Original Paper

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Comparison of the Hallucinogenic Indole Alkaloids Ibogaine and Harmaline for Potential Immunomodulatory Activity

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Key Words
Abuse of drugs
β-Carbolines
Cellular immunity
Hallucinogenic compounds
Harmaline
Ibogaine
Immunomodulation
Indole alkaloids
Natural immunity

Abstract
The immunomodulatory potential of the indole alkaloids ibogaine and harmaline was examined in a panel of in vitro immune function assays. These assays were chosen to assess T-cell regulatory and effector function, B-cell function, macrophage function, and natural killer-cell function. The in vitro exposure to either ibogaine or harmaline resulted in a dose-related suppression of all immune functions examined except macrophage function. This suppression was noted at various concentrations in different assays, but was generally only associated with high concentrations (10–100 μmol/l).

Ibogaine is an alkaloid compound isolated from the African shrub *Tabernanthe iboga*. At low doses, it produces central stimulatory effects; at higher doses, it produces hallucinations [1]. Although ibogaine enjoyed a brief career as a street drug in the 1960s, it was classified by the US Food and Drug Administration as a schedule-I drug in 1970 [2]. Ibogaine is of special note in that it is the subject of two US patents for the treatment of drug addiction [3]. It is also under investigation for treatment of abuse of both drugs and alcohol [4]. The structure of ibogaine hydrochloride, also known as Endabuse™, is illustrated in figure 1a.

Another indole alkaloid with hallucinogenic properties is harmaline. Harmaline is a member of the β-carbolines, a group of compounds with multiple activities on mammalian cells [5]. The β-carbolines are of particular interest in that, in addition to their presence in plant materials, they may be produced as metabolic byproducts. Although harmaline and its congeners produce physiological reac-

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Methods

Drugs

Ibogaine hydrochloride was
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harmaline hydrochloride was
Chemical Co. (St. Louis, Mo.)
solved in culture medium to yie
tions, and were warmed to 37°C
solved. These stock solutions
(0.4 μm), and log dilutions were
in culture medium.

Reagents

RPMI-1640 medium, Hank
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and Ultraculture medium wa
(Walkersville, Md.). Brewer th
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tions similar to those seen following ibogaine exposure, the two classes of compounds apparently function by different mechanisms [6]. The structure of harmaline hydrochloride is shown in figure 1b.

Whereas a considerable body of knowledge exists on the pharmacological properties of both ibogaine and harmaline, a careful review of the relevant literature reveals that neither drug has been examined for its potential effects on the immune system. Therefore, the purpose of the studies reported here was to examine the potential of in vitro exposure of the indole alkaloids ibogaine and harmaline to alter immune regulatory and effector functions. The results of these studies suggest that both indole alkaloid drugs exhibited immunomodulatory activity in a range of immune functional assays. This activity was most consistent at higher in vitro concentrations.

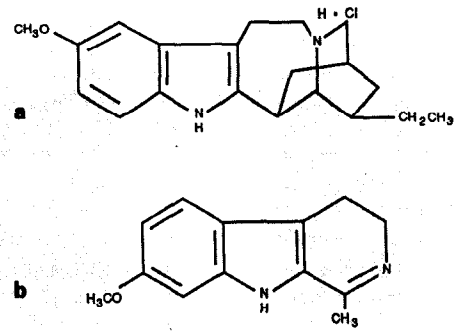

Fig. 1. Chemical structures for compounds examined in this study: ibogaine hydrochloride (**a**) and harmaline hydrochloride (**b**).

Methods

Drugs

Ibogaine hydrochloride was kindly supplied by the National Institute on Drug Abuse, Rockville, Md., and harmaline hydrochloride was obtained from Sigma Chemical Co. (St. Louis, Mo.). The drugs were dissolved in culture medium to yield 1-mmol/l stock solutions, and were warmed to 37°C until completely dissolved. These stock solutions were filter sterilized (0.4 μm), and log dilutions were subsequently prepared in culture medium.

