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Selective modulation of immune function resulting from in vitro exposure to methylenedioxymethamphetamine (Ecstasy)

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Abstract

Abuse of illicit analogs of methamphetamine (i.e., 'designer drugs') represents a growing problem. One of the most popular methamphetamine analogs is (±)-3,4-methylenedioxymethamphetamine (MDMA), commonly known as Ecstasy. The authors demonstrated previously that in vitro exposure to methamphetamine results in modulation of immune functional parameters necessary for host defense. The current study was performed to assess the potential direct (in vitro) immunomodulatory effect of exposure to a modified methamphetamine. Splenocytes or peritoneal macrophages from B6C3F1 mice were cultured in vitro at MDMA concentrations of 0.0001–100 μM. T-cell regulatory function was assessed by anti-CD3-mediated production of IL-2 and IL-4, B-cell function was assessed by quantitating cellular proliferation, natural immunity was assessed by quantitating natural killer (NK) cell activity, T-cell effector function was evaluated as a function of cytotoxic T-lymphocyte (CTL) activity, and macrophage function was assessed by IL-6 tumor necrosis factor (TNF) production. In vitro exposure to MDMA had no effect on B-cell proliferation at any concentration tested. In comparison, in the absence of direct cellular toxicity, production of IL-2 was enhanced at concentrations as low as 0.0001 μM. IL-4 production was not affected by exposure to any concentration of MDMA examined, suggesting a differential alteration in T-helper cell function by this compound. Basal and augmented NK cell function were enhanced at MDMA concentrations between 0.0001 and 1.0 μM when examined at an effector:target ratio of 100:1. CTL induction was significantly suppressed at a concentration of 100 μM. Finally, macrophage production of TNF was slightly suppressed at 10 and 100 μM MDMA, although this inhibition was not statistically significant.

Keywords: Methylenedioxymethamphetamine; Designer drugs; Immunosuppression; Methamphetamine; Cell-mediated immunity

1. Introduction

The term 'designer drug' generally denotes an illicit derivative of scheduled compounds; the novelty of these derivatives originally allowed them to escape regulation on their manufacture and use (Jerrard, 1990). Designer drugs are generally grouped into four major categories: synthetic opioids derived from fentanyl and meperidine; phencyclidine analogs (arylhexylamines); metha-

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qualone analogs; and the mescaline analogs (phenylethylamines) exemplified by methamphetamine and its analogs, including such hallucinogenic amphetamines as methylenedioxyamphetamine (MDA) (Buchanan and Brown, 1988; Ford et al., 1990). The chemical structure of methamphetamine is illustrated in Fig. 1a.

Currently, one of the most popular designer drugs is (±)-3,4-methylenedioxymethamphetamine (MDMA), commonly known as Ecstasy or Adam (Fig. 1b). MDMA is the methylated derivative of the amphetamine analog MDA, a prototype of the hallucinogenic amphetamine drugs (Bost, 1988). MDMA is relatively easy to synthesize in clandestine drug laboratories, although most of the precursor chemicals for its synthesis are now federally regulated. However, recent underground publications provide interested parties with complete directions for MDMA synthesis utilizing readily available over-the-counter ephedrine (Uncle Fester, 1994). Therefore, MDMA will probably remain a potential drug of abuse for the foreseeable future.

MDMA was first synthesized in 1914 (McKenna and Peroutka, 1990), but was only utilized therapeutically beginning in the 1980s. Although MDMA has been in use (both legally and illicitly) for a number of years, only limited information has been published on its potential health effects. Drug abuse has been associated with a number of medical complications, often depending upon a wide range of variables (Chiang and Goldfrank, 1990). One possible complication of drug abuse is suppression of the immune system, or immunotoxicity (Pillai and Watson, 1990). The immune system is a highly regulated organ system and, as such, presents a variety of potential targets for modulation. This modulation may take the form of immunosuppression, possibly leading to an enhanced susceptibility to infection; conversely, it may take the form of immunostimulation, possibly resulting in hypersensitivity (allergy) or autoimmune disease (Gleichmann et al., 1989).

