ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/13863679

Ibogaine and a Total Alkaloidal Extract of Voacanga africana Modulate Neuronal Excitability and Synaptic Transmission in the Rat Parabrachial Nucleus In Vitro

Article *in* Brain Research Bulletin · February 1997

DOI: 10.1016/S0361-9230(97)00284-0 · Source: PubMed

CITATIONS
11

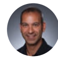
READS
206

7 authors, including:

Samuel Kombian
Kuwait University

75 PUBLICATIONS **1,735** CITATIONS

SEE PROFILE



Tarek M Saleh
University of Guelph

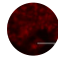
85 PUBLICATIONS **1,927** CITATIONS

SEE PROFILE

John Akabutu
University of Alberta

36 PUBLICATIONS **1,048** CITATIONS

SEE PROFILE



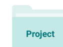
John Buolamwini
Rosalind Franklin University of Medicine and Science

38 PUBLICATIONS **1,850** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Neuropharmacological and related effects of Synedrella nodiflora View project



The effects of early life exposure to pathogen associated molecular patterns on susceptibility to autoimmunity in later life View project

All content following this page was uploaded by Tarek M Saleh on 25 October 2017.

The user has requested enhancement of the downloaded file.

ELSEVIER

Brain Research Bulletin, Vol. 44, No. 5, pp. 603–610, 1997
Copyright © 1997 Elsevier Science Inc.
Printed in the USA. All rights reserved
0361-9230/97 $17.00 + .00

PII S0361-9230(97)00284-0

Ibogaine and a Total Alkaloidal Extract of *Voacanga africana* Modulate Neuronal Excitability and Synaptic Transmission in the Rat Parabrachial Nucleus *In Vitro*

SAMUEL B. KOMBIAN,*[1] TAREK M. SALEH,*[2] N. I. YAO FIAGBE,† XIHUA CHEN,* JOHN J. AKABUTU,‡ JOHN K. BUOLAMWINI§ AND QUENTIN J. PITTMAN*

**NRG, University of Calgary, Faculty of Medicine, 3330 Hospital Drive NW, Calgary, Canada; †Faculty of Pharmacy, Department of Medicinal Chemistry, UST, Kumasi, Ghana; ‡University of Alberta, Edmonton, Canada; §Department of Medicinal Chemistry, University of Mississipi, University, MS, USA*

ABSTRACT: Ibogaine is a natural alkaloid of *Voacanga africana* that is effective in the treatment of withdrawal symptoms and craving in drug addicts. As the synaptic and cellular basis of ibogaine's actions are not well understood, this study tested the hypothesis that ibogaine and *Voacanga africana* extract modulate neuronal excitability and synaptic transmission in the parabrachial nucleus using the nystatin perforated patch-recording technique. Ibogaine and *Voacanga africana* extract dose dependently, reversibly, and consistently attenuate evoked excitatory synaptic currents recorded in parabrachial neurons. The ED_{50} of ibogaine's effect is 5 μM, while that of *Voacanga africana* extract is 170 μg/ml. At higher concentrations, ibogaine and *Voacanga africana* extract induce inward currents or depolarization that are accompanied by increases in evoked and spontaneous firing rate. The depolarization or inward current is also accompanied by an increase in input resistance and reverses polarity around 0 mV. The depolarization and synaptic depression were blocked by the dopamine receptor antagonist haloperidol. These results indicate that ibogaine and *Voacanga africana* extract 1) depolarize parabrachial neurons with increased excitability and firing rate; 2) depress non-NMDA receptor-mediated fast synaptic transmission; 3) involve dopamine receptor activation in their actions. These results further reveal that the *Voacanga africana* extract has one-hundredth the activity of ibogaine in depressing synaptic responses. Thus, ibogaine and *Voacanga africana* extract may produce their central effects by altering dopaminergic and glutamatergic processes. © 1997 Elsevier Science Inc.

KEY WORDS: Nystatin Patch-Recording, EPSC, Hallucinogen, Dopamine, Addiction.

INTRODUCTION

Ibogaine is one of several naturally occurring alkaloids found in many families of African shrubs including *Voacanga africana* (VA) [1,44,48]. Ibogaine and/or VA extracts are powerful hallucinogens [31,36] and various parts of the *Voacanga* genus have been used to induce hallucinations and trances in religious rituals in certain African societies (see [12] for review). Although ibogaine is known to produce several peripheral and central nervous system (CNS) effects (see [12] for review), its best known action is that of decreasing the severity of withdrawal symptoms from morphine, psychostimulants, nicotine and alcohol, and reducing drug craving [12,13,31,42]. This effect significantly differs from other addiction management approaches in that a single dose produces very prolonged abstinence from drug use or craving [31]. The mechanism by which ibogaine produces this effect has been the subject of intense research because of the potential for treating addicts of their drug seeking behavior or cravings [see [40] for review].

Neurochemical studies indicate that ibogaine interacts with several neurotransmitters and their receptors to produce its effects [22,40]. For example, ibogaine has been shown to modulate both dopamine and serotonin responses by blocking their reuptake [22,23,38–40] or by causing their release from cytoplasmic stores [14]. In addition, ibogaine also binds to several receptors in the CNS, including opioid receptors [3,6,19,27] and the MK-801 binding site of the NMDA receptor complex [24]. In behavioral studies, ibogaine has psychoactive and memory-altering effects by itself [17,25,28–30] and is tremorigenic at higher doses [7,13]. In addition to these direct effects, ibogaine also alters the actions of several drugs of abuse, including cocaine, amphetamine, morphine, alcohol, and nicotine [2,4,13,27,32,40]. In some studies, ibogaine antagonizes or attenuates several behavioral effects of these drugs [9,14,37,42] while enhancing or not affecting them in other reports [20,21,41]. It is, at present, not clear which of these actions of ibogaine mediate its "antiaddictive" effects.

