——CHAPTER 9——

SIGMA RECEPTORS AND IBOGA ALKALOIDS

WAYNE D. BOWEN

Unit on Receptor Biochemistry and Pharmacology
Laboratory of Medicinal Chemistry
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Bethesda, MD 20892

I. Introduction

Ibogaine, one of the naturally occurring indole alkaloids found in the shrub *Tabernanthe iboga* of central Africa, has been shown to have psychotropic effects, and was initially used for its hallucinogenic properties (*1,2*). Anecdotal reports of heroin and cocaine addicts suggested that taking ibogaine decreased drug craving, with the effects lasting for several months (*3,4*). This has been supported in several animal studies where ibogaine has been shown to reduce self-administration of both morphine and cocaine (*5-8*). On this basis, there has been interest in investigating ibogaine for its potential in treating drug abuse (*9*).

However, ibogaine has also been shown to have negative effects in animal studies that might potentially limit its clinical utility in humans. These effects include production of tremors and neurotoxicity (*1,2*). Specifically, treatment of rats with ibogaine at 100 mg/kg in one to three doses was found to cause

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activation of microglia and astrocytes and loss of Purkinje cells in the parasagittal zones of the cerebellar vermis (*10,11*). Harmaline was found to have similar effects. The receptor sites through which ibogaine mediates its antiaddictive and neurotoxic effects are not known with certainty, since it interacts with low affinity at a number of neurotransmitter and transporter sites including NMDA-glutamatergic and kappa- opioid receptors (*1,2*). Current evidence indicates that ibogaine and other *iboga* alkaloids might produce some of their neurotoxic effects by interaction with sigma-2 receptors.

II. Sigma Receptors

A. General Characteristics and Functions

Sigma receptors are membrane proteins that bind several psychotropic drugs with high affinity (*12*). They were initially proposed to be related to opioid receptors (*13*) and then confused with the phencyclidine binding site on the NMDA-glutamatergic receptor ionophore. Sigma receptors, as defined today, are unique binding sites, with a pharmacological profile unlike any other known neurotransmitter or hormone receptor (*14*). Initial interest in sigma receptors came mainly from their high affinity for typical neuroleptic drugs, such as haloperidol, and their potential as alternative targets for antipsychotic agents (*15,16*).

Two major subclasses of sigma receptors have been identified. These have been termed sigma-1 and sigma-2, and they are differentiated by their pharmacological profile, function, and molecular size (*17,18*). Both subtypes have high to moderate affinity for typical neuroleptics, with haloperidol exhibiting the highest affinity for both sites. However, sigma-1 receptors exhibit high affinity for (+)-benzomorphans, such as (+)-pentazocine, whereas sigma-2 receptors have low affinity for the (+)-benzomorphans. The (-)-isomers of benzomorphans do not strongly differentiate the two sites. Photoaffinity labeling revealed a molecular weight of 25 kDa for sigma-1 receptors and of 18- 21.5 kDa for sigma-2 receptors (*17,19*).

Sigma receptors are widely distributed throughout the brain, but occur in particularly high density in the motor regions. These include cerebellum, brainstem, motor nuclei, and substantia nigra (*12*). Sigma receptors are also found in high density in many tissues outside of the nervous system. Sigma receptors are present in endocrine, immune, and reproductive tissues (*20*). Both subtypes are expressed in high density in the liver and kidney (*19*). In addition, both subtypes of sigma receptors are found to be expressed in very high density

in tumor cell lines derived from various tissues (*21*). These include neuroblastomas, glioma, melanoma, and carcinoma cell lines of breast, prostate, and lung. Furthermore, the expression of sigma receptors in tumor cell lines increases when the cells are in a state of rapid proliferation (*22*), and tumor tissue has been found to express a higher density of sigma receptors than surrounding normal tissue (*23*). High sigma receptor expression in tumor cell lines and up regulation during rapid cell growth suggests a possible role of sigma receptors in cell growth and proliferation.

No endogenous functional ligand (agonist) for sigma receptors has been conclusively identified. There is evidence for the existence of sigma receptor binding substances in brain and tissue extracts (*24,25*), and for depolarization-induced release of a substance(s) from brain tissue slices that occupies sigma receptors (*26*). Progesterone has affinity for sigma-1 receptors (*27*) and certain neurosteroids have been shown to exhibit modulatory effects via sigma receptors (*28*). This has led to the proposal that certain steroids may be endogenous ligands for the sigma receptors.

The sigma-1 receptor has been cloned in guinea pig, mouse, rat, and human, and shown to be a novel protein with > 90% species homology (*29-32*). The sigma-1 protein is unrelated to any known receptor family. The protein sequence has substantial homology to the fungal sterol biosynthetic enzyme, $\Delta^{8,7}$-sterol isomerase (*29*). This has suggested a role of sigma-1 receptors in sterol metabolism, particularly in that of neurosteroids (*33*). However, the protein exhibits no enzymatic activity and is unrelated to the mammalian $\Delta^{8,7}$-sterol isomerase (*34*). Thus, the relevance of sigma-1 receptors to sterol metabolism is not yet clear. In light of the affinity of progesterone and some neurosteroids for sigma-1 receptors, it is possible that the homology represents a steroid binding activity. No information on the structure of the sigma-2 receptor is available at present.

Some of the functions attributed to sigma-1 receptors include: (1) modulation of synthesis and release of dopamine (*35,36*) and acetylcholine (*37*), (2) modulation of NMDA-type glutamatergic receptor electrophysiology (*38*), (3) modulation of NMDA-stimulated neurotransmitter release (*39,40*), (4) modulation of muscarinic receptor-stimulated phosphoinositide turnover (*41*), (5) neuroprotective and antiamnesic activity (*42*), (6) modulation of opioid analgesia (*43*), and (7) alteration of cocaine-induced locomotor activity and toxicity (*44*).

Less is known about the functions of sigma-2 receptors in the brain. As mentioned above, sigma receptors are highly expressed in regions of the brain that regulate posture or that are involved in motor control (*12*). Microinjection of sigma ligands into motor regions of the brain induces marked alterations in movement and posture. Microinjections of typical neuroleptics, as well as selective sigma ligands into the rat red nucleus, induces an acute dystonic reaction (*45*). Microinjection of sigma ligands into the facial nucleus, or spinal

trigeminal nucleus oralis, produced orofacial dyskinesias (vacuous chewing and facial tremors) in rats (*46*). Unilateral microinjection of sigma ligands into the substantia nigra results in contralateral circling (*47*). These effects on motor behavior and posture were described by a pharmacological profile generally consistent with mediation by sigma-2 receptors (*47,48*). These results suggest that sigma-2 receptors might be involved in the regulation of motor behavior and may contribute to some of the motor side effects of typical antipsychotic drugs, particularly tardive dyskinesias and acute dystonias (*12,49*).