In a previous study (House et al., 1994) we demonstrated that in vitro exposure to methamphetamine, the parent compound of MDMA, resulted in modulation of several key immune function parameters. The purpose of the study reported here was to examine the potential of the methamphetamine derivative MDMA to affect the function of these immune effector and regulatory mechanisms following in vitro exposure. This work demonstrates that in vitro exposure to MDMA is associated with a pattern of immunomodulation similar in most respects to that observed with methamphetamine. The implications of these findings are discussed in the context of human exposure to these drugs.

2. Materials and methods

2.1. Drug

The (±)-3,4-methylenedioxymethamphetamine HCl (MDMA) was furnished by the National Institute on Drug Abuse, Rockville, MD. The drug was dissolved in culture medium to yield a stock solution of 1 mM, and was warmed to 37°C with mixing until completely dissolved. This stock solution was filter-sterilized, and log dilutions were subsequently prepared in culture medium.

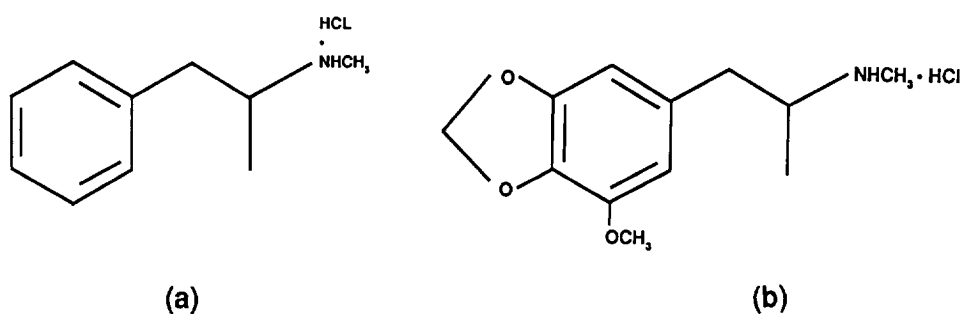

Fig. 1. Chemical structures of (a) methamphetamine HCl and (b) 3,4-methylenedioxymethamphetamine HCl.

2.2. Reagents

RPMI-1640 medium, Hank's balanced salt solution (HBSS), and Dulbecco's phosphate-buffered saline (PBS), and Ultraculture were from Biowhittaker (Walkersville, MD). Brewer thioglycolate was from Fisher Scientific (Chicago, IL), anti-murine IgM antibody was from Jackson Immuno-Research (West Grove, PA), bacterial lipopolysaccharide (LPS) was from List Biologicals (Campbell, CA), Eagle's minimal essential medium (E-MEM) was from Gibco (Grand Island, NY), and fetal bovine serum (FBS) was from Hyclone (Logan, UT). Mercaptoethanol, mitomycin C, actinomycin D, and phenazine methosulfate (PMS) were all obtained from Sigma Chemical Co. (St. Louis, MO). Anti-murine CD3 monoclonal antibody was from Pharmingen (San Diego, CA), recombinant human and murine interleukin-2 (IL-2) and rmIL-4 were both from PeproTech (Rocky Hill, NJ), rmIL-6 was obtained from R&D Systems (Minneapolis, MN), and rmTNF-α was purchased from Genzyme (Boston, MA). ^{51}Cr was from ICN (Costa Mesa, CA), and 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)-carbonyl]-2*H* -tetrazolium hydroxide (XTT) was obtained from Diagnostic Chemicals (Oxford, CT).

2.3. Preparation of murine cells

Six- to eight-week-old female B6C3F1 mice obtained from the National Cancer Institute (Frederick, MD) served as a source of cells for in vitro studies. The animals were euthanized, the spleens removed, and single-cell suspensions were prepared by rubbing the spleens through sterile nylon mesh (Spectrum Medical, Los Angeles, CA). Five individual pools consisting of 3–5 animals/pool were utilized for each assay employing spleen cells, and five individual animals served as a source of macrophages. Cells were washed once by centrifugation, and the cell pellets were resuspended in fresh medium. Cell concentrations were determined by Coulter counting, and cell viabilities were assessed by vital dye exclusion.