Reagents

RPMI-1640 medium, Hanks' balanced salt solution, and Dulbecco's phosphate-buffered saline were all purchased from JRH Biosciences (Lenexa, Kans.), and Ultraculture medium was from Biowhittaker (Walkersville, Md.). Brewer thioglycolate was from Fisher Scientific (Chicago, Ill.), anti-murine IgM antibody was from Jackson ImmunoResearch (West Grove, Pa.), bacterial lipopolysaccharide was from List Biologicals (Campbell, Calif.), Eagle's minimal essential medium was from Gibco (Grand Island, N.Y.), and fetal bovine serum was from Hyclone (Logan, Utah). Mercaptoethanol, mitomycin C, actinomycin D, and phenazine methosulfate were all obtained from Sigma Chemical Co. (St. Louis, Mo.). Anti-murine CD3 monoclonal antibody was from Pharmingen (San Diego, Calif.), interleukin-2 (IL-2) and interleukin-4 (IL-4) were both from PeproTech (Rocky Hill, N.J.), and interleukin-6 (IL-6) was purchased from R&D Systems (Minneapolis, Minn.). ^{51}Cr was from ICN (Costa Mesa, Calif.), and 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) was obtained from Polysciences (Warrenton, Pa.).

Preparation of Murine Cells

Six- to eight-week-old female B6C3F1 mice obtained from the National Cancer Institute (Bethesda, Md.) served as a source of splenic lymphocytes and peritoneal macrophages. The animals were euthanized, the spleens were removed, and single-cell suspensions were prepared by rubbing the spleens through sterile nylon mesh (Spectrum Medical, Los Angeles, Calif.). Five individual splenocyte pools consisting of 3–5 animals/pool were utilized for each assay.

Preparation of T-Cell Cytokines

Isolated lymphocytes were adjusted to a concentration of 1×10^6 viable cells/ml in serum-free Ultraculture medium and added to 24-well culture plates (Costar, Cambridge, Mass.). Anti-murine CD3 antibody was added to the cell cultures at a final concentration of 500 ng/ml. The plates were incubated at 37°C for 48 h following addition of drugs. After incubation, supernatant fluids were harvested by centrifugation and stored at –70°C until assay.

Cytokine Bioassays

Measurement of IL-2. IL-2 production was quantitated by a modification of the method of Gillis et al. [7]. Lymphocyte culture supernatants were cultured for 24 h with log-phase CTLL-2 indicator cells. Four hours prior to harvest, a colorimetric indicator reagent consisting of 1 mg/ml XTT and 0.25 mmol/l phenazine methosulfate in Dulbecco's phosphate-buffered saline was added to all wells in a volume of 50 µl/well. The optical density of the solution in each well was measured with a microplate spectrophotometer at 450 nm. Samples were compared with a reference curve constructed with recombinant human IL-2.

Measurement of IL-4. IL-4 production was quantitated using a modification of the method described by Hu-Li et al. [8]. Lymphocyte culture supernatants were cultured for 48 h with log-phase CT.4S cells. Four hours prior to harvest, the XTT reagent was added and the optical density was measured as described above. Test samples were compared using a reference curve constructed with recombinant murine IL-4.

In vitro Induction of Cytotoxic T Lymphocytes (CTLs)

CTL function was evaluated by a scrum-free in vitro method described by House et al. [9]. Splenocytes were cultured for 5 days in the presence of various dilutions of drug in Ultraculture medium containing mitomycin C-inactivated P815 stimulators. After the induction phase the effector cells were harvested and co-cultured at various dilutions for 4 h with radiolabeled P815 target cells. The supernatant fluids were collected (Skatron, Sterling, Va.), and radiolabel release was quantitated in a gamma counter. Percent cytotoxicity was calculated by the formula: % cytotoxicity = [(ER – SR)/(TR – SR)] × 100, where ER represents experimental release, SR represents spontaneous release, and TR represents total releasable counts.