To begin to understand the cellular basis of these CNS effects of ibogaine, its actions on neuronal responses have been examined. Intravenous injection of ibogaine has been shown to increase the firing rate of ventral tegmental area dopaminergic neurons [11]. Consistent with its binding to the MK-801 binding site of the NMDA receptor complex, ibogaine has been shown to block, in a voltage-dependent manner, currents induced by NMDA and to prevent glutamate-induced cell death in hippocampal cultures [5,29]. Furthermore, it has been shown to block NMDA-induced depolarization of frog spinal cord motorneurons [24]. To our knowledge, no study has examined the action of ibogaine and related alkaloids on synaptic transmission in the CNS, a likely locus for its behavioral effects. This study, therefore, examined the

[1] To whom requests for reprints should be addressed. e-mail: kombian@acs.ucalgary.ca
[2] Present address: Atlantic Veterinary College, University of PEI, Charlottetown, PEI, Canada.

effects of ibogaine and a total alkaloidal extract of VA on parabrachial nucleus (PBN) neuronal excitability and on non-NMDA receptor-mediated excitatory synaptic responses using *in vitro* electrophysiological recording techniques. The PBN was chosen for this study because the biophysical characteristics of cells in this nucleus, in addition to the excitatory afferent pathways and inputs to these cells, are quite well characterized, permitting selective activation of appropriate afferents [33,34]. Furthermore, excitatory synaptic transmission in this region has been thoroughly elucidated, thus making it attractive for examining the effects of substances on synaptic transmission [35,49,50]. Doing these experiments in a slice preparation also afforded us to apply known concentrations of drugs directly to neurons in a controlled environment.

MATERIALS AND METHODS

All experiments were carried out in accordance with guidelines established by the Canadian Council on Animal Care and were approved by the University of Calgary Animal Care Committee. The details of all the techniques used in this study have been reported elsewhere in previous studies conducted in this laboratory [18,35].

Slice Preparation

Coronal pontine slices (400-μm thick) containing the PBN were cut from a block of brain removed from halothane-anesthetized Sprague–Dawley rats (25–40 days of age) obtained from Charles River and the University of Calgary Biosciences Breeding Colony. The slices were hemisected and incubated at room temperature (22°C) in artificial cerebrospinal fluid (ACSF; pH 7.3–7.4) bubbled with carbogen (5% CO_2 and 95% O_2). The composition of the ACSF was (in mM): NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.4; $NaHCO_3$, 18; glucose, 11. A slice was then transferred into a 500 μl recording chamber where it was submerged and continuously perfused with prewarmed ACSF (32°C) at a rate of 2–3 ml/min. To evoke synaptic responses, a bipolar stimulating electrode connected to a stimulus isolation unit was placed ventral–lateral to the PBN, close to the ventral tip of the superior cerebellar peduncle.

Nystatin-Patch Recording

The details of this recording technique have been described elsewhere [18,35]. Briefly, nystatin perforated-patch whole-cell recordings from PBN neurons were made with glass micropipettes (Garner Glass Co.; tip resistance 4–8 MΩ) filled at the tip with a solution containing (in mM) K-Acetate, 120; HEPES, 40; $MgCl_2$, 5; EGTA, 10. The rest of the electrode was then backfilled with the same solution, to which nystatin (450 μg/ml) in dimethyl sulfoxide (DMSO) and Pluronic F127 were added. High resistance seals (1–3 GΩ) were made using an Axoclamp 2A amplifier.

Data Acquisition and Analysis

After adequate access to the cell was achieved (1–15 min), the resting membrane potential (RMP) was estimated. Cells were then characterized using standard biophysical criteria [35]. Synaptic currents were evoked by applying single pulses via bipolar stimulating electrodes ($\leq$10V, 40–400 μs) and a stimulus intensity that yielded a response 50–60% of the maximum response was used for the remainder of the experiment. Three successive synaptic samples were taken 10 s apart, digitally averaged, and stored for analysis. All experiments were performed in the presence of bicuculline (25 μM) to eliminate any $GABA_A$ receptor-mediated inhibitory postsynaptic current (IPSC).

Cells served as their own controls and only cells that showed significant (>80%) recovery from any drug effect were included in the analysis. Statistical comparisons were performed using analysis of variance (ANOVA) and the Student's t-test and $p < 0.05$ was considered significant. In all synaptic current experiments, a −20 mV, 100 ms square pulse was applied 200 ms after synaptic stimulation to monitor input and series/access resistance. Current–voltage relationships (I–V curves) were generated either by applying 500 ms step, square current pulses or by applying slow voltage ramps and recording the corresponding steady-state current (in voltage clamp). In addition to the computer-assisted data acquisition, continuous records of membrane potentials and currents were made on a chart recorder (Gould 2400S).

All acquired data were analyzed off-line using pClamp pro-

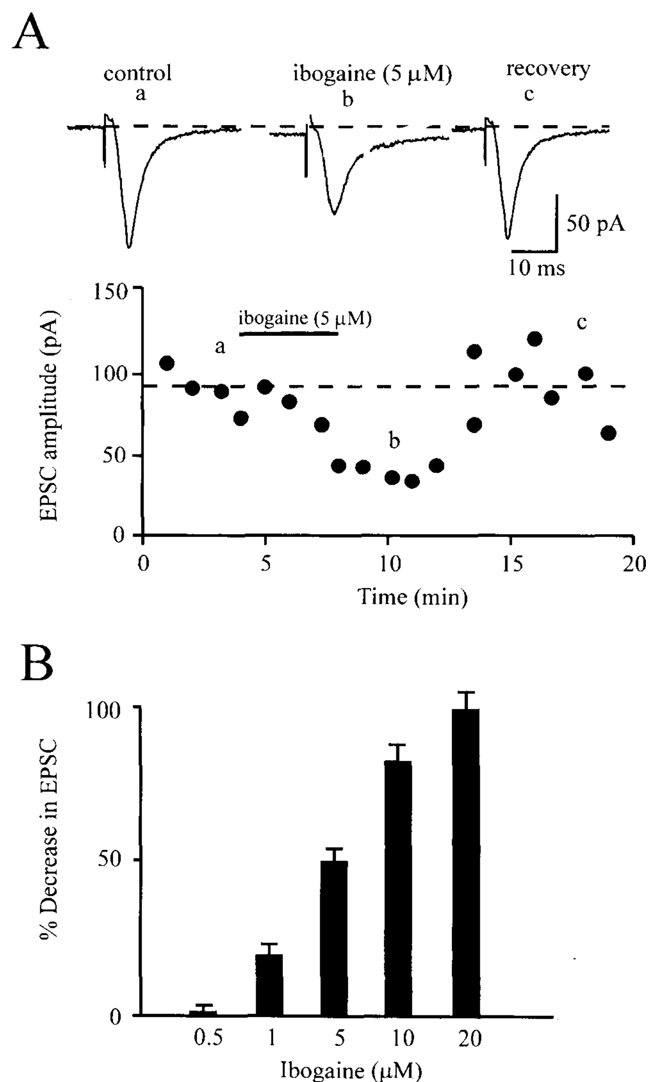


FIG. 1. Ibogaine reversibly depresses excitatory synaptic transmission. (A) An example of a typical time-dependent effect of ibogaine (5 μM) on EPSC amplitude (lower panel). Upper panel shows representative EPSC traces (average 3–4/trace) taken at the times indicated by letters in lower panel. (B) Concentration–response graph showing that the ibogaine-induced synaptic depression is concentration dependent with an estimated EC_{50} of 5 μM (n = 2–5 cells at each concentration).