2.4. B-lymphocyte proliferation

Evaluation of B-lymphocyte function was performed using a method modified from Abbas et al. (1990). Lymphocytes suspended in RPMI-1640/10% FBS were cultured in 96-well flat-bottom microculture plates at 1×10^5 cells/well. Recombinant mIL-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 MDMA 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 harvest a colorimetric indicator reagent consisting of 1 mg/ml XTT and 0.25 mM PMS in PBS 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.

2.5. Preparation of T-cell cytokines

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

2.6. Preparation of macrophage-derived cytokines

Mice were injected i.p. with thioglycolate to elicit peritoneal macrophages. Three days later, cells were harvested by peritoneal lavage with cold HBSS, and macrophages were enriched by adherence to plastic for 2 h. Adherent cells were removed with a cell scraper and washed once in PBS. Macrophages were adjusted to a concentration of 1×10^5 viable cells/ml in culture medium. Various dilutions of MDMA were added in the presence of 10 μg/ml LPS 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.

2.7. Cytokine bioassays

Measurement of IL-2. IL-2 production was quantitated by the method of Gillis et al. (1978). Lymphocyte culture supernatants were incubated for 24 h with log-phase CTLL-2 indicator cells. Four hours prior to assay termination, XTT/PMS

was added to all wells and optical density was determined at 450 nm. The IL-2 concentrations in the test samples were extrapolated from a reference curve constructed with rmIL-2.

Measurement of IL-4. IL-4 production was quantitated using a modification of the method of Hu-Li et al. (1989). Lymphocyte culture supernatants were incubated for 48 h with log-phase CT.4S cells. Four hours prior to harvest, XTT/PMS reagent was added and the optical density was measured. The IL-4 concentrations in the test samples were extrapolated from a reference curve constructed with rmIL-4.

Measurement of IL-6. Macrophage production of IL-6 was assessed by a modification of the method of Van Snick et al. (1986). Macrophage culture supernatants were cultured for 72 h with log-phase 7TD1 cells. XTT/PMS reagent was added, and 4 h later optical density was measured. Test samples were compared with a reference curve constructed with rmIL-6.

Measurement of tumor necrosis factor (TNF). Macrophage production of TNF was assessed by a modification of methods described by Meager et al. (1989). Macrophage culture supernatants were cultured for 20 h with L929 cell monolayers in the presence of actinomycin-D. XTT/PMS reagent was added, and 4 h later optical density was measured. Test samples were compared with a reference curve constructed with rmTNF-α.

2.8. In vitro induction of cytotoxic T-lymphocytes (CTL)

CTL function was evaluated as described previously (Thomas et al., 1993). Splenocytes were cultured for 5 days in the presence of various dilutions of MDMA in Ultraculture containing mitomycin C-inactivated P815 stimulators. Following the induction phase, the effector cells were harvested, resuspended to 5×10^6 viable cells/ml, and two serial dilutions of the cell suspension were prepared. The dilutions were added to 96-well, round-bottom microculture plates, and radiolabeled P815 cells were added to all wells. The plates were incubated at 37°C for 4 h, and the supernatant fluids were collected with a supernatant harvesting system (Skatron, Sterling, VA). Release of chromium by the target cells was quantitated, and percent cytotoxicity was calculated as the ratio of experimental radiolabel release to total releasable counts, corrected for spontaneous release.

2.9. Basal and augmented NK cell activity

Basal and augmented NK cell activity were measured by a modification (Thomas et al., 1993) of the method of Talmadge (1985). Splenocytes were cultured in duplicate for 24 h with and without rhIL-2 and various concentrations of MDMA. The effector cells were harvested and co-cultured with radiolabeled YAC-1 tumor target cells for 4 h, and the supernatant fluids were harvested. Released radiolabel was measured in a gamma counter, and percent cytotoxicity was calculated by the formula used for the CTL assay.