B-Lymphocyte Proliferation

Evaluation of B-lymphocyte function was performed using a method described by House et al. [9]. Lymphocytes suspended in RPMI-1640/10% fetal bovine serum were added to 96-well flat-bottom microculture plates at 1×10^5 cells/well. Recombinant murine IL-4 and anti-murine IgM antibody were added to each well at final concentrations of 400 pg/ml and 1 µg/ml, respectively. Dilutions of drugs were added to replicate wells, and control wells contained culture medium only. The plates were incubated at 37°C for 68 h. Four hours prior to assay termination, XTT was added to all wells and optical density was determined as for the cytokine bioassays.

Basal and Augmented Natural Killer (NK) Cell Activity

Basal and augmented NK cell activity was measured by a modification [9] of the method of Talmadge [10]. Splenocytes were cultured in duplicate in Eagle's minimal essential medium/10% fetal bovine serum for 24 h both with and without recombinant human IL-2 and various concentrations of drugs. The cells were then harvested and co-cultured at various dilutions with radiolabeled YAC-1 tumor target cells. The plates were incubated at 37°C for 4 h, harvested with a supernatant collection system, and released radiolabel was measured in a gamma counter. Percent specific cytotoxicity was calculated by the formula described for the CTL assay.

Preparation of Macrophage-Derived IL-6

Mice were injected intraperitoneally with Brewer thioglycolate medium to elicit peritoneal macrophages. Four days later, cells were harvested by peritoneal lavage with ice-cold Hanks' balanced salt solution, and macrophages were enriched by adherence to plastic for 2 h. Adherent monolayers were removed with a cell scraper and then washed once in Dulbecco's phosphate-buffered saline. Macrophages were adjusted to a concentration of 1×10^5 viable cells/ml in culture medium. Various dilutions of drugs were added in the presence of 10 µg/ml lipopolysaccharide to 1 ml macrophage cultures and incubated at 37°C for 48 h. Culture supernatants were harvested by centrifugation and stored at –70°C until assay.

Measurement of IL-6

Macrophage production of IL-6 was assessed by a modification of the method of Van Snick et al. [11]. Macrophage culture supernatants were cultured for 72 h with log-phase 7TD1 cells. Four hours prior to harvest, the XTT reagent was added and the optical density was measured as described above. Test samples were compared with a reference curve constructed with recombinant murine IL-6.

Results

T-Cell Regulatory Function

T-cell regulatory function was examined by quantitating production of cytokines, which are regulatory molecules produced by CD4 T-helper cells. Both T-helper-1 lymphocyte and T-helper-2 lymphocyte function

Table 1. Production of IL- exposed in vitro to hallucinog

Drug µmol/l	Mean abso ibogaine[b]
Control	0.359 ± 0.0
0.0001	0.337 ± 0.0
0.001	0.364 ± 0.0
0.01	0.369 ± 0.0
0.1	0.343 ± 0.0
1.0	0.352 ± 0.0
10	0.279 ± 0.0
100	0.092 ± 0.0

[a] Values expressed as mean 450 nm (n = 5 pools of 4 mice

[b] Half-maximal proliferati mIL-2 reference curve in th noted at 0.5 ng/ml (absorbanc

[c] Half-maximal proliferati mIL-2 reference curve in the noted at 1.0 ng/ml (absorbanc

* Significantly different fr $p \leq 0.05$.

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Table 1. Production of IL-2 by murine splenocytes exposed in vitro to hallucinogenic indole alkaloids

Drug µmol/l	Mean absorbance[a]	
	ibogaine[b]	harmaline[c]
Control	0.359±0.018	0.243±0.033
0.0001	0.337±0.015	0.255±0.008
0.001	0.364±0.025	0.265±0.010
0.01	0.369±0.018	0.242±0.023
0.1	0.343±0.017	0.276±0.008
1.0	0.352±0.024	0.256±0.010
10	0.279±0.022*	0.185±0.006*
100	0.092±0.006*	0.045±0.001*

[a] Values expressed as mean (± SEM) absorbance at 450 nm (n = 5 pools of 4 mice/pool).
[b] Half-maximal proliferation of the recombinant mIL-2 reference curve in the ibogaine bioassay was noted at 0.5 ng/ml (absorbance value of 0.645).
[c] Half-maximal proliferation of the recombinant mIL-2 reference curve in the harmaline bioassay was noted at 1.0 ng/ml (absorbance value of 0.287).
* Significantly different from respective control at $p \leq 0.05$.