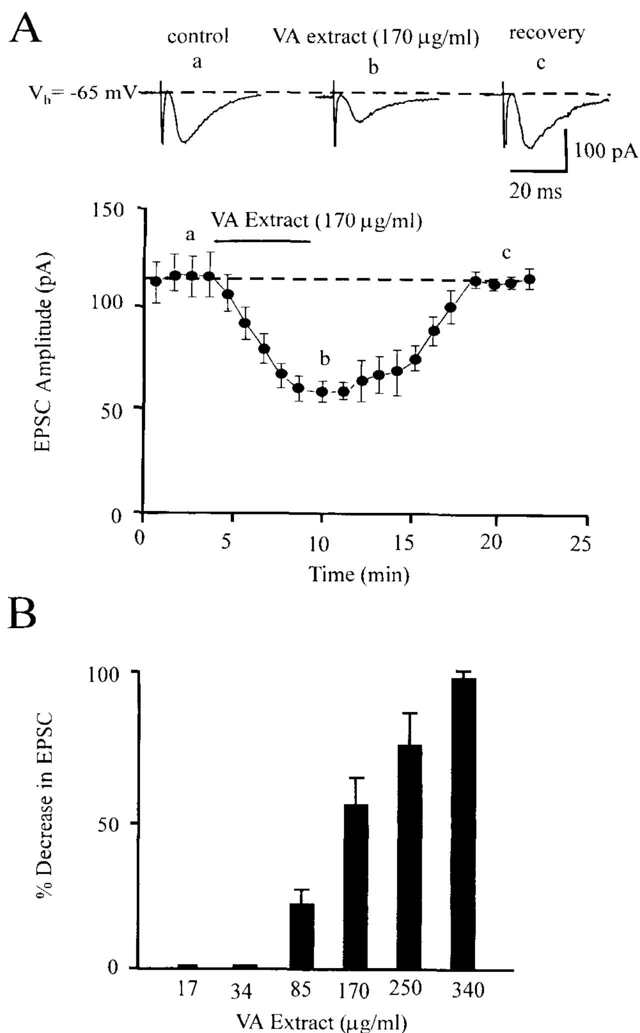


FIG. 2. VA extract reversibly depresses excitatory synaptic transmission. (A) A time-dependent effect of VA (170 μg/ml) on evoked EPSC amplitude (lower panel; average of four cells). Upper panel are sample EPSC traces taken at the times indicated by letters in the lower panel. (B) Concentration–response graph showing that the VA-induced synaptic depression is concentration dependent with an estimated EC_{50} of 170 μg/ml (n = 3–4 at each concentration).

grams (Axon Instruments) and a 386 personal computer. All data were corrected for any electrode offset observed at the end of each recording session. Input resistances for all cells were estimated from the step I–V protocol by determining the difference of the steady-state current (measured at ~150 ms into the pulse) at −70 and −50 mV. The amplitude of the excitatory postsynaptic current (EPSC) was taken as a measure of the magnitude of synaptic strength. Data are expressed as mean percentage change from control values ± standard error of the mean (SEM). All drugs were bath applied by perfusion with ACSF containing the final concentration of the drug. Appropriate stocks were made and diluted with ACSF just before application.

Preparation of VA Extract

Total alkaloidal extracts of *Voacanga africana* seeds (obtained from Valley Farms Ltd, Accra, Ghana) were prepared using standard extraction procedures [10,43]. Briefly, dried seeds were powdered and defatted using petroleum ether, and a crude concentrated extract was obtained by a series of ammonia basification and methanol extractions. This crude extract was then purified using solvent extraction, pH manipulation, and precipitation techniques [43].

Drugs Used and Their Sources

Pluronic F127 was obtained from BASF Wyandotte (Michigan); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was obtained from RBI, while ibogaine, bicuculline, nystatin, haloperidol, and all the salts in the ACSF were obtained from Sigma (St. Louis, MO). Stock solutions of ibogaine and VA extract were made in 63% ethanol and diluted 500–1000-fold prior to application.

RESULTS

The results reported in this study were obtained from >25 neuronal recordings from the dorsal, central, and external lateral aspects of the PBN. These cells had RMPs of −53 to −67 mV (−60 ± 2 mV). Because the stock solutions of both ibogaine and the VA extract were made in 63% alcohol, the possibility that some of the effects of these compounds were due to alcohol was examined. At low aliquot volumes, the 1000-fold dilutions that were applied to the bath resulted in between 0.06–0.08% final alcohol content, which had negligible effects. However, when large aliquots, such as those used to obtain the highest concentrations of VA extract (e.g., 340 μg/ml), the final alcohol content was about 1.2%. Control experiments (two cells) using 1.2% alcohol showed that this concentration of alcohol reduces the EPSC by only about 20%.

Ibogaine and VA Extract Depress Excitatory Synaptic Transmission

Electrical stimulation of the ventral–lateral aspect of the PBN in the presence of bicuculline (25 μM) elicited a pure non-NMDA receptor-mediated EPSP/EPSC, because it could be completely blocked by the non-NMDA receptor antagonist CNQX (10 μM;

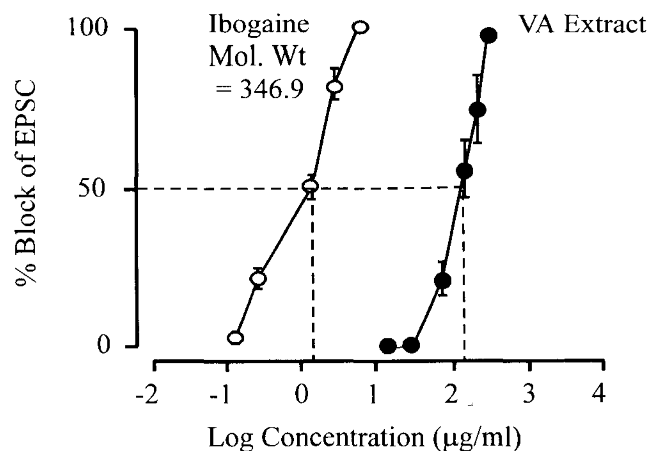


FIG. 3. Comparison of ibogaine and VA extract effects on synaptic transmission. Concentration–response values from Figs. 1 and 2 have been replotted using a log transformation of the concentration for comparison. The concentration of ibogaine (molecular weight = 346.9) has been converted to μg/ml for this purpose. Dotted lines show points where ED_{50} was estimated (1.7 μg/ml for ibogaine and 170 μg/ml for VA extract).