2.10. Statistical analysis

Data were analyzed for statistical significance by Dunnett's multicomparison test and analysis of variance (ANOVA) using an RS1 statistical package (BBN Software Products Corp., Cambridge, MA). Data were considered to be significantly different from respective controls at $P \leq 0.05$.

3. Results

3.1. B-cell function

B-cell function was measured by proliferation in response to stimulation with the antigen analog anti-IgM/IL-4. In vitro exposure to MDMA had no observable effect on proliferation of B-cells at any concentration examined in this study (data not shown).

3.2. 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 TH1 and TH2 function were assessed by evaluating production of IL-2 and IL-4, respectively, which are cytokines characteristic of those populations. In the current study, exposure to MDMA resulted in a biphasic response in IL-2 production by T-cells. IL-2 production at MDMA concentrations between 10 and 0.001 μM was nor-

Table 1
Production of cytokines by murine T-lymphocytes exposed in vitro to methylenedioxymethamphetamine

Exposure concentration of MDMA (μM)	Cytokine production[a] (ng/ml)	
	IL-2	IL-4
Control	0.127 ± 0.010	0.046 ± 0.002
0.0001	0.214 ± 0.023[b]	0.050 ± 0.004
0.001	0.137 ± 0.011	0.046 ± 0.006
0.01	0.108 ± 0.008	0.043 ± 0.002
0.1	0.118 ± 0.007	0.047 ± 0.005
1.0	0.134 ± 0.014	0.046 ± 0.003
10.0	0.104 ± 0.005	0.044 ± 0.003
100.0	0.074 ± 0.002[b]	0.040 ± 0.002

[a]Values expressed as mean (± S.E.M.) cytokine concentration as extrapolated from a recombinant reference curve.
[b]Significantly different from respective control at $P \leq 0.05$.

mal as compared with control values. However, in vitro exposure to 0.0001 μM was associated with a statistically significant 169% enhancement in IL-2 production (Table 1). Conversely, cells exposed to MDMA at 100 μM (the highest concentration examined) exhibited a profound, statistically significant 42% suppression in IL-2 production (Table 1). This suppression was not associated with any appreciable alteration in cellular viability at this concentration (data not shown). Exposure to T-cells to MDMA had no demonstrable effect on production of IL-4 (Table 1).

3.3. T-cell effector function

T-lymphocyte effector function was assessed by in vitro induction of cytotoxic T-lymphocytes (CTL), which require a sensitization with antigen prior to acquisition of cytolytic function. In vitro exposure to MDMA significantly suppressed in vitro induction of CTL only at 100 μM (Fig. 2). This suppression was not associated with any alteration in viability of the effector cell population (data not shown).

3.4. NK cell function

Natural resistance was evaluated by means of the NK cell assay; this assay was enhanced by also evaluating the ability of cytokines (e.g., IL-2) to augment the basal activity of these cells. In vitro exposure to MDMA resulted in an enhancement in basal NK cell activity at concentrations between 0.0001 and 1.0 μM when evaluated at an effector:target cell ratio of 100:1 (Fig. 3a); this enhancement-exhibited a bell-shaped dose-response curve, with significant enhancement noted at 0.1 μM, and maximal enhancement noted

