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ORIGINAL INVESTIGATION

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Receptor binding profile suggests multiple mechanisms of action are responsible for ibogaine's putative anti-addictive activity

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Abstract The indole alkaloid ibogaine (NIH 10567, Endabuse) is currently being examined for its potential utility in the treatment of cocaine and opioid addiction. However, a clearly defined molecular mechanism of action for ibogaine's putative anti-addictive properties has not been delineated. Radioligand binding assays targeting over 50 distinct neurotransmitter receptors, ion channels, and select second messenger systems were employed to establish a broad in vitro pharmacological profile for ibogaine. These studies revealed that ibogaine interacted with a wide variety of receptors at concentrations of 1–100 μM. These included the mu, delta, kappa, opiate, $5HT_2$, $5HT_3$, and muscarinic$_{1 \text{ and } 2}$ receptors, and the dopamine, norepinephrine, and serotonin uptake sites. In addition, ibogaine interacted with *N*-methyl-D-aspartic acid (NMDA) associated ion and sodium ion channels as determined by the inhibition of [^{3}H]MK-801 and [^{3}H]bactrachotoxin A 20-α-benzoate binding (BTX-B), respectively. This broad spectrum of activity may in part be responsible for ibogaine's putative anti-addictive activity.

Key words Ibogaine · Drug abuse · Addiction · Neurotransmitter receptors · Radioligand binding

Introduction

Indolealkylamines are one branch of a large number of centrally active compounds that produce stimulatory and anxiogenic effects in animals (Schneider and Sigg 1957; Gershon and Lang 1962). One indolealkylamine derivative, ibogaine, has been shown to elicit both actions in man (Naranjo 1969). It has been suggested that ibogaine may have therapeutic potential in the treatment of opiate (heroin) addiction, stimulant (cocaine) abuse, and ethanol dependence by disrupting some aspect of physiological or psychological addiction (US Patents 4,499,096, 4,587,243, 4,857,523, respectively). Recent studies have attempted to substantiate these claims by using in vivo animal models, and results appear to support ibogaine's use in substance abuse therapy. Briefly, ibogaine administration decreased morphine self-administration (Glick et al. 1991), blocked morphine- and cocaine-induced dopamine turnover (Maisonneuve et al. 1991), reduced morphine-induced motor activity, and antagonized cocaine-induced locomotor stimulation (Sershen et al. 1992). From these aforementioned studies three neurotransmitter systems, e.g., the bioaminergic (dopamine/serotonin), peptidergic (opioid), amino acidergic (GABA), appear to be potential molecular targets responsible for ibogaine's therapeutic action.

By employing over 50 different radioligand binding assays we have been able to establish a broad receptor selectivity and potency profile for ibogaine, thus extending prior observations (Deecher et al. 1992; Sershen et al. 1992; Popik et al. 1994; Repke et al. 1994). Our results revealed that ibogaine is a nonselective agent interacting with a number of receptor systems. These include the serotonergic, dopaminergic, muscarinic, opiate, and the amino acid-ergic systems. Many of the interactions demonstrated were in the 1 to 100-μM range and no receptors examined have demonstrated nanomolar affinity for ibogaine. The potential in vivo significance of micromolar ibogaine interactions, as revealed in preliminary pharmacokinetic studies (Zetler et al. 1972) and the broad range of micromolar CNS activities revealed in this study suggest that any one or combination of these activities may be involved in ibogaine's putative therapeutic action. Therefore a clear delineation of ibogaine's therapeutic mechanisms of

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action must be determined before the efficacy of such a compound in the treatment of addiction can be accurately evaluated.

Materials and methods

Chemicals

Ibogaine HCL was purchased from Sigma Chemicals (St Louis, Mo). All [^{3}H] or [^{125}I] radioligands used were commercially available from NEN DuPont (Boston) or Amersham (Illinois). Unlabeled ligands and peptides were purchased from a variety of commercial sources.

Radioligand binding assays

Competition assays were performed in 250-µl volumes containing 200 µl receptor preparation, 25 µl radioligand, and 25 µl cold ligand (non-specific binding determinant) or ibogaine. All compounds were solubilized in neat DMSO diluted to a final concentration of 0.4% in the assay (Sweetnam et al. 1993). Assays were terminated by rapid filtration over Whatman glass fiber filters (GFC and GFB) followed by washing with 12 ml cold assay buffer. Radioactivity was determined by either liquid scintillation or gamma spectrometry. Nonspecific binding was defined as the radioactivity remaining in the presence of a saturating concentration of cold ligand. For specific assay conditions (i.e., tissue preparation, buffers, incubation times and temperatures, filter treatments) in assays in which ibogaine was run in concentration-response format the reader is referred to references noted in Table 1.