B. Sigma-2 Receptors and Cell Death

Results from some of the brain microinjection studies described above suggested that some sigma ligands might be neurotoxic. Reduced haloperidol (a major haloperidol metabolite and a potent sigma ligand) and the cyclohexane diamine, BD614, caused extensive gliosis and loss of magnocellular neurons in and around the injection site (*50,51*). Further investigation *in vitro* revealed that some ligands were cytotoxic to tumor cell lines of both neuronal and nonneuronal origin (e.g., SK-N-SH neuroblastoma and C6 glioma), as well as to primary cultures of rat central nervous system (e.g., cerebellar granule cells, cortical neurons, superior cervical ganglion cells) (*52-54*). Sigma ligands initially caused damage to cell processes, followed by a loss of processes, assumption of a spherical shape ("rounding"), and detachment from the surface. Continued exposure to sigma compounds ultimately resulted in cell death. The effect was dose dependent, with higher doses causing morphological changes and death at shorter time periods. In primary cultures, effects could be seen in relatively low doses (1 to 3 μM) for the most active compounds, with effects occurring over a course of up to 21 days with some cultures. This confirms the chronic nature of the effect, where the effective dose decreases as the period of exposure increases.

Detailed assessment of the pharmacology of this effect indicated the involvement of sigma-2 receptors. Compounds binding to both sigma-1 and sigma-2 sites, such as haloperidol, were active, whereas sigma-1-selective compounds such as (+)-pentazocine and compounds, which lack significant sigma affinity, but which are agonists or antagonists at other receptors, were inactive (*52-54*). Sigma-2 receptor specificity was confirmed using the sigma-2-selective ligands CB-64D and CB-184 (*55*), which were quite potent at producing cytotoxicity. Thus, chronic activation of sigma-2 receptors results in morphological changes and cell death.

Cell death may occur by either necrosis or apoptosis (*56-58*). Necrosis is thought to result from physical or chemical injury to the cell. It is typified by cell swelling, destruction of cytoplasmic organelles, and loss of membrane integrity, and is not controlled by a genetic program. Necrosis in tissues is accompanied by an inflammatory response. Apoptosis (or programmed cell death) can result from

various and specific developmental or environmental stimuli. It is typified by cell shrinkage, membrane blebbing and cytoplasmic boiling, chromatin condensation, and nuclear DNA fragmentation, all with maintenance of membrane integrity (*58*). In tissues, apoptotic cells are removed by macrophages or adjacent epithelial cells, without generating an inflammatory response. Apoptosis is a highly regulated process, involving several signaling pathways, transcription factors, proteolytic enzymes (caspases), nucleases, and other intracellular molecules that both promote and prevent the death of the cell (*56,58*). Induction of apoptotic cell death or dysregulation of apoptosis plays a key role in several physiological and pathological processes (57). These include development, immune responses, carcinogenesis and tumor progression, hypoxia, viral infection, and degenerative disorders. Furthermore, many cytotoxic agents cause cell death via apoptosis.

The mode of cell death induced by sigma-2 ligands in various cell types was found to be apoptotic (5*9,60*). Treatment of SK-N-SH neuroblastoma cells or breast tumor cell lines with sigma-2 agonists, including CB-64D and CB-184, caused inversion of phosphatidyl serine, DNA fragmentation, and nuclear condensation, as measured by annexin-V binding, TdT-mediated dUTP nick-end labeling (TUNEL), and bisbenzimide (Hoechst 33258) staining, respectively. All of these are known hallmarks of apoptosis (*58*). Similar results were observed using primary cultures of rat cerebellar granule cells (*59*). Treatment of cells with sigma-1 selective ligands (e.g. (+)-pentazocine) produced no change in the cells. Thus, activation of sigma-2 receptors subsequently activates the cellular machinery, which results in programmed cell death.

C. Sigma-2 Receptors and Calcium Signaling

The ability of sigma ligands to induce morphological changes and apoptosis led to an investigation of the signaling mechanisms that are utilized by sigma-2 receptors. It is well established that calcium plays a role in cytotoxicity and that alterations in cell calcium levels play a role in the induction of apoptosis in various cell types (*61-63*). Thus, the ability of sigma receptors to modulate intracellular calcium was investigated using indo-1-loaded human SK-N-SH neuroblastoma cells. Sigma receptor ligands from various structural classes produced two types of increases in intracellular (cytosolic) calcium concentration ($[Ca^{++}]i$) (*64,65*). Sigma receptor-inactive compounds structurally similar to the most active sigma ligands produced little or no effect. Mediation of the effect on $[Ca^{++}]i$ by sigma-2 receptors was strongly indicated by (1) the high activity of the sigma-2-selective ligand CB-64D, (2) the greater activity of CB-64D ((+)-isomer) over CB-64L ((-)-isomer), and (3) the very low activity of the sigma-1-selective (+)-benzomorphans, (+)-pentazocine, (±)-SKF-10,047, and dextrallorphan (*65*).

The two types of rise in [Ca^{++}]i produced by sigma-2 receptor ligands were distinguishable both temporally and by source (*65*). The compounds all produced an immediate, dose-dependent, and transient rise in [Ca^{++}]i, which usually returned to near baseline within 7 to 10 minutes. This transient rise in [Ca^{++}]i occurred in the absence of extracellular calcium and was virtually eliminated by pretreatment of cells with thapsigargin. Thus, sigma-2 receptors stimulate a transient release of calcium from the endoplasmic reticulum. Prolonged exposure of cells to sigma receptor ligands resulted in a latent and sustained rise in [Ca^{++}]i. This sustained rise in [Ca^{++}]i was affected neither by removal of extracellular calcium nor by thapsigargin pretreatment. This indicates that sigma-2 receptor ligands also induce release of calcium from mitochondrial stores or from some other calcium store that is insensitive to thapsigargin, such as golgi apparatus. These findings indicate that sigma-2 receptors may utilize calcium signals in producing cellular effects.

The fact that production of a rise in [Ca^{++}]i, changes in cellular morphology, and induction of apoptosis all have the same pharmacological profile suggests that these processes are linked, and that sigma-2 receptors coordinate the events leading to apoptotic cell death. In view of the ability of sigma-2 receptors to induce cytotoxicity, and in light of the lack of information regarding the receptor sites(s) that might mediate ibogaine-induced neurotoxicity, we investigated whether ibogaine might interact with sigma receptors. *Iboga* alkaloids were found to interact selectively with sigma-2 receptors and to induce a rise in intracellular calcium levels, morphological changes, and apoptosis (*66-71*).