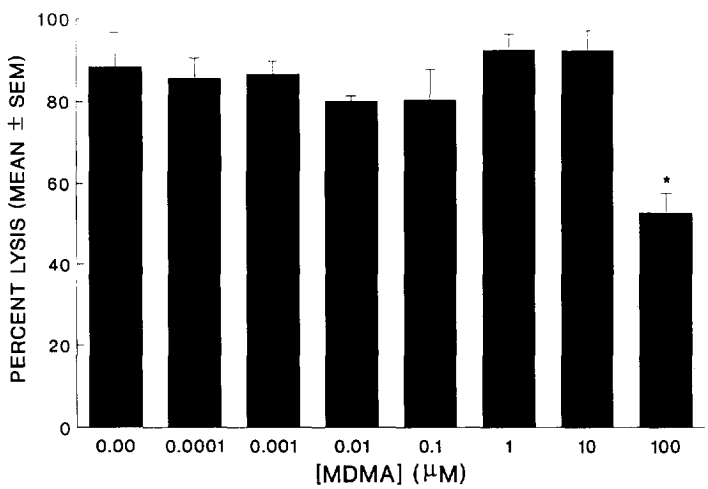

Fig. 2. Induction of CTL in murine splenic lymphocytes exposed in vitro to MDMA. Values represent mean (± S.E.M.) percent lysis of radiolabeled P815 target cells in a 4-h chromium release at an effector:target ratio of 25:1. Asterisk indicates significant difference from respective control value at $P \leq 0.05$. $N = 5$ pools of 4 mice/pool.

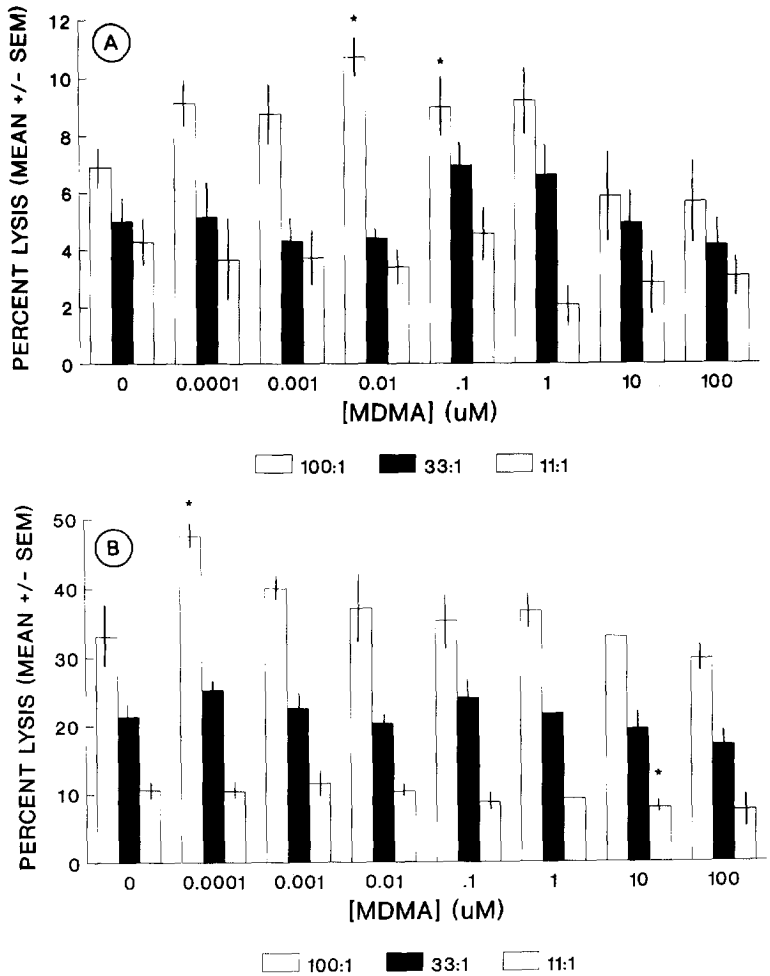

Fig. 3. NK cell activity in murine splenic lymphocytes exposed in vitro to MDMA. Values represent mean lysis of radiolabeled YAC-1 target cells in a 4-h chromium release assay at various effector:target cell ratios. (a) Basal NK cell activity; (b) IL-2 augmented NK cell activity. Asterisks indicate significant difference from respective control value at $P \leq 0.05$. $N = 5$ cools of 4 mice/pool.

at 0.01 μM (Fig. 3a). In comparison, NK cells stimulated by exogenous hIL-2 did not exhibit either the same pattern or degree of enhanced activity as noted in the basal NK cultures. Augmented NK cell activity was significantly increased as compared to controls only at 0.0001 μM MDMA at an effector:target cell ratio of 100:1, with activity rapidly returning to control levels at higher concentrations (Fig. 3b). This pattern of enhanced activity was not apparent at lower effector:target ratios for either the basal or augmented NK cell cultures.