Table 2. Production of IL-4 by murine splenocytes exposed in vitro to hallucinogenic indole alkaloids

Drug µmol/l	Mean absorbance[a]	
	ibogaine[b]	harmaline[c]
Control	0.154±0.005	0.186±0.016
0.0001	0.172±0.010	0.169±0.008
0.001	0.176±0.004*	0.160±0.020
0.01	0.164±0.003	0.167±0.020
0.1	0.152±0.005	0.190±0.010
1.0	0.156±0.010	0.182±0.022
10	0.152±0.007	0.170±0.006
100	0.049±0.003*	0.089±0.002*

[a] Values expressed as mean (± SEM) absorbance at 450 nm (n = 5 pools of 4 mice/pool).
[b] Half-maximal proliferation of the recombinant mIL-4 reference curve in the ibogaine bioassay was noted at 100 pg/ml (absorbance value of 0.194).
[c] Half-maximal proliferation of the recombinant mIL-4 reference curve in the harmaline bioassay was noted at 1.0 ng/ml (absorbance value of 0.228).
* Significantly different from respective control at $p \leq 0.05$.

were assessed by evaluating production of IL-2 and IL-4, respectively, which are cytokines characteristic of those populations. Exposure to either ibogaine or harmaline in vitro resulted in suppression of IL-2 at 10–100 µmol/l (table 1). IL-4 production was slightly less sensitive to the suppressive effects of each of these drugs; both ibogaine and harmaline significantly suppressed IL-4 production only at 100 µmol/l (table 2).

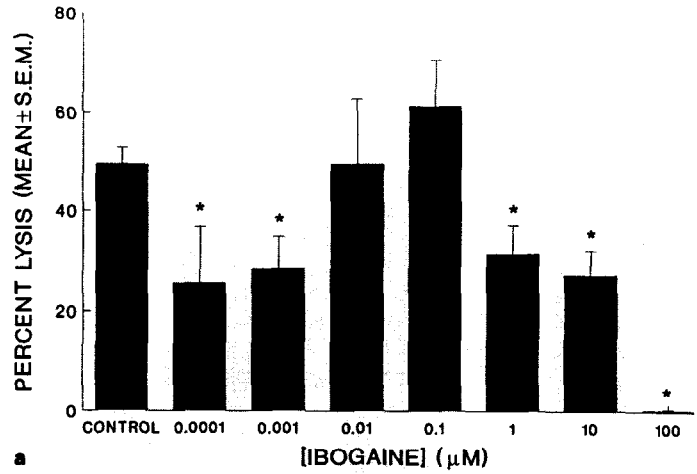
T-Cell Effector Function

T-lymphocyte effector function was assessed by in vitro induction of CTLs, which require a sensitization with antigen prior to acquisition of cytolytic function. In vitro exposure to ibogaine resulted in a significant suppression of CTL activity at concentrations between 0.0001 and 0.001 µmol/l (fig. 2a).

Table 3. Proliferation of murine B lymphocytes exposed in vitro to hallucinogenic indole alkaloids

Drug µmol/l	Mean absorbance[a]	
	ibogaine	harmaline
Control	0.356±0.030	0.313±0.013
0.0001	0.379±0.034	0.322±0.018
0.001	0.377±0.027	0.284±0.023
0.01	0.394±0.029	0.257±0.017*
0.1	0.375±0.025	0.251±0.017*
1.0	0.379±0.028	0.251±0.015*
10	0.369±0.030	0.256±0.018*
100	0.194±0.033*	0.090±0.004*

[a] Values expressed as mean (± SEM) absorbance at 450 nm (n = 5 mice per group).
* Significantly different from respective control at $p \leq 0.05$.