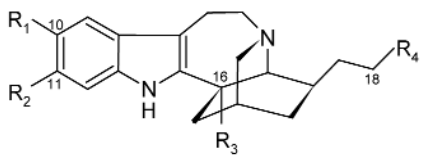
III. Binding of Iboga Alkaloids to Sigma Receptors

Table I shows the binding affinities of ibogaine and various related *iboga* alkaloids at sigma-2 receptors. Sigma-1 receptor affinities are given in the following text. Sigma-1 receptors were labeled with the sigma-1-selective probe, [^{3}H](+)-pentazocine, in guinea pig brain membranes (*72*). Sigma-2 receptors were labeled with [^{3}H]DTG using rat liver membranes, in the presence of dextrallorphan to mask binding to sigma-1 sites (*19*). Ibogaine exhibited moderate affinity for sigma-2 sites (K_i = 201 ± 24 nM), but had very low affinity for sigma-1 receptors (K_i = 8,554 ± 1,134 nM), resulting in 43-fold selectivity for sigma-2 sites over sigma-1. Mach *et al.* (*67*) obtained similar results with ibogaine. Although the affinity of ibogaine for sigma-2 receptors is only moderate, this is none the less quite significant, since ibogaine generally has much lower affinity for other neurotransmitter receptors studied thus far (*73-78*). Although there is variation across studies, ibogaine is reported to bind with K_i values in the range

of 1 - 15 μM to subtypes of muscarinic cholinergic, α-adrenergic, kappa-opioid, ionophore site of NMDA-glutamatergic receptor, as well as the dopamine and serotonin transporters. Ibogaine is reported to be inactive (K_i > 100 μM) at serotonergic, dopaminergic, metabotropic glutamatergic, benzodiazepine, γ-aminobutyric $acid_A$, and cannabinoid receptors. Furthermore, ibogaine turns out to be one of the rare sigma-2-selective ligands, since most compounds binding to sigma receptors either interact selectively with sigma-1 sites or bind to both sites with high affinity (*17-19, 65*). Interestingly, in addition to ibogaine, all of the ibogaine analogs shown in Table I also have a low affinity for sigma-1 receptors.

For discussion of the structure-activity relationships for affinity at sigma receptors, (±)-ibogamine will be considered as the parent compound for those shown in Table I. (±)-Ibogamine has an unsubstituted indole moiety, with a sigma-2 K_i = 137 ± 13 nM and sigma-1 K_i = 1,835 ± 131 nM. A methoxy group in the 10-position (ibogaine) did not markedly change the sigma-2 affinity, but decreased the sigma-1 affinity (K_i = 8,554 ± 1,134 nM). A methoxy group in the 11-position (tabernanthine) produced little change in sigma-2 affinity, and only a small decrease in sigma-1 affinity (K_i = 2,872 ± 37 nM), resulting in 14.8-fold selectivity for sigma-2 receptors. An *O-t*-butyl group in the 10-position also did not dramatically change the sigma-2 receptor affinity or the sigma-1 affinity (K_i = 4,859 ± 682 nM), resulting in 20-fold selectivity for sigma-2 sites. Thus, the

TABLE I.
AFFINITIES OF IBOGAINE AND RELATED INDOLE ALKALOIDS AT SIGMA-2 RECEPTORS



Alkaloid	R_1	R_2	R_3	R_4	Sigma-2 K_i (nM)
(±)-Ibogamine	H	H	H	H	137 ± 13
Ibogaine	OCH_3	H	H	H	201 ± 24
Tabernanthine	H	OCH_3	H	H	194 ± 10
10-*t*-Butoxy-ibogamine	*O-t*-Bu	H	H	H	247 ± 26
Noribogaine	OH	H	H	H	5,226 ± 1,426
(±)-Coronaridine	H	H	CO_2CH_3	H	>100,000
(±)-MC	H	H	CO_2CH_3	OCH_3	8,472 ± 1,237

Portions adapted from data in Bowen *et al.* (66). Sigma-1 receptor affinities are given in the text. Ibogaine was purchased from Sigma Chemicals (St. Louis, MO). See acknowledgments section for sources of other alkaloids. Alkaloids here and throughout the text without stereochemical designation are derived from natural ibogaine and are (-)-enantiomers.

presence or position of the methoxy group on the aromatic ring of the indole moiety is not critical for sigma-2 affinity. Furthermore, the size of the substituent appears not to be critical since the *O*-*t*-butyl group is just as well tolerated at the sigma-2 receptor as the methoxy group. However, a phenolic hydroxyl group in the 10-position (noribogaine) results in a 38-fold loss of binding affinity at sigma-2 receptors and an 8-fold loss of affinity at sigma-1 receptors (K_i = 15,006 ± 898 nM). Thus, a phenolic hydroxyl group appears not to be tolerated in the sigma-2 receptor binding site.

The effect of substitution in the saturated ring system was also examined. The presence of a carbomethoxy group in the 16-position ((±)-coronaridine) resulted in complete loss of sigma-2 receptor binding affinity and a 20-fold loss in sigma-1 affinity (K_i = 35,688 ± 2,858 nM) compared to (±)-ibogamine. Addition of a methoxy group at the 18-position of the 16-carbomethoxy analog, (±)-18-methoxycoronaridine ((±)-MC), led to a marked improvement of sigma-2 binding affinity compared to (±)-coronaridine, but was still of low affinity. Compared to (±)-ibogamine, (±)-MC had 62-fold lower sigma-2 binding affinity. (±)-MC had slightly improved sigma-1 binding affinity (K_i = 28,687 ± 283 nM) compared to (±)-coronaridine, but had 16-fold lower sigma-1 affinity compared to (±)-ibogamine. Thus, a carbomethoxy group at the 16-position is not tolerated in the sigma-2 receptor binding site. All of these analogs had a very low affinity at sigma-1 sites.

IV. Effect of Iboga Alkaloids on Intracellular Cytosolic Calcium

As described above, we have shown that sigma-2 receptors mediate a rise in cytosolic calcium levels (*64,65*). In view of the sigma-2 binding affinity of ibogaine and its analogs, we investigated whether *iboga* alkaloids could affect the levels of intracellular calcium in human SK-N-SH neuroblastoma cells. Human SK-N-SH neuroblastoma cells were loaded with Indo-1 calcium indicator dye, and $[Ca^{++}]i$ of individual cells was measured using the fluorescence ratio at 410 nm/485 nm (*65*).

The *iboga* alkaloid being tested was added to Indo-1-loaded SK-N-SH neuroblastoma cells, and the change in $[Ca^{++}]i$ was monitored for about 10 minutes. Ibogaine produced a dose-dependent rise in $[Ca^{++}]i$. The calcium levels began to rise almost immediately after addition of the alkaloid to the cells. Table II shows the effect of 100 μM of various *iboga* alkaloids on $[Ca^{++}]i$. The percent increase in $[Ca^{++}]i$ was calculated by determining the peak level of $[Ca^{++}]i$ relative to the starting basal level. In addition to ibogaine, (±)-ibogamine and 10-*t*-butoxy-ibogamine also produced a rise in $[Ca^{++}]i$. Noribogaine, (±)-coronaridine, and

TABLE II.
EFFECT OF IBOGAINE AND ITS ANALOGS ON $[Ca^{++}]I$

Alkaloid (100 μM)	Percentage increase in $[Ca^{++}]i$ above basal at 100 μM
Ibogaine	40.5 ± 2.0
(±)-Ibogamine	102 ± 14
10-*t*-Butoxy-ibogamine	100 ± 6.4
Noribogaine	0 ± 0
(±)-Coronaridine	0 ± 0
(±)-MC	5.0 ± 0.5
THAP (150 nM)/Ibogaine	0 ± 0
THAP (150 nM)/(±)-Ibogamine	0 ± 0

(±)-MC had little or no effect on $[Ca^{++}]i$. This pharmacological profile is consistent with mediation by sigma-2 receptors, since only those *iboga* alkaloids with significant sigma-2 affinity (Table I) are active at increasing $[Ca^{++}]i$.