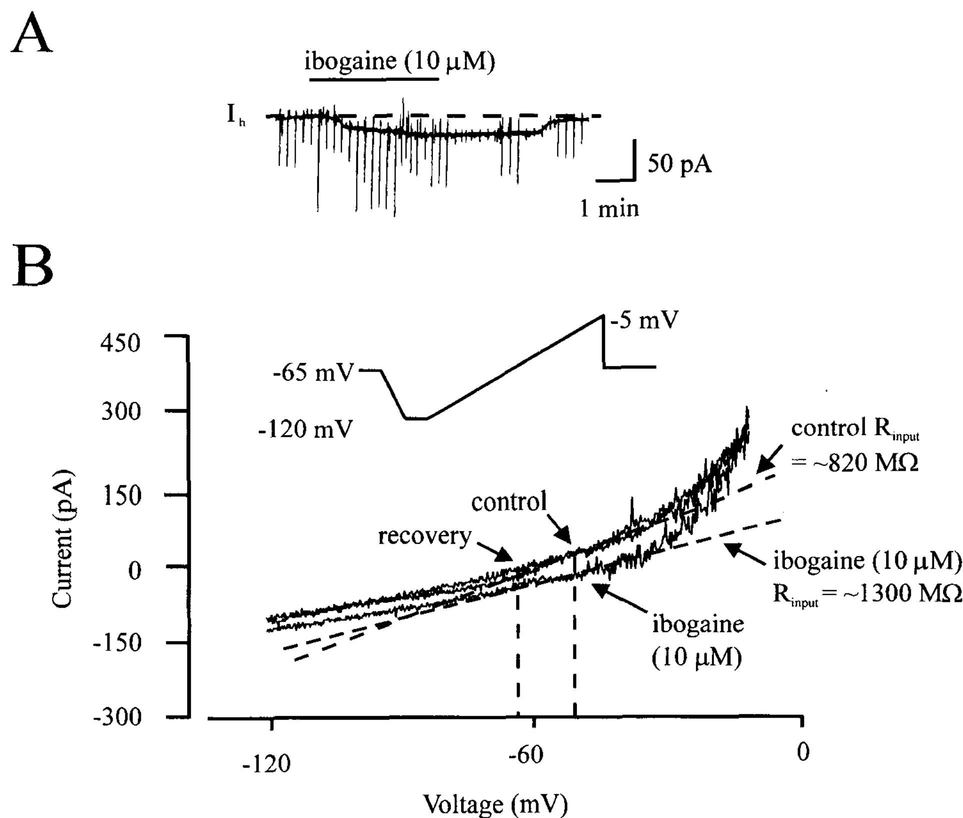


FIG. 4. A high concentration of ibogaine induces an inward current in PBN neurons. (A) A chart record showing that 10 μM ibogaine reversibly induces an inward current in neurons voltage clamped around resting potential (holding potential = −65 mV). Note that some of the downward deflections are breakthrough spikes. (B) Superimposed steady-state I–V curves [generated by application of slow voltage ramps (insert) to the membrane of this cell] obtained in control (TTX 1 μM), in the presence of 10 μM ibogaine and following washout. Broken vertical line mark the region (−50 to −65 mV) where estimation of input resistance was made. Note that the I–V curves in control and in the presence of ibogaine cross around −5 mV.

[35,49,50]). In voltage clamp, 4–5-min bath application of ibogaine (0.1–30 μM) consistently depresses the non-NMDA receptor-mediated EPSC in a concentration-dependent manner. Ibogaine has a threshold concentration above 0.5 μM and a maximal synaptic depressant effect at 20 μM (Fig. 1A and B). The estimated ED_{50} of this action is 5 μM. VA extract (17–340 μg/ml) similarly reversibly depresses the evoked EPSC. The threshold for this action is about 34 μg/ml with a maximal depressant action at 340 μg/ml and an estimated ED_{50} close to 170 μg/ml (Fig. 2A and B). The actions of both drugs are reversible upon washing out the drug (5–30 min; Figs. 1A and 2A) and are repeatable following multiple applications to the same cell. To compare the actions of ibogaine with those of VA extract, the concentration of ibogaine was converted from micromolar (μM) to microgram per milliliter (μg/ml) and the two concentration–response curves were plotted on the same graph (Fig. 3). This plot reveals that the VA curve lies to the right of the ibogaine curve, a difference of about 2 base units or 100 units. This indicates that VA extract has only 1/100th the potency of ibogaine in depressing excitatory synaptic responses in the PBN.

Ibogaine and VA Extract Depolarize PBN Neurons

In voltage clamp, bath application of ibogaine (0.1–30 μM) or VA extract (17–340 μg/ml) induces an inward current that reverses upon washout (Fig. 4A). This effect is more robust at higher concentrations than at lower concentrations. When steady-state I–V curves are generated in control [in the presence of tetrotoxin (TTX) 1 μM] and in the presence of ibogaine, the two curves intersect around 0 mV (reversal potential; Fig. 4B). Estimates of the input resistance (R_{input}) of these cells near resting potential shows that 10 μM ibogaine causes a 58% increase in R_{input} (n = 2; Fig. 4B). In current clamp, the inward current becomes a depolarization accompanied by increase in evoked and spontaneous firing frequency (70–300% increase) of all PBN neurons tested (Fig. 5A and C). These postsynaptic effects are also concentration dependent, being very small at lower concentrations but quite prominent at higher concentrations, i.e., near concentrations eliciting maximal synaptic depression (see Fig. 3). A plot of an I–V curve from the data in Fig. 5A reveals that the depolarization in this cell is accompanied by an increase in R_{input} from 407 MΩ to 630 MΩ (55% increase; Fig. 5B) estimated around the resting potential.