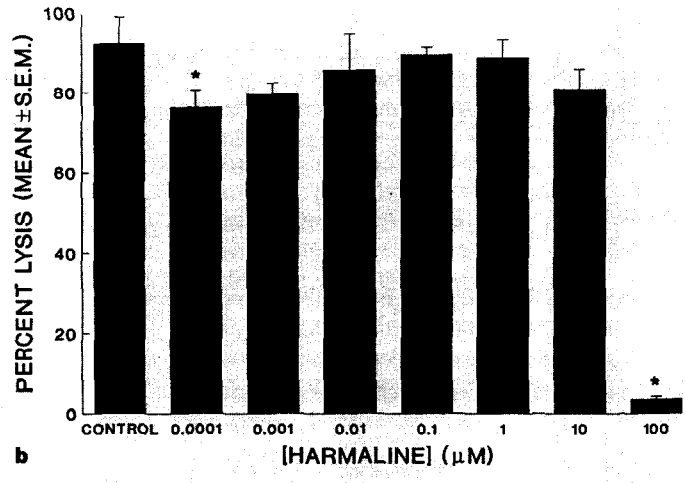


Fig. 2. Cytotoxic T-lymphocyte induction in murine splenocytes exposed in vitro to ibogaine (**a**) or harmaline (**b**). Bars represent mean (± SEM) percent cytotoxicity against radiolabeled P815 mastocytoma cells in a 4-hour co-culture assay (n = 5 pools of 4 mice/pool). Asterisks indicate significant difference from control at $p \leq 0.05$.

Fig. 3. IL-2-augmented NK-cel activity in murine splenocytes ex posed in vitro for 24 h to ibogain (**a**) or harmaline (**b**). Bars represen mean (± SEM) percent cytotoxici ty against radiolabeled YAC-1 tu mor cells in a 4-hour co-culture as say at an effector:target ratio o 11:1 (n = 5 pools of 4 mice/pool) Asterisks indicate significant dif ference from control at $p \leq 0.05$.

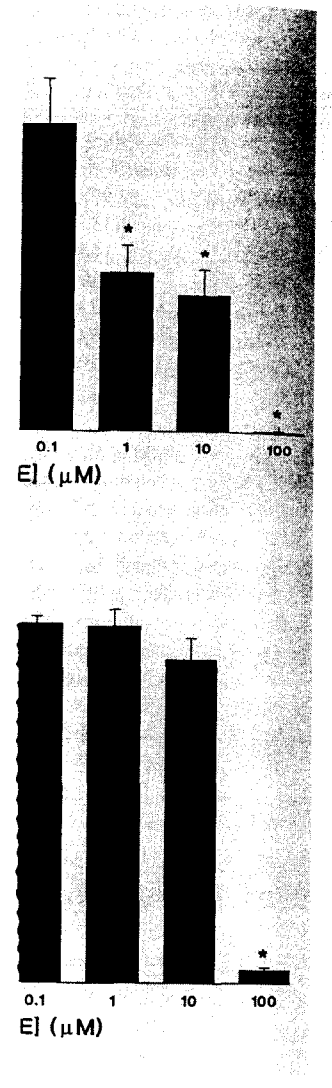
This response returned to control levels at ibogaine concentrations between 0.01 and 0.1 μmol/l, and was subsequently followed by a statistically significant suppression in CTL activity at concentrations of 1 μmol/l and greater (fig. 2a). In vitro exposure to harmaline significantly suppressed CTL activity only at concentrations of 0.0001 and 100 μmol/l (fig. 2b).