To determine the source of calcium contributing to the *iboga* alkaloid-induced rise in $[Ca^{++}]i$, SK-N-SH neuroblastoma cells were pretreated for 10 minutes with 150 nM thapsigargin (THAP) to deplete the store of calcium in the endoplasmic reticulum. Table II shows that thapsigargin-pretreatment completely eliminated the rise in $[Ca^{++}]i$ produced by ibogaine and (±)-ibogamine. These results show that, like other sigma-2 receptor ligands, such as CB-64D and BD737 (*64,65*), ibogaine and related *iboga* alkaloids that have sigma-2 receptor affinity act as sigma-2 receptor agonists to gate calcium from the endoplasmic reticulum. Whether or not *iboga* alkaloids also produce a latent, sustained, and thapsigargin-insensitive rise in $[Ca^{++}]i$, like that produced by other sigma-2 agonists on long-term exposure, was not examined.

V. Effect of Iboga Alkaloids on Cellular Morphology and Induction of Apoptosis

As mentioned above, sigma-2 receptors were found to mediate morphological changes and apoptotic cell death in a number of cell types, including tumor cell lines and primary cultures of neuronal cells (*52-54,59,60*). The ability of *iboga* alkaloids to cause cytotoxicity was examined *in vitro* using rat C6 glioma cells and human SK-N-SH neuroblastoma cells. The cytotoxic effect of *iboga* alkaloids was also examined in primary cultures of rat cerebellar granule cells.

Cells were exposed to various concentrations (3 to 30 μM) of ibogaine or its analogs and the morphology of the cells examined by phase contrast microscopy.

The morphological state was given a score after the indicated time of exposure. Scoring of cell morphology was similar to that described previously (*52*): N, normal cells; A, loss or damage to cell processes; B, initial stages of cell rounding; C, complete rounding with or without detachment from substratum; D, cell death with presence of cell debris. Effects on rat C6 glioma cells and human SK-N-SH neuroblastoma cells are shown in Tables III and IV. The sigma-2 receptor-active compounds, ibogaine, (±)-ibogamine, and 10-*t*-butoxy-ibogamine produced dose- and time-dependent changes in cellular morphology. In C6 glioma cells, 30 μM ibogaine produced significant changes in cell morphology within 72 hours. 10-*t*-Butoxy-ibogamine was more potent, producing significant morphology changes within 24 hours and cell death within 72 hours of exposure. In SK-N-SH cells, 30 μM (±)-ibogamine and 10-*t*-butoxy-ibogamine induced cell death within 72 hours of exposure, with ibogaine producing significant cell rounding by this time point. Again, 10-*t*-butoxy-ibogamine was most potent, producing significant morphological change in as little as 6 hours at 30 μM, followed by (±)-ibogamine, and then ibogaine. Effects on rat cerebellar granule cells are shown in Table V. In cerebellar granule cells, 10-*t*-butoxy-ibogamine produced significant changes in cells within 72 hours at a concentration of 10 μM and induced cell death by 10 days at 30 μM. Ibogaine at a concentration of 30 μM induced cell rounding by 10 days.

Iboga alkaloids lacking sigma-2 affinity did not exhibit cytotoxic effects in these cells. Noribogaine and (±)-MC failed to produce any effect on cells. (±)-Coronaridine was inactive in C6 glioma cells at 30 μM, but did produce morphologic effects in SK-N-SH neuroblastoma cells at 30 μM. However, (±)-coronaridine-induced toxicity was distinct from that produced by the other *iboga* alkaloids and other sigma-2 receptor ligands. This alkaloid caused the appearance of abundant intracellular bodies with a granular appearance (indicated by "gran" in Table IV), which did not occur with the other *iboga* alkaloids or with other sigma-2 receptor agonists such as CB-64D and BD737. In addition, harmaline, an indole alkaloid that is also sigma receptor-inactive (*66*), caused morphological changes similar to those of (±)-coronaridine (not shown). Thus, these effects of (±)-coronaridine and harmaline on neuroblastoma cells appear not to be mediated by sigma 2 receptors and are due to some other mechanism.

DNA fragmentation is one hallmark of apoptotic cell death (*58*). DNA fragmentation occurring during apoptosis can be detected by incorporating fluorescein-12-dUTP at the 3'-OH DNA ends using the enzyme, terminal deoxynucleotidyl transferase (TdT). TUNEL (TdT-mediated dUTP Nick-End Labeling) was previously used to detect sigma-2 receptor-induced apoptotic cell death in both SK-N-SH neuroblastoma cells and cerebellar granule cells (*59*). SK-N-SH neuroblastoma cells were treated with a 100 μM concentration of various *iboga* alkaloids for 24 to 72 hours and then prepared for TUNEL staining and analysis by fluorescence microscopy. Treatment of SK-N-SH neuroblastoma

TABLE III.
EFFECT OF IBOGA ALKALOIDS ON RAT C6 GLIOMA CELLS

Alkaloid	Concentration	Time of exposure 6 hours	24 hours	48 hours	72 hours
Ibogaine	30 μM	N	N	N	A-B
10-*t*-Butoxy-ibogamine	30 μM	N	A-B	B-C	C-D
Noribogaine	30 μM	N	N	N	N
(±)-Coronaridine	30 μM	N	N	N	N

TABLE IV.
EFFECT OF IBOGA ALKALOIDS ON HUMAN SK-N-SH NEUROBLASTOMA CELLS

Alkaloid	Concentration	Time of exposure 6 hours	24 hours	48 hours	72 hours
Ibogaine	10 μM	N	N	N	A
	30 μM	N	N	A-B	B-C
(±)-Ibogamine	10 μM	N	N	N	A
	30 μM	N	A	B-C	C > D
10-*t*-Butoxy-ibogamine	10 μM	N	A	A	A-B
	30 μM	A-B	B-C	C	D
Noribogaine	30 μM	N	N	N	N
(±)-MC	30 μM	N	N	N	N
(±)-Coronaridine	10 μM	N	N	N-A (gran)	A (gran)
	30 μM	N	A-B (gran)	B-C (gran)	B-C (gran)