3.5. Production of macrophage-derived cytokines

Macrophage function was assessed by production of the regulatory cytokines IL-6 and TNF. Exposure of macrophages to MDMA had no significant effect on LPS-induced production of IL-6 at any concentration examined in this study (Fig. 4). Murine macrophages exposed in vitro to MDMA exhibited a generalized trend toward lower TNF production with increased MDMA concentration (Fig. 5), although this decrease was not statistically significant at any concentration examined.

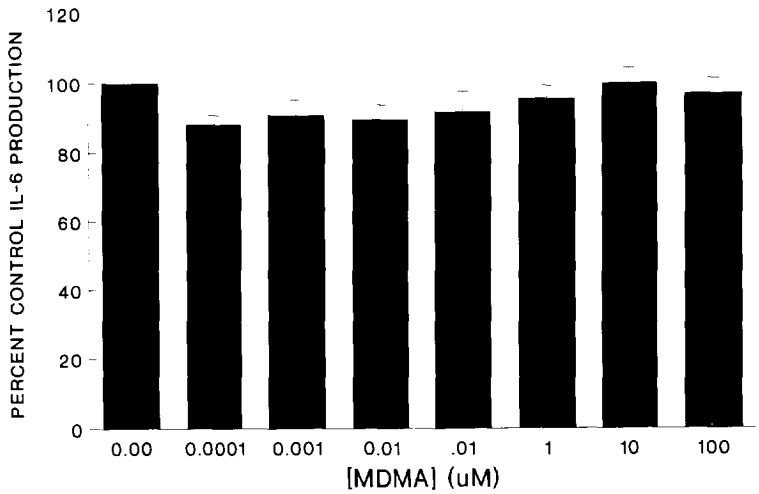

Fig. 4. Production of IL-6 by thioglycolate-elicited, LPS-stimulated peritoneal murine macrophages following in vitro exposure to MDMA. Bars represent percent control IL-6 production by macrophages. $N = 5$ individual mice.

4. Discussion

MDMA (Ecstasy) is a ring-substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. Positive subjective effects of the drug include euphoria, increased self-esteem and empathy, whereas negative effects include jaw-clenching, teeth-grinding, and hyperactivity. MDMA use may also have more serious side effects including cardiac dysrhythmias, hyperthermia, seizures, and intracranial hemorrhage (Sternbach and Varon, 1992). One of the most serious effects of MDMA exposure is neurotoxicity, both acute and long-term (McKenna and Peroutka, 1990). This neurotoxicity is apparently related to MDMA's ability to damage serotonin neurons; this neurotoxicity has been associated with long-term neuropsychological disturbances

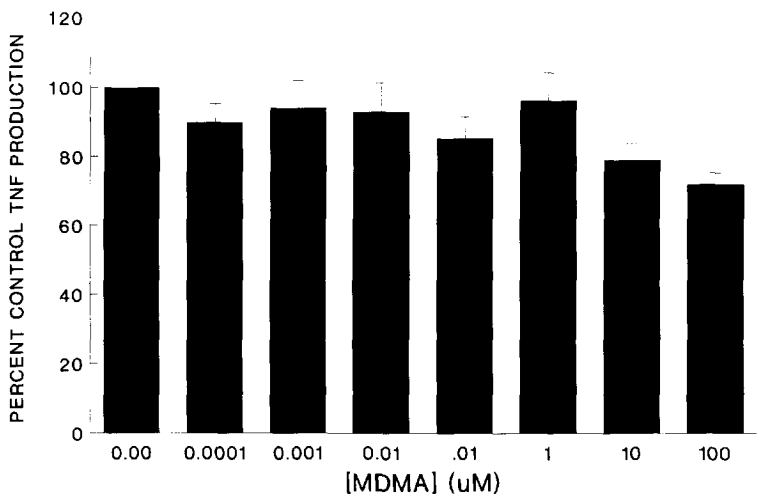

Fig. 5. Production of TNF by thioglycolate-elicited, LPS-stimulated peritoneal murine macrophages following in vitro exposure to MDMA. Bars represent percent control TNF production by macrophages. $N = 5$ individual mice.

