone, 0.057 ± 0.001 μM and 13.5 ± 0.3 M⁻¹ s⁻¹; 5-HT, 0.015 ± 0.001 μM and 49.7 ± 2 M⁻¹ s⁻¹; cocaine, 0.116 ± 0.002 μM and 6.63 ± 0.1 M⁻¹ s⁻¹; ibogaine, 8.3 ± 0.3 nM and 93 ± 4 M⁻¹ s⁻¹.

FIGURE 4. Modulation of MTSEA-induced inactivation by ibogaine, cocaine, and 5-HT. A, membranes from cells expressing SERT mutants X5C, K279C, and S277C were treated with 50 μM MTSEA in the presence of the indicated concentrations of ibogaine. After washing membranes free of MTSEA and ibogaine, residual binding activity was determined by incubating with [125I]β-CIT as described under “Experimental Procedures.” B, membranes from cells expressing S277C were treated as in A with 50 μM MTSEA in the presence of the indicated concentrations of cocaine, ibogaine, and 5-HT. Maximal protection from MTSEA inactivation required 0.58 ± 0.11 μM cocaine, and half-maximal potentiation of inactivation required 0.98 ± 0.18 μM ibogaine or 0.68 ± 0.11 μM 5-HT.

FIGURE 5. MTSEA dependence of SERT S277C inactivation and effects of ibogaine, cocaine, and 5-HT. Membranes from cells expressing SERT S277C were treated for 10 min with the indicated concentrations of MTSEA in the presence of 10 μM ibogaine, 5-HT, or cocaine. The MTSEA concentration giving half-maximal inactivation of β-CIT binding (after washing to remove MTSEA and drugs) was used to calculate inactivation rate constants as plotted in Fig. 6. In this experiment, half-maximal MTSEA concentrations and corresponding rate constants were as follows: MTSEA alone, 0.057 ± 0.001 μM and 13.5 ± 0.3 M⁻¹ s⁻¹; 5-HT, 0.015 ± 0.001 μM and 49.7 ± 2 M⁻¹ s⁻¹; cocaine, 0.116 ± 0.002 μM and 6.63 ± 0.1 M⁻¹ s⁻¹; ibogaine, 8.3 ± 0.3 nM and 93 ± 4 M⁻¹ s⁻¹.
Ibogaine Increases Accessibility in Other TM5 Cysteine Mutants—Using data similar to the data shown in Fig. 5, we tested the effect of ibogaine on eight additional cysteine mutants. In three, like S277C, cysteine replaced residues on the face of TM5 in contact with the cytoplasmic permeation pathway (G273C, V281C, and T284C) (16). Three other cysteine replacements were made on the opposite face of TM5 (K272C, T276C, and W282C). Finally, two positions likely to lie in the extracellular pathway (Y107C and S404C), where cysteine was shown previously to be protected by 5-HT (24, 25), were also tested. Fig. 6A shows that for all of the cysteine replacements on the reactive face of TM5, the rate constant for inactivation was increased by ibogaine. As shown previously, cocaine decreased the rate constant for all of these mutants, whereas 5-HT increased the rate for all except for T284C (16). Cysteine replacement mutants at positions extracellular to the binding site that became more reactive in the presence of ibogaine (red), those for which ibogaine did not increase accessibility (blue), and those in the extracellular pathway that became much less accessible in the presence of ibogaine (green). Color coding of TM helices is as in Ref. 12.

DISCUSSION

The evidence presented here supports the proposal that ibogaine binds to and stabilizes the cytoplasmic conformation of SERT, in which the substrate binding site is accessible from the cytoplasm. We previously identified positions on one face of TM5 that became more reactive in the presence of 5-HT and less reactive in the presence of cocaine (16). We interpret these reactivity changes as resulting from changes in accessibility. Because the corresponding positions are inaccessible in the crystal structure of LeuT, a bacterial homologue of SERT (12), we proposed that the increase in accessibility with 5-HT represented a conformational change required for bound 5-HT to dissociate to the cytoplasm. Consistent with this proposal, the 5-HT-induced accessibility increase required the presence of Na+ and Cl−, which are co-transported with 5-HT and are predicted by the alternating access model of transport to be essential for translocation (16).

In this experimental approach, we used inactivation of β-CIT binding as a measure of cysteine reactivity. We proposed that after MTSEA reacts with TM5 cysteines in the cytoplasmic permeation pathway, the modified transporter cannot exist in the extracellular state and thus cannot bind cocaine or β-CIT (16). Results from our previous studies are most consistent with the
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proposal that the reactive TM5 residues are accessible in the cytoplasmic state of SERT but not in the extracellular state. Therefore, it follows that insertion of a 2-thioethanolamine group within this pathway by MTSEA might prevent the pathway from closing and therefore prevent conversion of SERT to the extracellular state that binds β-CIT (16).