TABLE V.
EFFECT OF IBOGA ALKALOIDS ON RAT CEREBELLAR GRANULE CELLS

Alkaloid	Concentration	Time of exposure 1 day	3 days	7 days	10 days
Ibogaine	3 μM	N	N	N	N
	10 μM	N	N	N	A
	30 μM	N	A	A-B	B > C
10-*t*-Butoxy-ibogamine	3 μM	N	A	A	A-B
	10 μM	A	A > B	A-B	B-C
	30 μM	A-B	B-C	B < C	C-D
Noribogaine	3 μM	N	N	N	N
	10 μM	N	N	N	N
	30 μM	N	N	N	A-B

cell cultures with 100 μM ibogaine (48 hours), (±)-ibogamine (24 hours), and 10-*t*-butoxy-ibogamine (24 hours) resulted in TUNEL-positive cells, indicating apoptotic cell death. Treatment with 100 μM noribogaine for 72 hours failed to produce any TUNEL-staining cells, consistent with no change in morphology relative to untreated controls as observed above (Table IV). Similarly, TUNEL-positive cells were evident after treatment of rat cerebellar granule cells with 30 μM ibogaine (72 hours), (±)-ibogamine (48 hours), and 10-*t*-butoxy-ibogamine (48 hours). No TUNEL-positive cells were present after treatment with 30 μM noribogaine for up to 7 days. Thus, consistent with the profile for production of morphological changes, only those *iboga* alkaloids with affinity for sigma-2 receptors produced DNA fragmentation and apoptotic cell death.

VI. Summary and Discussion

The specific receptor sites at which ibogaine interacts to produce neurotoxicity *in vivo* have not yet been delineated with certainty, and the exact relevance of the cytotoxicity of ibogaine as demonstrated *in vitro* with regard to administration of the drug *in vivo* is not clear. O'Hearn and Molliver (*79*) have proposed an indirect toxicity model for ibogaine-induced cerebellar toxicity whereby acute administration of ibogaine (100 mg/kg, i.p., once) activates neurons in the inferior olive, resulting in sustained release of glutamate from climbing fiber synapses onto the Purkinje cells. This results in excitotoxic degeneration of the Purkinje cells in the cerebellum. This notion is strongly supported by the observation that ablation of the inferior olive abolishes the neurotoxic effect of an acute dose of ibogaine (*79*). Furthermore, ibogaine can potentiate neuronal glutamatergic activity, as evidenced by its ability to slightly increase the electrophysiological response to NMDA in the CA_3 region of the rat dorsal hippocampus (*80*). This enhancing effect was proposed to be mediated *via* a sigma-2 receptor-related site (*80*). Interestingly, an effect of ibogaine involving glutamate might appear paradoxical, since ibogaine has been shown to be a noncompetitive antagonist at the NMDA-glutamatergic receptor (*75,81*) and thus would be expected to have neuroprotective activity in models of glutamate-induced excitotoxicity. It is possible, however, that glutamatergic receptors other than the NMDA-type contribute to the cerebellar excitotoxicity. Also, the redundancy of the synaptic input onto Purkinje cells could make them exquisitely sensitive to glutamate-induced neurotoxicity (*79*).

It at first appears unlikely that sigma-2 receptors are solely responsible for the highly selective Purkinje cell toxicity produced by ibogaine, since harmaline, which lacks sigma-2 affinity (*66*), produces the same effect (*11*). The most

parsimonious explanation for this is that ibogaine and harmaline both act at some other site to activate the olivocerebellar projection. However, it remains possible that ibogaine and harmaline act through different mechanisms to activate the same pathway, with ibogaine acting at sigma-2 receptors and harmaline acting through a different site (see below).

Based on the *in vitro* results currently described, an additional model to consider is one where ibogaine causes activation of sigma-2 receptors and results in a direct cytotoxic effect on neuronal and/or glial cells through an apoptotic mechanism. It is possible that this direct neurotoxicity combines with excitotoxicity due to enhanced response to glutamate, both effects being mediated by sigma-2 receptors. In conjunction with the greater vulnerability of Purkinje cells to excitotoxic injury, this could result in the cerebellar degeneration caused by ibogaine. This would also explain the apparent paradox of ibogaine-induced excitotoxicity, despite ibogaine's properties as an NMDA-glutamatergic antagonist. Furthermore, it was observed in the *in vitro* model that harmaline also caused cell morphology changes, but these effects were clearly distinct from the effects produced by ibogaine and other sigma-2 receptor agonists. This suggests that harmaline and ibogaine act *via* different mechanisms *in vitro*, and might do so *in vivo*.

Whereas the climbing fiber model accounts for the specificity of ibogaine toxicity for cerebellar Purkinje cells, the direct toxicity model would apply to any ibogaine-induced cytotoxicity that might be observed in other brain regions or in peripheral tissues due to the wide tissue distribution of sigma-2 receptors (*19-21*). Such widespread cytotoxicity of ibogaine has not yet been reported in the brain or the periphery. No significant pathological effects were observed in liver, kidney, heart, or brain following chronic treatment of rats with ibogaine (10 mg/kg for 30 days or 40 mg/kg for 12 days, i.p.) (*82*). However, it should be noted that the neurotoxic effect of ibogaine is reported to be highly dependent on dose, whereby a single dose that is effective at reducing morphine and cocaine self-administration (40 mg/kg, i.p.) does not produce cerebellar neurotoxicity in the rat (*83*). Also, chronic administration of a behaviorally active dose of ibogaine (10 mg/kg, i.p., every other day for 60 days) failed to produce loss of cerebellar Purkinje cells in rats (*84*). Thus, it is conceivable that an acute dose of ibogaine higher than that used by O'Hearn and Molliver (*79*), a different route of administration, or a chronic paradigm at a dose greater than 40 mg/kg might produce widespread, direct toxicity to rat brain neurons as well as to peripheral tissues expressing high densities of sigma-2 receptors such as rat liver and kidney (*19*).

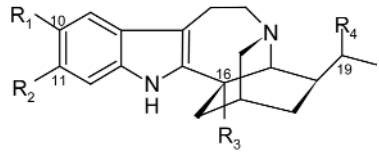
Noribogaine has been shown to be the major ibogaine metabolite in humans and results from *O*-demethylation (*85, 86*). Interestingly, noribogaine lacks affinity for sigma-2 receptors (Table I), produces no effects on $[Ca^{++}]i$ (Table II), and is devoid of cytotoxicity *in vitro* (Tables III-V). Therefore, after administration of a dose of ibogaine, *O*-demethylation to noribogaine would eliminate the

sigma-2 receptor binding affinity and therefore would abolish its potential cytotoxicity. This could have important implications for the treatment of drug abusers with ibogaine, since subjects with a low level of hepatic *O*-demethylase activity ("slow metabolizers") might be more susceptible to the potential cytotoxic effects of ibogaine than "rapid metabolizers." Differences in the rate of ibogaine demethylation could also explain the observed species differences in sensitivity to the neurotoxic effects of ibogaine. For example, ibogaine clearly produces neurotoxicity in rats at a dose of 100 mg/kg (*10,11,79*), but no neurotoxicity was observed in African green monkeys after treatment for 5 days with repeated doses of either 25 mg/kg (p.o.) or 100 mg/kg (s.c.) of ibogaine (*9*). Furthermore, no cerebellar degeneration or degeneration in any other brain area was observed on postmortem neuropathological examination of a female patient who had received four doses of ibogaine ranging from 10 to 30 mg/kg over a 15-month period (*9*). Thus, ibogaine may be neurotoxic in rodents, but not in primates, and this could conceivably be due to differences in its rate of conversion to the much less cytotoxic metabolite, noribogaine. This notion deserves further study.