Initial inhibitory binding determinations were performed in duplicate. Activity of >30% at a concentration of 10 µM was verified using a freshly prepared ibogaine solution. Total and nonspecific binding tubes, positive controls tubes, and 14-point reference curves

Table 1 Ibogaine selectivity and potency

Receptor/selectivity*	Radioligand	IC_{50}(µM)
Excitatory amino acids		
NMDA	[^{3}H]CGS-19755	>100
PCP	[^{3}H]TCP (ref 1)	50.5±100
MK-801	[^{3}H]MK-801 (ref 2)	5.6±0.8
Quisqualate	[^{3}H]AMPA	>100
Kainate	[^{3}H]kainic acid	>100
Glycine	[^{3}H]glycine	>100
Opiate		
Mu	[^{3}H]DAGO (ref 3)	26±7
Delta	[^{3}H]DPDPE (ref 4)	>100
Kappa	[^{3}H]U-69593 (ref 5)	16±2.1
Sigma	[^{3}H]DTG (ref 6)	38±4.0
Cholinergics		
M_1	[^{3}H]pirenzepine (ref 7)	7.6±0.7
M_2	[^{3}H]AF-DX384 (ref 8)	5.9±1.4
Nicotinic (nonselective)	[^{3}H]NMCI	>100
Catecholamines		
$Dopamine_1$	[^{3}H]SCH-23390	>100
$Dopamine_2$ (clone)	[^{3}H]spiperone	>100
$Dopamine_3$ (clone)	[^{3}H]spiperone	>100
$Dopamine_4$ (clone)	[^{3}H]spiperone	>100
$Serotonin_1$	[^{3}H]5-HT	>100
$Serotonin_2$	[^{3}H]ketanserin (ref 9)	4.8±1.4
$Serotonin_3$	[^{3}H]GR-75558 (ref 10)	3.9±1.1
Alpha $adrenergic_1$	[^{3}H]prazosin (ref 11)	7.2±3
Alpha $adrenergic_2$	[^{3}H]RX-781094	>100
Neurotransmitter reuptake sites		
Dopamine	[^{3}H]WIN-35428 (ref 12)	3.5±0.6
Serotonin	[^{3}H]citalopram (ref 13)	49±3.2
$Norepinephrine_3$	[^{3}H]DMI (ref 14)	15±4.4
Ion channel proteins		
$Sodium\ site_1$	[^{3}H]saxitoxin	>100
$Sodium\ site_2$	[^{3}H]BCTX (ref 15)	9±3.0

* Rat forebrain membranes used except where note. For the D_2, D_3 and D_4 assays human receptor clones were used. Vignon et al. 1983; Javitt and Zukin 1989; Gillan and Koster 1982; Akiyam et al. 1985; Lahti et al. 1985; Weber et al. 1986; Watson et al. 1986; Richards et al. 1990; Leysen et al. 1982; Lummins et al. 1990; Timmermans et al. 1991; Madras et al. 1989; D'Amato et al. 1987; Raisman et al. 1982; Creveling 1983

Table 2 Receptors at which ibogaine had no demonstrated activity at 10 µM

Receptor/selectivity	Radioligand
Catecholamines	
$Histamines_1$	[^{3}H]pyrilamine
Beta Adrenergic (nonselective)	[^{3}H]DHA
Neurotransmitters	
$Adenosine_1$	[^{3}H]CPX
$Adenosine_2$	[^{3}H]CGS 21680
Adenosine (uptake)	[^{3}H]nitrobenzylthionosine
Inhibitory amino acids and related sites	
$GABA_A$	[^{3}H]GABA
$GABA_B$	[^{3}H]GABA + isoguvacine
GABA uptake site	[^{3}H]GABA
Glycine (strychnine sensitive)	[^{3}H]strychnine
Benzodiazepine	[^{3}H]flunitrazepam
GABA associated chloride channel	[^{3}H]TBOB
Brain gut peptides	
Angiotensin 2	[^{125}I]angiotensin II
Arg-vasopressin	[^{3}H]Manning compound
Bombesin	[^{125}I]gastric releasing peptide
$CCK_{A/B}$	[^{3}H]CCK
EndothelinA/B	[^{125}I]endothelin
Substance P	[^{3}H]substance P
Neuropeptide Y	[^{125}I]NPY
Neurotensin	[^{3}H]Neurotensin
Somatostatin	[^{125}I]Somatostatin
Vasoactive intestinal peptide	[^{125}I]VIP
Growth factors	
Atrial Natriuretic factor 1	[^{125}I]ANP
Epidermal growth factor	[^{125}I]EGF
Nerve growth factor	[^{125}I]NGF
Ion channels	
Calcium (type N)	[^{125}I]omega conotoxin
Calcium (type T & L)	[^{3}H]nitrendipine
Potassium (low conductance-Ca^{++} activated)	[^{125}I]apamin
Prostaglandins	
Leukotriene B_4	[^{3}H]LTB4
Leukotriene D_4	[^{3}H]LTD4
Thromboxane A_2	[^{3}H]SQ 29548
Second messengers	
Forskolin	[^{3}H]forskolin
Phorbol ester	[^{3}H]PDBU
Inositol triphosphate	[^{3}H]IP3