including paranoid psychosis (Sternbach and Varon, 1992; McCann and Ricaurte, 1993). The possibility of neurotoxicity is, to be sure, a major consideration in evaluating the toxicity of an abused drug. To date, however, few studies have dealt with the effect of hallucinogenic amphetamines on other organ systems, especially the immune system. The purpose of the study reported here was to examine the potential of MDMA, a hallucinogenic amphetamine analog, to produce direct immunomodulation.

Earlier, we reported the results of a comparative study on the potential immunomodulatory effects of in vitro exposure to natural and synthetic amphetamines, including methamphetamine (House et al., 1994). That study found that in vitro exposure to amphetamines resulted in a differential modulation of the immune system depending on the compound and the parameter examined. In the context of this discussion, it is important to note that the present study examined potential immunopathology resulting from in vitro exposure to a relatively pure sample of MDMA. However, due to the ease of preparing the drug, as well as the limited clinical trials that have been conducted (and the consequent lack of divertable pharmaceutical or research sources), most street MDMA is probably of clandestine manufacture. Such preparations are often contaminated with various by-products or adulterants (Buchanan and Brown, 1988; Soine, 1990); therefore, exposure to homemade MDMA would not necessarily produce the same effects noted in the present study. An additional point of concern is the close interaction of the nervous and immune systems (Pillai and Watson, 1990). Since MDMA (and arguably, most drugs of abuse) acts on the nervous system, it is conceivable that in vivo exposure to MDMA may alter the immune system indirectly; this possibility was not addressed in the present study.

The first parameter to be examined was lymphocyte function as assessed by B-cell proliferation. One of the principal functions of B-cells is the production of specific antibodies; in addition, they also participate in antigen presentation as well as cytokine production following cognate interactions with T-cells and antigen-presenting cells. An early step in both of these processes is the proliferation of stimulated B-cells, which was evaluated in the present study following stimulation with an antigen analog. B-cell proliferation was unaffected by any concentration of MDMA examined here. This finding is similar to that observed following in vitro exposure of murine splenocytes to methamphetamine (House et al., 1994).

The next parameter examined was T-cell regulatory function. Cytokine production was quantitated since this is an important physiological response following T-cell activation and proliferation. Cytokines are required for almost all immunoregulatory activities, and thus represent important potential targets for drug-related immunomodulation. The $CD4^+$ helper T-cell is primarily responsible for cytokine production, and is further categorized into two major subpopulations based upon the observed pattern of cytokine secretion following activation (Mosmann et al., 1991). These subpopulations, TH1 and Th2, are differentiated by their exclusive production of IL-2/interferon-gamma and IL-4/IL-10, respectively. The present study demonstrated that in vitro exposure to MDMA results in a biphasic effect on the production of IL-2, with pronounced stimulation of IL-2 production at very low MDMA concentrations, and suppression of IL-2 production at the highest MDMA concentration examined (i.e., 100 μM).

This finding is dissimilar to that observed following methamphetamine exposure (House et al., 1994) in that methamphetamine did not display the same degree of suppression at high concentrations; more importantly, methamphetamine did not stimulate IL-2 production at any concentration, unlike MDMA. Conversely, similar to methamphetamine, MDMA had no observable effect on IL-4 production by TH2 cells. These observations suggest an interesting differential effect on cytokine production by MDMA. The nature of this differential effect currently remains uncharacterized.