Haloperidol Blocks Ibogaine and VA Extract Effects

Some of the central effects of ibogaine and VA are known to occur through an alteration in dopaminergic neurotransmission [14,22,38,39]. We, therefore, tested if the observed effects of ibogaine and VA were indirect via modulation of dopaminergic

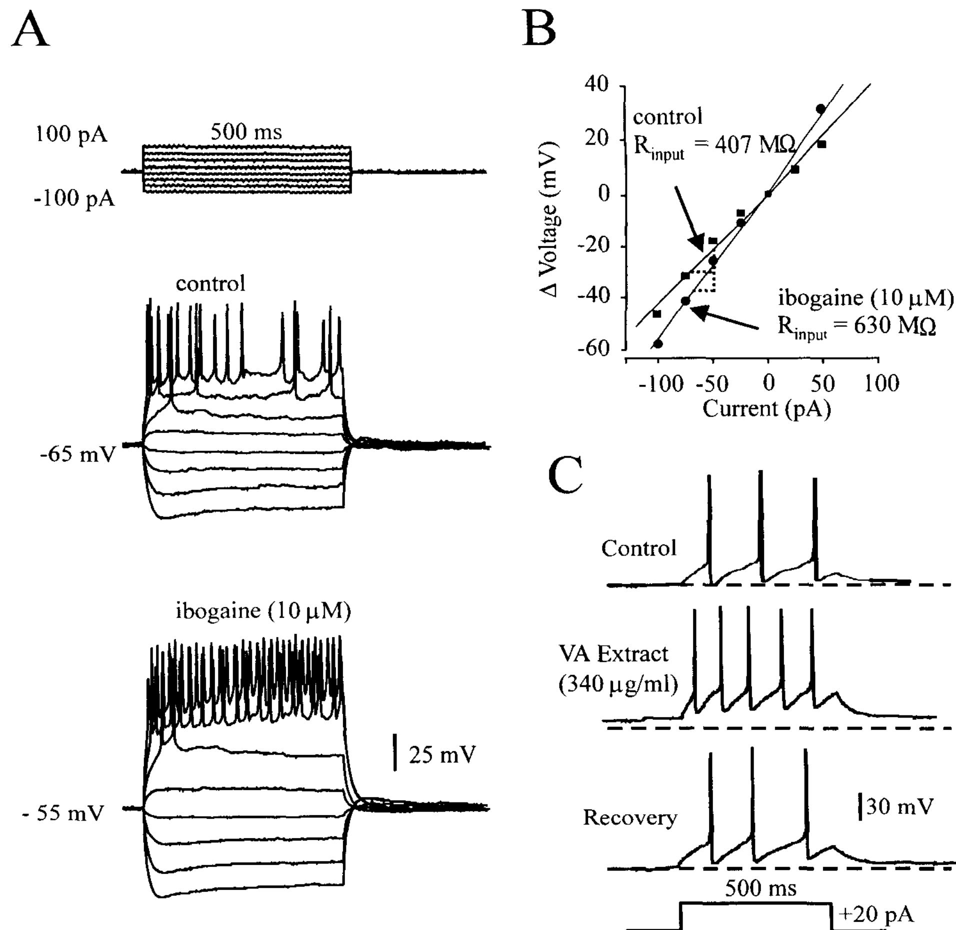


FIG. 5. In current clamp, both ibogaine and VA extract depolarize PBN neurons and increase firing rate. (A) Upper panel represents the current steps used to induce the voltage responses in control (middle) and in the presence of 10 μM ibogaine (bottom). (B) Superimposed I–V curves generated from A in control and in the presence of 10 μM ibogaine. Note the change in input resistance (R_{input}) from 407 MΩ to 630 MΩ (taken at the points indicated by dotted lines). (C) Responses of a PBN cell to injection of +20 pA current in control and in the presence of 340 μg/ml of VA extract.

processes in this nucleus by using the dopamine receptor antagonist haloperidol. In four cells, haloperidol 50 μM (which by itself had no effect, Fig. 6A) blocks the inward current induced by 5 μM ibogaine. At this concentration, ibogaine alone depresses the evoked EPSC by 50 ± 10% ($n = 7$), but in the presence of 50 μM haloperidol, ibogaine depresses the evoked EPSC only by 5 ± 5% ($n = 4$, $p < 0.05$; Fig. 6A). In two cells, haloperidol also blocks the VA extract (170 μg/ml)-induced inward current and synaptic depression (Fig. 6B).

DISCUSSION

The results of the present study show that both ibogaine and a total alkaloidal extract of VA increase the excitability of PBN neurons while depressing the non-NMDA receptor-mediated excitatory synaptic transmission in the PBN *in vitro*. Ibogaine and VA depolarized PB neurons with an increase in the excitability and the firing rate of PBN neurons. These postsynaptic effects of ibogaine and VA were most likely due to the closure of nonselective cationic channels because their actions resulted in increased input resistance of the cells and the I–V curves generated in their presence reversed polarity around 0 mV. These observed *in vitro* postsynaptic effects of ibogaine and VA are similar to those seen *in vivo*, where intravenous injection of ibogaine was shown to increase the firing rate of ventral tegmental area (VTA) neurons recorded extracellularly [11]. Because ibogaine and/or VA produce excitation of PBN and VTA neurons, this may be a common action of these compounds on CNS neurons in general and may be responsible for their tremorigenic effects as postulated by Deecher et al. [7]. Thus, in addition to the possible action on sodium channels [7], ibogaine and VA may act at a nonselective cation channel to increase excitation.

The synaptic effect of ibogaine and VA revealed by this study adds another dimension to the repertoire of possible mechanisms by which ibogaine and related compounds may alter CNS function to produce their central effects. Because glutamate is a ubiquitous transmitter in the CNS and acts on non-NMDA receptors to cause fast synaptic transmission, modulation of this effect by ibogaine would profoundly affect several other central actions of ibogaine. The micromolar EC_{50} of this effect also indicates that ibogaine is quite potent at modulating the non-NMDA receptor-mediated re-