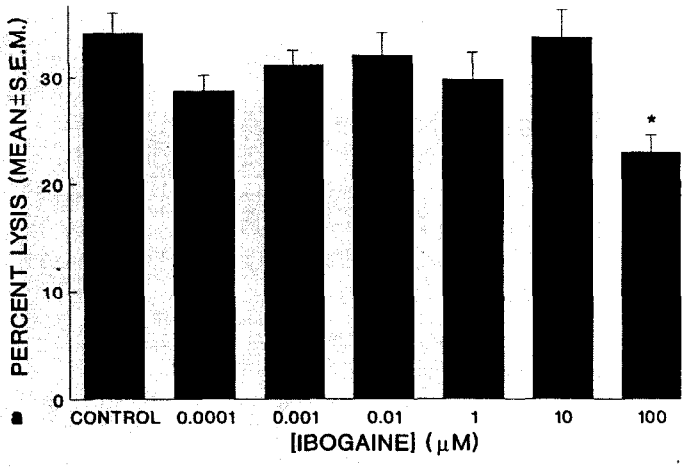
B-Cell Function

B-cell function was measured by proliferation in response to stimulation with anti-IgM/IL-4. Ibogaine was found to display the least immunomodulatory activity, with a significant suppression of B-cell proliferation only at a concentration of 100 μmol/l (table 3). Harmaline exhibited greater activity against B cells, with significant suppression of B-cell proliferation at concentratic and 100 μmol/l (table 3).

NK-Cell Function

In vitro exposure to iboga suppress augmented NK-cel a concentration of 100 μmo lar to CTL-mediated lysis, ited a biphasic activity, wit





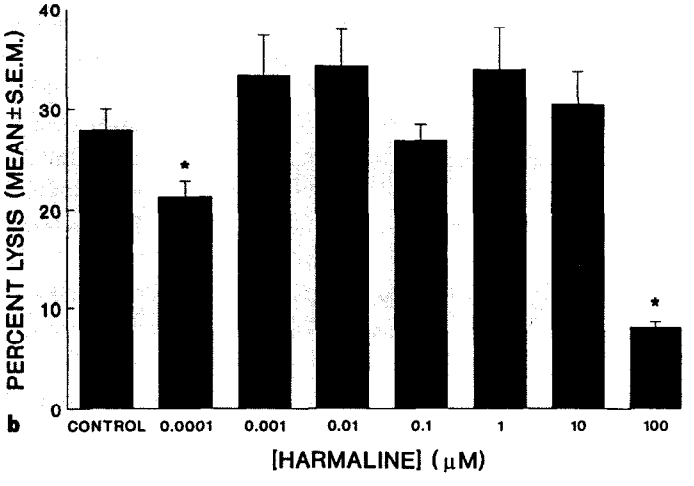


Fig. 3. IL-2-augmented NK-cell activity in murine splenocytes exposed in vitro for 24 h to ibogaine (**a**) or harmaline (**b**). Bars represent mean (± SEM) percent cytotoxicity against radiolabeled YAC-1 tumor cells in a 4-hour co-culture assay at an effector:target ratio of 11:1 (n = 5 pools of 4 mice/pool). Asterisks indicate significant difference from control at $p \leq 0.05$.

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NK-Cell Function

In vitro exposure to ibogaine was found to suppress augmented NK-cell function only at a concentration of 100 μmol/l (fig. 3a). Similar to CTL-mediated lysis, harmaline exhibited a biphasic activity, with significant suppression of NK-cell activity observed at both 0.0001 and 100 μmol/l (fig. 3b).

Production of IL-6

Macrophage function was assessed by production of the regulatory cytokine IL-6. In vitro exposure to ibogaine had no significant effect on IL-6 production at any concentration examined (fig. 4). In vitro exposure of