In the alternating access model, transport proteins are proposed to switch between at least two conformational states, which bind substrate from opposite sides of the membrane. As a tool for studying substrate permeation pathways, conformational states, and the structural mechanism of transporters, it is extremely useful to have ligands that stabilize transporters in each of these two conformations. To date, we are aware of only two other transporters, the adenine nucleotide exchanger of mitochondria and the red cell glucose transporter, where separate inhibitors are known to bind to the two states (17, 18). SERT now becomes only the third transporter and the first co-transporter in this category. The efficacy of ibogaine to stabilize the cytoplasmic state of SERT was demonstrated not only by its ability to increase accessibility of positions in the cytoplasmic pathway but also by its ability to decrease the accessibility of positions 107 and 404 in the extracellular pathway, as predicted if the extracellular pathway closed when the cytoplasmic pathway opened.

The evidence that ibogaine stabilizes the cytoplasmic state of SERT comes from both kinetic and structural approaches. Ibogaine is unique in its noncompetitive inhibition of SERT (Fig. 2). Inhibition by other known inhibitors was overcome by increased 5-HT concentrations. Although there are other mechanisms that lead to noncompetitive inhibition, we can understand the mechanism of ibogaine inhibition in the context of its ability to stabilize the cytoplasmic conformation of SERT. In this interpretation, ibogaine inhibition of transport is due to biasing the equilibrium between extracellular and cytoplasmic states of SERT toward the cytoplasmic state. In the transport studies shown in Figs. 1 and 2, 5-HT was added to the cell exterior, depleted of SERT binding sites by ibogaine. Because the ibogaine-inhibited state of SERT had no ability to bind extracellular 5-HT, adding more 5-HT could not reverse the conformational bias induced by ibogaine.

In contrast, equilibrium binding studies with β-CIT showed ibogaine to be a competitive inhibitor, as expected if binding of ibogaine and β-CIT, a cocaine analog, were mutually exclusive and SERT could not bind both compounds simultaneously. Competitive binding inhibition would most directly be explained by competition for the same binding site. However, noncompetitive inhibition of transport suggested that ibogaine and 5-HT did not bind to the same site, and competition between 5-HT and cocaine analogues suggested that those two did bind at the same or overlapping site(s) (26, 27). To resolve this apparent contradiction, we speculate that ibogaine and cocaine bind to sites that are mutually exclusive, because they exist on different conformational states of SERT. This interpretation is supported by the finding that ibogaine and cocaine induce or stabilize different SERT conformations, as measured by their opposing effects on the reactivity of cysteine residues in the extracellular and cytoplasmic permeation pathways (Fig. 6).

An alternative explanation, that ibogaine caused internalization of SERT, is unlikely, because ibogaine inhibition was maximal immediately after addition and did not increase with a 15-min preincubation. In other experiments (not shown), ibogaine failed to alter surface expression of SERT as measured by biotinylation.

The behavior of T284C provides an important insight into the difference between the effects of 5-HT and ibogaine. This mutant was, in contrast to all of the other mutants containing a cysteine on the reactive face of TM5, less reactive toward MTSEA in the presence of 5-HT. Because 5-HT must bind to both extracellular and cytoplasmic states of SERT as it is transported across the membrane, it is not a priori more likely to bind to one state or the other. Moreover, it is not unexpected that SERT mutants, particularly mutations close to the substrate binding site, might differ in their affinity for 5-HT in the two states. A likely explanation for the difference between the effect of 5-HT on T284C and its effect on the other reactive TM5 cysteine mutants is that the other mutants (G273C, S277C, and V281C in this study) bound 5-HT more avidly to the cytoplasmic state of SERT, but T284C bound 5-HT more avidly to the extracellular state. If ibogaine bound to SERT only in the cytoplasmic state, we would expect it to stabilize that state, in which the entire reactive region of TM5, including positions 273, 277, 281, and 284, were more accessible to MTSEA. Thus, the ability of ibogaine, but not 5-HT, to increase T284C reactivity (Fig. 6) is an expected consequence of 5-HT binding to both states and ibogaine binding to just the cytoplasmic state.

Like 5-HT, ibogaine contains an indole nucleus, with an amino group separated by two carbons from the 3-position of the indole ring and a substituted 5-position (Fig. 8). In 5-HT, the 5-substituent is a hydroxyl group, and the amino group is unsubstituted, but in ibogaine, a methoxy group replaces the hydroxyl, and the amino group is at the bridgehead of a con-
strained tricyclic system (Fig. 8). It may not be coincidental that the structure of 5-HT is essentially contained within the iboga-
mine structure. The constrained orientation of the aminoethyl side chain of 5-HT may allow ibogaine to occupy the 5-HT binding site of SERT only in the cytoplasmic conformation. We plan further studies to determine if ibogaine indeed binds to the 5-HT site and from which side of the plasma membrane it binds.

Ibogaine is unique in its ability to stabilize the cytoplasmic conformation of SERT. Because it is also a hallucinogen with putative anti-addiction properties, it is pertinent to consider the possibility that these effects are due, in some part, to the interaction of ibogaine with SERT. Selective SERT inhibitors, such as fluoxetine, have none of the dramatic behavioral con-
sequences of ibogaine, and ibogaine is known to have moderate affinity for other targets, such as serotonin 5-HT1A, 5-HT2A, 5-HT2C, 5-HT3, or dopamine D1 and D2 receptors (29). Given the present state of uncertainty with respect to ibogaine action, it may be that some consequence of its interaction with SERT, such as the possibility that it activates one of the many ionic currents that SERT conducts (30), could play a role in its pharmacologic actions.

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