Another implication of these findings is that it appears possible to dissociate the neurotoxic effects from the beneficial effects of *iboga* alkaloids. In rats, noribogaine (40 mg/kg) has effects similar to ibogaine in suppressing morphine and cocaine self-administration, but does not have the tremorigenic effects of an equal dose of ibogaine (also, see below) (*87*). 18-Methoxycoronaridine (MC) is a synthetic analog of ibogaine (*88*). MC suppresses morphine and cocaine self-administration. However, rats treated with up to 100 mg/kg MC showed no evidence of cerebellar neurotoxicity (*88*). This absence of *in vivo* neurotoxicity with MC is consistent with the lack of sigma-2 receptor binding affinity, lack of effect on $[Ca^{++}]i$, and lack of cytotoxicity *in vitro* (Tables I, II, and IV). Thus, sigma-2 receptors appear not to be involved in the positive effects of ibogaine and may specifically contribute to the neurotoxic effects. It should be possible to develop synthetic ibogaine analogs that have low sigma-2 receptor affinity and low neurotoxicity, but that remain potent at blocking drug self-administration. This could be accomplished by incorporating hydroxyl groups on the aromatic ring of the indole moiety, as in noribogaine, or by making substitutions at the 16-position of the saturated ring system, as in the case of MC.

Sigma-2 receptors may contribute to other toxic effects of *iboga* alkaloids. Ibogaine and some of its congeners are known to cause tremors with marked ataxia in both mice and rats (*89-91*). Singbartl and colleagues (*89,90*) have examined the structure-activity relationships for the tremorigenic effect of a number of *iboga* alkaloids. They found that a carbomethoxy group had a clear negative effect on tremorigenic activity, and that an aromatic methoxy group enhanced, whereas a hydroxyl group decreased, tremorigenic activity. They concluded that due to this defined structure-activity relationship, indole

TABLE VI.
TREMORIGENIC STRUCTURE-ACTIVITY RELATIONSHIP AND SIGMA BINDING AFFINITIES OF IBOGA ALKALOIDS



Alkaloid	R_1	R_2	R_3	R_4	Tremors (ED_{50}, μmol/kg s.c.)	Sigma-2 K_i (nM) or *predicted affinity
Ibogaine	OCH_3	H	H	H	34.8	201
Tabernanthine	H	OCH_3	H	H	4.5	194
Ibogaline	OCH_3	OCH_3	H	H	7.6	High*
Iboxygaine	OCH_3	H	H	OH	80.4	High*
Noribogaine	OH	H	H	H	176	5,226
Voacangine	OCH_3	H	CO_2CH_3	H	Inactive	Low*
Conopharyngine	OCH_3	OCH_3	CO_2CH_3	H	Inactive	Low*
Voacristine	OCH_3	H	CO_2CH_3	OH	Inactive	Low*

Adapted from data in Singbartl and colleagues (*89,90*) and Bowen *et al.* (*66*).

derivatives must interact with a specific receptor site for the generation of tremors (*90*).

In view of the high density of sigma receptors in brain motor control regions, and the effects of sigma-2 receptor ligands on movement and posture (*12,45-49*), it is interesting to note that the pharmacological profile for the tremorigenic effect of *iboga* alkaloids is also consistent with mediation by sigma-2 receptors. Table VI shows the structure-activity relationship for tremors described by Singbartl and colleagues (*89,90*), along with the observed sigma-2 binding K_i value, or a prediction of whether or not the alkaloid would exhibit high or low sigma-2 binding activity based on the structure-activity relationship described in Table I. The sigma-2 receptor-active alkaloids, ibogaine and tabernanthine, both produced tremors. The *iboga* alkaloids iboxygaine and ibogaline are predicted to have good sigma-2 affinity, since the position of the aromatic methoxy group does not affect sigma-2 binding activity. Both of these alkaloids had tremorigenic activity. Noribogaine, which has very weak sigma-2 binding affinity due to the presence of a phenolic hydroxyl group, also had relatively weak tremorigenic activity. Table I shows that a carbomethoxy group at the 16-position, greatly reduces or eliminates sigma-2 receptor binding affinity. All of the *iboga* alkaloids that have a carbomethoxy group at the 16-position (voacangine, voacristine, and

conopharyngine) were all inactive at producing tremors. Furthermore, Glick and colleagues have shown that MC is devoid of tremorigenic activity (*88*). Thus, the tremorigenic activity of *iboga* alkaloids, like the neurotoxic effect, is consistent with binding to sigma-2 receptors.

Further study will be needed in order to determine whether sigma-2 receptors contribute to the neurotoxic and/or tremorigenic effects of ibogaine and other *iboga* alkaloids observed *in vivo*. As pointed out earlier, harmaline, a β-carboline indole alkaloid structurally related to ibogaine, but devoid of sigma-2 binding affinity (*66*), also causes cerebellar neurotoxicity and tremors (*11, 79*). This suggests that sigma-2 receptors do not explain all of the neurotoxic actions of these indole alkaloids and that other receptor sites may also be involved. However, as relatively selective sigma-2 receptor ligands, *iboga* alkaloids may serve as templates on which to design selective agonists and antagonists for further study of sigma-2 receptor function. Designing ibogaine derivatives that lack sigma-2 receptor affinity may result in effective and nontoxic agents for the treatment of drug abuse.

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References

1. P. Popik, R. T. Layer, and P. Skolnick, *Pharmacol. Rev.* **47,** 235 (1995).
2. P. Popik and S.D. Glick, *Drugs of the Future* **21,** 1109 (1996).
3. H.S. Lotsof, *U. S. Pat.* **4,499,096**; *Chem. Abstr.* **102,** 160426w (1985).
4. H.S. Lotsof, *U. S. Pat.* **4,587,243**; *Chem. Abstr.* **106,** 12967r (1986).
5. S.D. Glick, K. Rossman, S. Steindorf, I.M. Maisonneuve, and J.N. Carlson, *Eur. J. Pharmacol.* **195,** 341 (1991).
6. S.D. Glick, M.E. Kuehne, J. Raucci, T.E. Wilson, D. Larson, R.W. Keller, Jr., and J.N. Carlson, *Brain Res.* **657,** 14 (1994).
7. S.L.T. Cappendijk and M.R. Dzoljic, *Eur. J. Pharmacol.* **241,** 261 (1993).
8. H. Sershen, A. Hashim, and A. Lajtha, *Pharmacol. Biochem. Behav.* **47,** 13 (1994).
9. D.C. Mash, C.A. Kovera, B.E. Buck, M.D. Norenberg, P. Shapshak, W.L. Hearn, and J. Sanchez-Ramos, *Ann. N.Y. Acad. Sci.* **844,** 274 (1998).