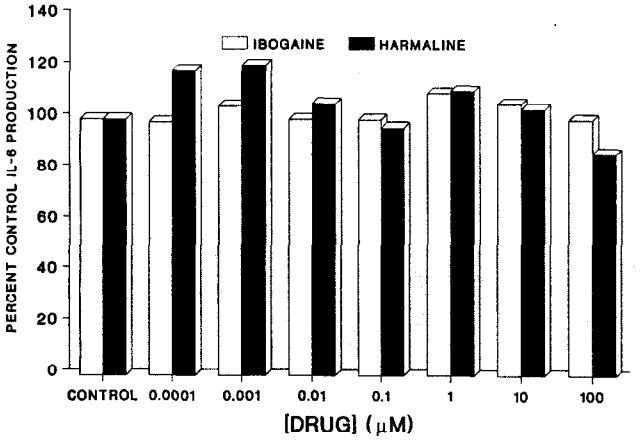

Fig. 4. Production of IL-6 (expressed as percent control) by thioglycolate-elicited peritoneal macrophages exposed in vitro to ibogaine or harmaline (n = 5 mice/pool).

macrophages to harmaline was found to result in a modest, nonsignificant increase in IL-6 production at concentrations of 0.0001 and 0.001 μmol/l only (fig. 4).

Discussion

Ibogaine, an indole alkaloid derived from the root bark of an African shrub, is the subject of two US patents for the treatment of drug addiction. It is also under consideration as a treatment for abuse of a variety of other substances including tobacco and alcohol [4]. Any drug considered for use in this wide range of conditions must not display any adverse effects on the immune system of patients receiving therapy. A review of the pertinent literature for the past 20 years revealed no investigations in this area; therefore, the purpose of the studies described here was to assess the potential for in vitro exposure to ibogaine to alter a panel of immune effector and regulatory parameters.

A second compound examined in this study was harmaline, which, like ibogaine, is a plant-derived indole alkaloid with tremorigenic and hallucinogenic effects [12]. Although structurally similar, ibogaine and harmaline appear to exert their psychoactive effects via different mechanisms [6]. Comparing the potential immunological effects of harmaline with those of ibogaine was therefore of interest.

Modulation of the immune response may be manifested in one of two principal ways. The first is immunosuppression, in which one or more immune functional parameters is suppressed. Such suppression may then result in an enhanced host susceptibility to infectious or neoplastic disease. The second form of immunomodulation is immunostimulation (immunoenhancement), in which one or more immune parameters are significantly elevated above normal background levels. Unless resolved, this situation may ultimately result in such conditions as allergy or autoimmune disease [13].

One of the principal components of the immune system in vertebrates is the lymphocyte. Lymphocytes represent a diverse group of cells, with the major classification being

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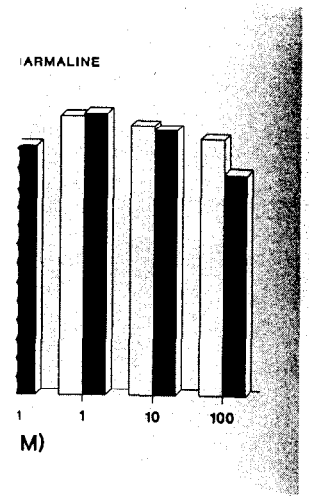

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division into B lymphocytes (derived from bone marrow or bursa) and T lymphocytes (derived from the thymus). The function of both B and T lymphocytes was examined in this study. The first parameter to be examined was T-lymphocyte function as assessed by differential cytokine production. Recent studies have suggested the existence of CD4 (helper) T-cell subpopulations based upon their pattern of cytokine secretion following activation [14]. Two of these subpopulations, T-helper-1 and T-helper-2 lymphocytes, are differentiated by their exclusive production of IL-2 and IL-4, respectively. The present study demonstrated that in vitro exposure to either ibogaine or harmaline resulted in suppression of IL-2 production at concentrations of 10–100 μmol/l. IL-4 production was not as sensitive to suppression, with significant differences noted only at 100 μmol/l for each drug. This finding suggests that the immunosuppressive effects exhibited by each of these compounds was not selective for T-cell subsets, but rather appeared to work via a broader mechanism.