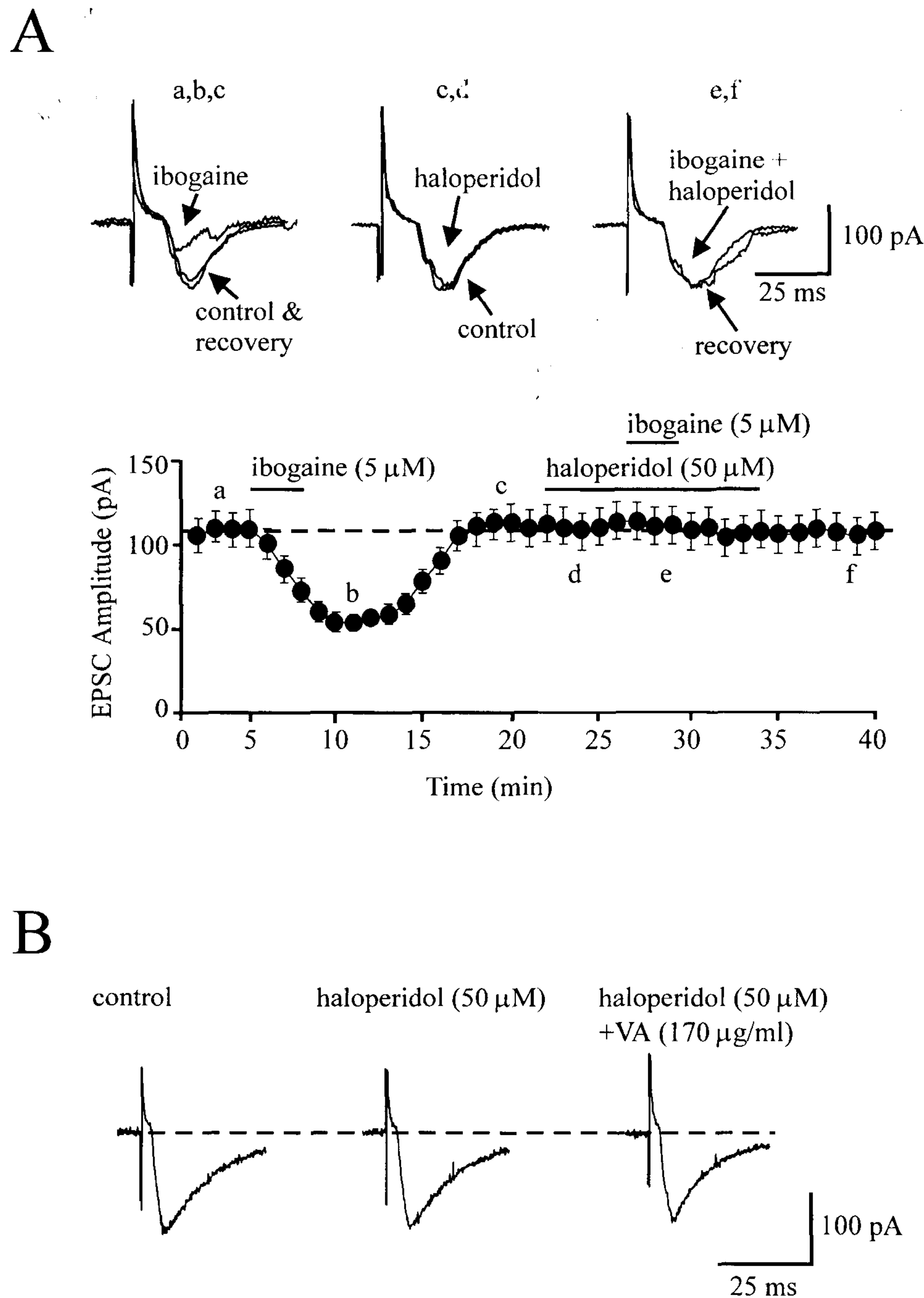


FIG. 6. Ibogaine and VA effects are blocked by haloperidol. (A) A summary graph showing the block by haloperidol of ibogaine's effects on synaptic transmission (lower panel; $n = 5$ cells). In the upper panel are superimposed synaptic traces taken at the times indicated by letters in the lower panel. (B) Sample synaptic responses showing haloperidol blockade of VA-induced synaptic depression.

sponse in this nucleus. This contrasts with the lack of effect of ibogaine on non-NMDA receptor-mediated responses in cultured hippocampal neurons [5]. This difference in effect may be due to several reasons: first of all, both the postsynaptic and synaptic effects of ibogaine in the present study are probably indirect via dopamine. Because the other study [5] was done in a pure culture system that only examined the direct actions of ibogaine, such indirect effects of ibogaine could not be examined. Second, it is possible that the synaptic responses recorded in the PBN are mediated purely by AMPA, rather than by a mixture of AMPA and kainate receptors, of which only the latter was examined in the hippocampus. Third, it is possible that ibogaine may selectively modulate synaptically located non-NMDA receptors rather than the extrasynaptic receptors that were likely examined under culture

conditions. Finally, although not thoroughly examined in this study, a possible presynaptic action of ibogaine to decrease glutamate release can explain the observed synaptic depression. In the culture studies [5], presynaptic actions of ibogaine on terminals from other cell types could not be examined. Such a presynaptic action is most likely the case, as ibogaine is reported not to affect AMPA/kainate binding [30] and AMPA-induced postsynaptic responses in frog spinal cord motorneurons [24]. One of the currently popular mechanisms by which ibogaine may produce its antiaddictive action is by modulating NMDA receptor responses through interference at the MK-801 binding site (reviewed by [31]). A presynaptic decrease in glutamate release will be predicted to be additive to its reported blockade of NMDA receptors [24,29] because less glutamate will be available to activate NMDA receptors. Conversely, it is possible that such an effect will, in the long run, make less effective ibogaine's NMDA receptor blockade by decreasing the initial depolarization (provided by non-NMDA receptor activation) required to open NMDA channels to permit ibogaine to bind to the MK-801 site, which is an open channel binding site and blocker [5].

Both the postsynaptic and synaptic effects of ibogaine and VA observed in the PBN may be indirectly mediated by their action to alter dopamine levels, because we could block them with haloperidol, a dopamine receptor antagonist. Although the literature is replete with opposing reports of ibogaine's effect on dopamine turnover, such differences may be due to time- and dose-dependent effects as suggested by Glick et al. [12]. More recently, it was demonstrated that ibogaine caused dopamine release from nonvesicular (cytoplasmic) pool without affecting reuptake [14]. There is also evidence that ibogaine does not directly interact with dopamine receptors [7,46]. The haloperidol blockade of the actions of ibogaine and VA would suggest that these compounds act (in a time frame of 1–5 min) to induce sufficient increase in extracellular dopamine, which then acts on presynaptic dopamine receptors to cause decreases in glutamate release and, hence, a decrease in non-NMDA receptor-mediated fast EPSC. This is quite likely the case, as there is evidence of dopaminergic projection to this nucleus arising from dopaminergic cells in the VTA [16]. Consistent with this hypothesis, we found that direct application of dopamine to this preparation produced similar depression of the EPSC (unpublished observation). Similarly, in the nucleus accumbens, dopamine has been reported to act presynaptically to decrease non-NMDA receptor-mediated EPSCs [15,26].