10. E. O'Hearn, D.B. Long, and M.E. Molliver, *NeuroReport* **4,** 299 (1993).
11. E. O'Hearn and M.E. Molliver, *Neurosci.* **55,** 303 (1993).
12. J.M. Walker, W.D. Bowen, F.O. Walker, R.R. Matsumoto, B.R. de Costa, and K.C. Rice, *Pharmacological Rev.* **42,** 355 (1990).
13. W.R. Martin, C.G. Eades, J.A. Thompson, R.E. Huppler, and P.E. Gilbert, *J. Pharmacol. Exp. Ther.* **197,** 517 (1976).
14. R. Quirion, R. Chicheportiche, P.C. Contreras, K.M. Johnson, D. Lodge, S.W. Tam, J.H. Woods, and S.R. Zukin, *Trends Neurosci.* **10,** 444 (1987).
15. T.-P. Su, *J. Pharmacol. Exp. Ther.* **223,** 284 (1982).
16. S.W. Tam and L. Cook, *Proc. Natl. Acad. Sci. USA* **81,** 5618 (1984).
17. S.B. Hellewell and W.D. Bowen, *Brain Res.* **527,** 244 (1990).
18. R. Quirion, W.D. Bowen, Y. Itzhak, J.L. Junien, J.M. Musacchio, R.B. Rothman, T.-P. Su, S.W. Tam, and D.P. Taylor, *Trends Pharmacol. Sci.* **13,** 85 (1992).
19. S.B. Hellewell, A.Bruce, G. Feinstein, J. Orringer, W. Williams, and W.D. Bowen, *Eur. J. Pharmacol. - Mol. Pharmacol. Sect.* **268,** 9 (1994).
20. S.A. Wolfe, Jr. and E.B. De Souza, *in* "Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection?" (J.M. Kamenka and E.F. Domino, eds.), p. 927. NPP Books, Ann Arbor, MI, 1992.
21. B.J. Vilner, C.S. John, and W.D. Bowen, *Cancer Res.* **55,** 408 (1995).
22. R.H. Mach, C.R. Smith, I. al-Nabulsi, B.R. Whirrett, S.R. Childers, and K.T. Wheeler, *Cancer Res.* **57,** 156 (1997).
23. W.T. Bem, G.E. Thomas, J.Y. Mamone, S.M. Homan, B.K. Levy, F.E. Johnson, and C.J. Coscia, *Cancer Res.* **51,** 6558 (1991).
24. T.-P. Su, A.D. Weissman, and S.-Y. Yeh, *Life Sci.* **38,** 2199 (1986).
25. P.C. Contreras, D.A. DiMaggio, and T.L. O'Donohue, *Synapse* **1,** 57 (1987).
26. J.F. Neumaier and C.Chavkin, *Brain Res.* **500,** 215 (1989).
27. T.-P. Su, E.D. London, and J.H. Jaffe, *Science* **240,** 219 (1988).
28. F.P. Monnet, V. Mahe, P. Robel, and E.E. Baulieu, *Proc. Natl. Acad. Sci. USA* **92,** 3774 (1995).
29. M. Hanner, F.F. Moebius, A. Flandorfer, H.G. Knaus, J. Striessnig, E. Kempner, and H. Glossmann, *Proc. Natl. Acad. Sci. USA* **93,** 8072 (1996).
30. R. Kekuda, P.D. Prasad, Y.-J. Fei, F.H. Leibach, and V. Ganapathy, *Biochem. Biophys. Res. Commun.* **229,** 553 (1996).
31. P. Seth, F.H. Leibach, and V. Ganapathy, *Biochem. Biophys. Res. Commun.* **241,** 535 (1997).
32. P. Seth, Y.-J. Fei, H.W. Li, W. Huang, F.H. Leibach, and V. Ganapathy, J. *Neurochem.* **70,** 922 (1998).
33. F.F. Moebius, J. Striessnig, and H. Glossmann, *Trends Pharmacol. Sci.* **18,** 67 (1997).
34. S. Silve, P.H. Dupuy, C. Labit-Lebouteiller, M. Kaghad, P. Chalon, A. Rahier, M. Taton, J. Lupker, D. Shire, and G. Loison, *J. Biol. Chem.* **271,** 22434 (1996).
35. R.G. Booth and R.J. Baldessarini, *Brain Res.* **557,** 349 (1991).
36. S.L. Patrick, J.M. Walker, J.M. Perkel, M. Lockwood, and R.L. Patrick, *Eur. J. Pharmacol.* **231,** 243 (1993).
37. K. Matsuno, T. Senda, T. Kobayashi, and S. Mita, *Brain Res.* **690,** 200 (1995).
38. F.P. Monnet, G. Debonnel, and C. De Montigny, *J. Pharmacol. Exp. Ther.* **261,** 123 (1992).
39. G.M. Gonzalez-Alvear and L.L. Werling, *Brain Res.* **673,** 61 (1995).
40. F.P. Monnet, B.R. de Costa, and W.D. Bowen, *Brit. J. Pharmacol.* **119,** 65 (1996).
41. W.D. Bowen, P.J. Tolentino, K.K. Hsu, J.M. Cutts, and S.S. Naidu, *in* "Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection?" (J.M. Kamenka and E.F. Domino, eds.), p. 155. NPP Books, Ann Arbor, MI, 1992.
42. T. Maurice and B.P. Lockhart, *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* **21,** 69 (1997).
43. M. King, Y.-X. Pan, J. Mei, A. Chang, J. Xu, and G.W. Pasternak, *Eur. J. Pharmacol.* **331,** R5 (1997).
44. K.A. McCracken, W.D. Bowen, B.R. de Costa, and R.R. Matsumoto, *Eur. J. Pharmacol.* **370,** 225 (1999).