Lymphocyte function was also assessed by means of B-cell proliferation. The primary function of B cells is the production of specific antibodies; in addition, they also participate in antigen presentation as well as the production of cytokines. An early step in these processes is the proliferation of stimulated B cells. In the present study, B-cell proliferation was suppressed by ibogaine only at very high concentrations (100 μmol/l). In contrast, in vitro exposure to harmaline significantly suppressed B-cell proliferation at concentrations as low as 0.01 μmol/l. The exact nature of this differential sensitivity is unknown.

Another vital function of T lymphocytes is represented by the CTLs, which are a population of CD8 lymphocytes capable of directly destroying target cells after a prior exposure [15, 16]. Induction of CTL activity requires the interaction of various cell types, and is therefore an excellent target for evaluating potential immunomodulation. In the present study, in vitro exposure to either of the alkaloid drugs tested resulted in a significant suppression of CTL induction. Of particular interest was the observation of a biphasic response in all cases; for example, the least suppression of CTL induction was noted at 0.01 and 0.1 μmol/l, with a greater degree of suppression noted at both lower and higher drug concentrations. Although both ibogaine and harmaline strongly suppressed CTL induction at 100 μmol/l, this suppression in CTL activity was not due to any decrease in cellular viability or recovery of cells following the 5-day induction culture. Rather, this appears to represent a functional alteration in the induction process by each of the drugs. The extremely high concentration required for this degree of suppression suggests a form of nonspecific toxicity rather than a specific immunomodulatory activity for either of these drugs.

Another immune-related parameter evaluated in this study was basal and cytokine-augmented NK-cell activity. NK cells are a population of lymphocytes unrelated to either T or B cells, and are able to nonspecifically destroy certain target cells without prior exposure. As such, they represent an important form of host resistance [17]. The present study demonstrated that exposure to either of the alkaloid drugs had minimal effect on basal NK-cell function. To examine the effect of drugs on NK-cell function in more detail, we also examined the effect on augmented NK-cell function. Certain cytokines, including IL-2 and interferon-γ, are capable of enhancing the cytotoxic potential of NK cells [10]. To evaluate the possible effects on this function, cells were cultured for 24 h in the presence or absence of recombinant IL-2, followed by assessment of antitumor cytotoxicity. In this experiment, in vitro exposure to ibogaine sup-

pressed NK-cell activity only at a concentration of 100 μmol/l, suggesting that this drug was not strongly interacting with the cells. Interestingly, in vitro exposure to harmaline suppressed NK-cell activity at both 0.0001 and 100 μmol/l, similar to harmaline's effect on CTL-mediated cytolysis. The mechanism for this biphasic activity is unknown.

Finally, the effect of alkaloid exposure on macrophage function was assessed by quantitating the production of the cytokine IL-6, which is a pivotal cytokine with a multitude of effector and regulatory functions [18]; as such, it represents an appropriate target for determination of potential alterations in macrophage function. The present study showed that IL-6 production by thioglycolate-elicited peritoneal macrophages was not affected in a consistent manner by in vitro exposure to the indole alkaloids. Although exposure to harmaline exhibited some slight stimulatory activity at low concentrations, the biological significance of this stimulation is doubtful.

The slight differences in specificity of the two compounds (e.g., biphasic dose response, differential sensitivity) on B lymphocytes may possibly be related to the demonstration by Deecher et al. [6] that ibogaine interacts with the κ-opiate receptor whereas harmaline does not. Ongoing studies in our laboratory (data not shown) have demonstrated differential effects on cellular immune function exerted by compounds interacting with the various opiate receptors. The role of these receptors in ibogaine- and harmaline-mediated immune modulation remains to be characterized.

In summary, in vitro exposure to the hallucinogenic indole alkaloids ibogaine and harmaline resulted in suppression of a variety of immune effector and regulatory mechanisms. However, this suppression was generally observed only at extremely high in vitro concentrations (i.e., 10–100 μmol/l), albeit cellular viability was unaffected by either of these drugs at any concentration examined. Therefore, although these drugs may provide interesting tools to examine immunomodulatory mechanisms, **it is doubtful whether they would exert significant immunotoxicity at pharmacological concentrations.**

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