The present study reveals a hitherto unreported effect of ibogaine in the CNS that may contribute to its central effects. This effect is due to a known action of ibogaine, i.e., the modulation of dopaminergic levels (see [40] for review). Because most drugs of abuse are known to act in mesolimbic dopaminergic areas to produce their effects [8,47], if ibogaine and related alkaloids act similarly in psychotropic brain regions such as the nucleus accumbens and the limbic cortical areas, then this action may contribute to its hallucinogenic and antiaddictive actions/effects. If, however, this action is unique to the PBN, then it would be toxicologically relevant information because the PBN, although not proven to be involved in the action of drugs of abuse, is an important regulatory site for visceral functions. Such an action may underly the visceral effects of ibogaine and related alkaloids [31,45].

Our investigation also examined the effects of a total alkaloidal extract of VA that contains ibogaine as one of its major constituents. Comparison of the actions of ibogaine with those of the extract did not reveal any qualitative differences in their actions. Quantitatively, the extract appears to be a hundred times less potent than ibogaine in depressing the evoked EPSCs. This finding suggests that the VA extract can serve as a natural herbal source of ibogaine capable of producing similar neuronal effects to ibogaine.

ACKNOWLEDGEMENTS

We thank J. W. Wilson of Valley Farms Ltd, Ghana, for kindly providing VA seeds for the extraction. S.B.K. is supported by MRC (Canada) Centennial and Alberta Heritage Foundation for Medical Research Fellowships. X.C. is supported by MRC (Canada) Fellowship, and Q.J.P. is supported by MRC and Alberta Heritage Foundation for Medical Research Senior Scientist Award.

REFERENCES

1. Battersby, R. T.; Brown, R. T.; Kapil, R. S.; Plunkett, A. O.; Taylor, J. B. Biosynthesis of indole alkaloids. Chem. Commun. 2:46–47; 1966.
2. Benwell, M. E.; Holtom, P. E.; Moran, R. J.; Balfour, D. J. Neurochemical and behavioural interactions between ibogaine and nicotine in the rat. Br. J. Pharmacol. 117:743–749; 1996.
3. Bowen, W. D.; Vilner, B. J.; Williams, W.; Bertha, C. M.; Kuehne, M. E.; Jacobson, A. E. Ibogaine and its congeners are sigma 2 receptor-selective ligands with moderate affinity. Eur. J. Pharmacol. 279:R1–R3; 1995.
4. Cappendijk, S. L.; Fekkes, D.; Dzoljic, M. R. The inhibitory effect of norharman on morphine withdrawal syndrome in rats: Comparison with ibogaine. Behav. Brain Res. 65:117–119; 1994.
5. Chen, K.; Kokate, T. G.; Donevan, S. D.; Carroll, F. I.; Rogawski, M. A. Ibogaine block of the NMDA receptor: *In vitro* and *in vivo* studies. Neuropharmacology 35:423–431; 1996.
6. Codd, E. E. High affinity ibogaine binding to a *mu* opioid agonist site. Life Sci. 57:PL315–PL320; 1995.
7. Deecher, D. C.; Teitler, M.; Soderlund, D. M.; Bornmann, W. G.; Kuehne, M. E.; Glick, S. D. Mechanisms of action of ibogaine and harmaline congeners based on radioligand binding studies. Brain Res. 571:242–247; 1992.
8. Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85:5274–5278; 1988.
9. Dzoljic, E. D.; Kaplan, C. D.; Dzoljic, M. R. Effect of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. Arch. Int. Pharmacodyn. Ther. 294:64–70; 1988.
10. Fiagbe, N. I. Y. Proposals for investigation of *Voacanga africana* (Apocynaceae): An anti-addictive drug? 11th Pharmaceut. Technol. Conf. 1:334–338; 1992.
11. French, E. D.; Dillon, K.; Ali, S. F. Effects of ibogaine, and cocaine and morphine after ibogaine, on ventral tegmental dopamine neurons. Life Sci. 59:PL199–PL205; 1996.
12. Glick, S. D.; Gallagher, C. A.; Hough, L. B.; Rossman, K. L.; Maisonneuve, I. M. Differential effects of ibogaine pretreatment on brain levels of morphine and (+)-amphetamine. Brain Res. 588:173–176; 1992.
13. Glick, S. D.; Rossman, K.; Rao, N. C.; Maisonneuve, I. M.; Carlson, J. N. Effects of ibogaine on acute signs of morphine withdrawal in rats: Independence from tremor. Neuropharmacology 31:497–500; 1992.
14. Harsing, L. G., Jr.; Sershen, H.; Lajtha, A. Evidence that ibogaine releases dopamine from the cytoplasmic pool in isolated mouse striatum. J. Neural Transm. [Gen. Sect.] 96:215–225; 1994.
15. Harvey, J.; Lacey, M. G. Endogenous and exogenous dopamine depress EPSCs in rat nucleus accumbens *in vitro* via D_1 receptor activation. J. Physiol. (Lond.) 492:143–154; 1996.
16. Hedreen, J. Terminal degeneration demonstrated by the Fink–Heimer method following lateral ventricular injection of 6-hydroxydopamine. Brain Res. Bull. 5:425–436; 1980.
17. Kesner, R. P.; Jackson-Smith, P.; Henry, C.; Amann, K. Effects of ibogaine on sensory-motor function, activity, and spatial learning in rats. Pharmacol. Biochem. Behav. 51:103–109; 1995.
18. Kombian, S. B.; Zidichouski, J. A.; Pittman, Q. J. $GABA_B$ receptors presynaptically modulate excitatory synaptic transmission in the rat supraoptic nucleus *in vitro*. J. Neurophysiol. 76:1166–1179; 1996.
19. Mach, R. H.; Smith, C. R.; Childers, S. R. Ibogaine possesses a selective affinity for sigma 2 receptors. Life Sci. 57:PL57–PL62; 1995.
20. Maisonneuve, I. M.; Glick, S. D. Interactions between ibogaine and