45. J.M. Walker, R.R. Matsumoto, W.D. Bowen, D.L. Gans, K.D. Jones, and F.O. Walker, *Neurology* **38,** 961 (1988).
46. T.T. Tran, B.R. de Costa, and R.R. Matsumoto, *Psychopharmacol.* **137,** 191 (1998).
47. J.M. Walker, W.D. Bowen, S.L. Patrick, W.E. Williams, S.W. Mascarella, X. Bai, and F.I. Carroll, *Eur. J. Pharmacol.* **231,** 61 (1993).
48. R.R. Matsumoto, M.K. Hemstreet, N.L. Lai, A. Thurkauf, B.R. de Costa, K.C. Rice, S.B. Hellewell, W.D. Bowen, and J.M. Walker, *Pharmacol. Biochem. Behav.* **36,** 151 (1990).
49. J.M. Walker, W.J. Martin, A.G. Hohmann, M.K. Hemstreet, J.S. Roth, M.L. Leitner, S.D. Weiser, S.L. Patrick, R.L. Patrick, and R.R. Matsumoto, *in* "Sigma Receptors, Neuroscience Perspectives Series" (Y. Itzhak, ed.), p. 205. Academic Press, London, 1994.
50. W.D. Bowen, E.L. Moses, P.J. Tolentino, and J.M. Walker, *Eur. J. Pharmacol.* **177,** 111 (1990).
51. W.D. Bowen, J.M. Walker, B.R. de Costa, R. Wu, P.J. Tolentino, D. Finn, R.B. Rothman, and K.C. Rice, *J. Pharmacol. Exp. Ther.* **262,** 32 (1992).
52. B.J. Vilner, B.R. de Costa, and W.D. Bowen, *J. Neurosci.* **15,** 117 (1995).
53. W.D. Bowen and B.J. Vilner, *Soc. Neurosci. Abstr.* **20,** 747, #314.10 (1994).
54. B.J. Vilner and W.D. Bowen, *Soc. Neurosci. Abstr.* **22,** 2006, #787.6 (1996).
55. W.D. Bowen, C.M. Bertha, B.J. Vilner, and K.C. Rice, *Eur. J. Pharmacol.* **278**, 257 (1995).
56. R.A. Schwartzman and J.A. Cidlowski, *Endocrine Rev.* **14,** 133 (1993).
57. I. Vermes and C. Haanan, *Adv. Clin. Chem.* **31,** 177 (1994).
58. A.H. Wyllie, *Brit. Med. Bull.* **53,** 451 (1997).
59. B.J. Vilner and W.D. Bowen, *Soc. Neurosci. Abstr.* **23,** 2319, #905.6 (1997).
60. K.W. Crawford, B.J. Vilner, and W.D. Bowen, *Proc. Am. Assoc. Cancer Res.* **40,** 166, #1104 (1999).
61. M.J. Berridge, M.D. Bootman, and P. Lipp, *Nature* **395,** 645 (1998).
62. P. Nicotera, B. Zhivotovsky, and S. Orrenius, *Cell Calcium* **16,** 279 (1994).
63. D.J. McConkey and S. Orrenius, *J. Leuk. Biol.* **59,** 775 (1996).
64. B.J. Vilner and W.D. Bowen, *Soc. Neurosci. Abstr.* **21,** 1608, #631.3 (1995).
65. B.J. Vilner and W.D. Bowen, *J. Pharmacol. Exp. Ther.* **292,** 900 (2000).
66. W.D. Bowen, B.J. Vilner, W. Williams, C.M. Bertha, M.E. Kuehne, and A.E. Jacobson, *Eur. J. Pharmacol.* **279,** R1 (1995).
67. R.H. Mach, C.R. Smith, and S.R. Childers, *Life Sci.* **57,** PL57 (1995).
68. W. Williams, U.K. Bandarage, M.E. Kuehne, C.M. Bertha, and W.D. Bowen, *in* "Problems of Drug Dependence, 1997: Proceedings of the 59th Annual Scientific Meeting, National Institute on Drug Abuse Research Monograph 178" (L.S. Harris, ed.), p. 236. U.S. Government Printing Office, Washington, DC, 1998.
69. W.D. Bowen, B.J. Vilner, U.K. Bandarage, and M.E. Kuehne, *Soc. Neurosci. Abstr.* **22,** 2006, #787.5 (1996).
70. B.J. Vilner, U.K. Bandarage, M.E. Kuehne, C.M. Bertha, and W.D. Bowen, *in* "Problems of Drug Dependence, 1997: Proceedings of the 59th Annual Scientific Meeting, National Institute on Drug Abuse Research Monograph 178" (L.S. Harris, ed.), p. 235. U.S. Government Printing Office, Washington, DC, 1998.
71. W.D. Bowen, B.J. Vilner, W. Williams, U.K. Bandarage, and M.E. Kuehne, *Soc. Neurosci. Abstr.* **23,** 2319, #905.7 (1997).
72. W.D. Bowen, B.R. de Costa, S.B. Hellewell, J.M. Walker, and K.C. Rice, *Mol. Neuropharmacol.* **3,** 117 (1993).
73. D.C. Deecher, M. Teitler, D.M. Soderlund, W.G. Bornmann, M.E. Kuehne, and S.D. Glick, *Brain Res.* **571,** 242 (1992).
74. H. Sershen, A. Hashim, L. Harsing, and A. Lajtha, *Life Sci.* **50,** 1079 (1992).
75. P. Popik, R.T. Layer, and P. Skolnick, *Psychopharmacol.* **114,** 672 (1994).
76. D.B. Repke, D.R. Artis, J.T. Nelson, and E.H.F. Wong, *J. Org. Chem.* **59,** 2164 (1994).
77. P.M. Sweetnam, J. Lancaster, A. Snowman, J. Collins, S. Perschke, C. Bauer, and J. Ferkany, *Psychopharmacol.* **118,** 369 (1995).
78. M.E.M. Benwell, P.E. Holtom, R.J. Moran, and D.J.K. Balfour, *Brit. J. Pharmacol.* **117,** 743

(1996).
79. E. O'Hearn and M.E. Molliver, *J. Neurosci.* **17,** 8828 (1997).
80. S. Couture and G. Debonnel, *Synapse* **29,** 62 (1998).
81. D.C. Mash, J.K. Staley, J.P. Pablo, A.M. Holohean, J.C. Hackman, and R.A. Davidoff, *Neurosci. Lett.* **192,** 53 (1995).
82. H.I. Dhahir, "A Comparative Study of the Toxicity of Ibogaine and Serotonin," *Diss. Abstr. Int.* 32/04-B, 2311, 1971.
83. H.H. Molinari, I.M. Maisonneuve, and S.D. Glick, *Brain Res.* **737,** 255 (1996).
84. S. Helsley, C.A. Dlugos, R.J. Pentney, R.A. Rabin, and J.C. Winter, *Brain Res.* **759,** 306 (1997).
85. D.C. Mash, J.K. Staley, M.H. Baumann, R.B. Rothman, and W.L. Hearn, *Life Sci.* **57,** PL 45 (1995).
86. J.K. Staley, Q. Ouyang, J. Pablo, W.L. Hearn, D.D. Flynn, R.B. Rothman, K.C. Rice, and D.C. Mash, *Psychopharmacol.* **127,** 10 (1996).
87. S.D. Glick, S.M. Pearl, J. Cai, and I.M. Maisonneuve, *Brain Res.* **713,** 294 (1996).
88. S.D. Glick, M.E. Kuehne, I.M. Maisonneuve, U.K. Bandarage, and H.H. Molinari, *Brain Res.* **719,** 29 (1996).
89. G. Zetler, G. Singbartl, and L. Schlosser, *Pharmacol.* **7,** 237 (1972).
90. G. Singbartl, G. Zetler, and L. Schlosser, *Neuropharmacol.* **12,** 239 (1973).
91. S.D. Glick, K. Rossman, N.C. Rao, I.M. Maisonneuve, and J.N. Carlson, *Neuropharmacol.* **31,** 497 (1992).