cocaine in rats: *In vivo* microdialysis and motor behavior. Eur. J. Pharmacol. 212:263–266; 1992.
21. Maisonneuve, I. M.; Keller, R. W., Jr.; Glick, S. D. Interactions of ibogaine and D-amphetamine: In vivo microdialysis and motor behavior in rats. Brain Res. 579:87–92; 1992.
22. Maisonneuve, I. M.; Rossman, K. L.; Keller, R. W., Jr.; Glick, S. D. Acute and prolonged effects of ibogaine on brain dopamine metabolism and morphine-induced locomotor activity in rats. Brain Res. 575:69–73; 1992.
23. Mash, D. C.; Staley, J. K.; Baumann, M. H.; Rothman, R. B.; Hearn, W. L. Identification of a primary metabolite of ibogaine that targets serotonin transporters and elevates serotonin. Life Sci. 57:PL45–PL50; 1995.
24. Mash, D. C.; Staley, J. K.; Pablo, J. P.; Holohean, A. M.; Hackman, J. C.; Davidoff, R. A. Properties of ibogaine and its principal metabolite (12-hydroxyibogamine) at the MK-801 binding site of the NMDA receptor complex. Neurosci. Lett. 192:53–56; 1995.
25. Naranjo, C. Psychotherapeutic possibilities of new fantasy-enhancing drugs. Clin. Toxicol. 2:209–224; 1973.
26. Nicola, S. M.; Kombian, S. B.; Malenka, R. C. Psychostimulants depress excitatory synaptic transmission in the nucleus accumbens via presynaptic D1-like dopamine receptors. J. Neurosci. 16:1591–1604; 1996.
27. Pearl, S. M.; Herrick–Davis, K.; Teitler, M.; Glick, S. D. Radioligand-binding study of noribogaine, a likely metabolite of ibogaine. Brain Res. 675:342–344; 1995.
28. Popik, P. Facilitation of memory retrieval by the "anti-addictive" alkaloid, ibogaine. Life Sci. 59:L379–L384; 1996.
29. Popik, P.; Layer, R. T.; Fossom, L. H.; Benveniste, M.; Geter–Douglass, B.; Witkin, J. M.; Skolnick, P. NMDA antagonist properties of the putative anti-addictive drug, ibogaine. J. Pharmacol. Exp. Ther. 275:753–760; 1995.
30. Popik, P.; Layer, R. T.; Skolnick, P. The putative anti-addictive drug ibogaine is a competitive inhibitor of [^{3}H]MK-801 binding to the NMDA receptor complex. Psychopharmacology (Berlin) 114:672–674; 1994.
31. Popik, P.; Layer, R. T.; Skolnick, P. 100 years of ibogaine: Neurochemical and pharmacological actions of a putative anti-addictive drug. Pharmacol. Rev. 47:235–253; 1995.
32. Rezvani, A. H.; Overstreet, D. H.; Lee, Y. W. Attenuation of alcohol intake by ibogaine in three strains of alcohol-preferring rats. Pharmacol. Biochem. Behav. 52:615–620; 1995.
33. Saleh, T. M.; Cechetto, D. F. Neurotransmitters in the parabrachial nucleus mediating visceral input to the thalamus in rats. Am. J. Physiol. 266:R1287–1296; 1994.
34. Saleh, T. M.; Cechetto, D. F. Neurochemical interactions in the parabrachial nucleus mediating visceral inputs to visceral thalamic neurons. Am. J. Physiol. 268:R786–R795; 1995.
35. Saleh, T. M.; Kombian, S. B.; Zidichouski, J. A.; Pittman, Q. J. Peptidergic modulation of synaptic transmission in the parabrachial nucleus *in vitro*: Importance of degradative enzymes in regulating synaptic efficacy. J. Neurosci. 16:6046–6055; 1996.
36. Schneider, J. A.; Sigg, E. B. Neuropharmacological studies of ibogaine, an indole alkaloid with central stimulant properties. Ann. NY Acad. Sci. 66:765–776; 1957.
37. Sershen, H.; Harsing, L. G., Jr.; Hashim, A.; Lajtha, A. Ibogaine reduces amphetamine-induced locomotor stimulation in C57BL/6By mice, but stimulates locomotor activity in rats. Life Sci. 51:1003–1011; 1992.
38. Sershen, H.; Hashim, A.; Lajtha, A. Effect of ibogaine on serotonergic and dopaminergic interactions in striatum from mice and rats. Neurochem. Res. 19:1463–1466; 1994.
39. Sershen, H.; Hashim, A.; Lajtha, A. Effect of ibogaine on cocaine-induced efflux of [^{3}H] dopamine and [^{3}H] serotonin from mouse striatum. Pharmacol. Biochem. Behav. 53:863–869; 1996.
40. Sershen, H.; Hashim, A.; Lajtha, A. Ibogaine and cocaine abuse: Pharmacological interactions at dopamine and serotonin receptors. Brain Res. Bull. 42:161–168; 1997.
41. Sharpe, L. G.; Jaffe, J. H. Ibogaine fails to reduce naloxone-precipitated withdrawal in the morphine-dependent rat. NeuroReport 1:17–19; 1990.
42. Sheppard, S. G. A preliminary investigation of ibogaine: Case reports and recommendations for further study. J. Subst. Abuse Treat. 11: 379–385; 1994.
43. Thomas, D. W.; Bieman, K. Alkaloids of *Voacanga africana*. Lloydia 31:1–8; 1968.
44. Thomas, R. A possible biosynthetic relationship between the cyclopentanoid monoterpenes and the indole alkaloids. Tetrahedron Lett. 16:544–553; 1961.
45. Vincent, D.; Sero, M. Action inhibitrice de *Tabernanthe iboga* sur la cholinesterase du serum. C. R. S. Soc. Biol. 136:612–614; 1942.
46. Whitaker, P. M.; Seeman, P. Hallucinogenic binding to dopamine/neuroleptic receptors. J. Pharm. Pharmacol. 29:506–507; 1977.
47. Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469–492; 1987.
48. Yamasaki, M.; Leete, E. Biosynthesis of the iboga alkaloids: The incorporation of tryptophan-3C14 into ibogaine. Tetrahedron Lett. 23:1499–1501; 1964.
49. Zidichouski, J. A.; Easaw, J. C.; Jhamandas, J. H. Glutamate receptor subtypes mediate excitatory synaptic responses of rat lateral parabrachial neurons. Am. J. Physiol. 270:H1557–H1567; 1996.
50. Zidichouski, J. A.; Jhamandas, J. H. Electrophysiological characterization of excitatory amino acid responses in the rat lateral parabrachial neurons *in vitro*. Brain Res. 611:313–321; 1993.

View publication stats