Another important function of T-cells is their role as cytolytic effector cells. This capability is exemplified best by the CTL, a population of $CD8^+$ T-lymphocytes capable of directly destroying target cells following a period of sensitization with specific antigen (Berke, 1989; Taylor and

Cohen, 1992). CTL induction requires the interaction of various-cells, and therefore represents an excellent target for evaluating drug-mediated immunomodulation. In the present study, in vitro exposure to MDMA had no apparent effect on a 5-day in vitro CTL induction except at 100 μM; this suppression was not the result of alteration in cellular viability or effector cell recovery, since neither of these parameters appeared to be affected by MDMA exposure. However, IL-2 production by THI cells was also significantly suppressed at this same concentration of MDMA. Since IL-2 is known to be vital for CTL induction (Skinner et al., 1986), a potential mechanism for the suppression observed at 100 μM may be related to a modulation in IL-2 production. It should be noted here that MDMA was added at the initiation of the CTL cultures only, although the induction of CTL from the CTL precursors is the result of a complex cellular interaction over time. Conceivably, then, appreciable MDMA may not be present at those critical phases of the assay that may be susceptible to the drug's effects. The exact mechanism of the CTL suppression observed in this study remains uncharacterized.

A non-specific host resistance mechanism evaluated in this study was basal and cytokine-augmented NK cell activity. NK cells are a population of lymphocytes that are unrelated to either T- or B-cells, able to destroy certain target cells without prior antigen exposure (Lotzová, 1993). Thus, NK cells act as mediators of non-specific host resistance which may be modulated by immune mechanisms. To examine the effect of drugs on NK cell function in broader detail, we also studied the effect on augmented NK cell function. Certain cytokines, especially IL-2, are known to be capable of enhancing the cytotoxic potential of NK cells (Talmadge, 1985). In the present study, MDMA-exposed cells were cultured for 24 h in the presence or absence of rIL-2, followed by assessment of cytotoxicity against NK-sensitive target cells. In general, in vitro exposure to MDMA resulted in an elevation in NK cell function. One hypothesis for this stimulation is based on the results with IL-2 production discussed earlier. Both the IL-2 production and NK cell experiments utilized whole splenocyte populations; therefore, the same cell populations would have been exposed to MDMA. Exposure to MDMA could induce TH1 to produce IL-2, which in turn could augment NK cell function as was observed in the basal NK cell cultures. Alternatively, cultures that were already augmented by the addition of exogenous IL-2 would not be as sensitive to this effect; consequently, MDMA was found not to enhance NK function to the same degree in the augmented cultures. Based on this hypothesis, a point of interest is that the augmented NK cultures were enhanced most at the lowest concentration of MDMA, which was also observed to significantly enhance IL-2 production. It should also be noted that this pattern is similar to that reported previously for methamphetamine (House et al., 1994), in which both basal and IL-2-augmented NK cell function were enhanced by in vitro drug exposure. Of potential significance is the fact that the most pronounced enhancement of NK cell function resulting from methamphetamine exposure was observed at higher concentrations of methamphetamine, whereas MDMA appeared to exert its effects at the lower concentrations.

The clinical pharmacology of MDMA has not been extensively characterized (Karch, 1993). However, Bost (1988) reported blood levels in intoxicated drivers of 110–590 ng/ml (~0.0005–0.003 μM), with higher blood levels usually associated with acute toxicity or death.

These levels are in the range of NK cell modulation as demonstrated here. The present study, however, evaluated only in vitro exposure to MDMA; in vivo exposure could perhaps yield different results, for example through metabolic breakdown products of MDMA (e.g., MDA). The nature of the immunostimulatory effects produced in vitro is not currently understood.

Finally, the effect of drug exposure on macrophage function was assessed by quantitating the production of the regulatory cytokines IL-6 and TNF. These cytokines, in conjunction with IL-1 and other factors, modulate a regulatory network involved in regulation of a wide range of immune and non-immune host-defense mechanisms (Akira et al., 1990). As such, these cytokines represent appropriate targets for screening potential alterations in macrophage function following drug

exposure. Exposure to MDMA had no effect on the production of IL-6 by peritoneal macrophages at any concentration examined in the present study. Production of TNF, while displaying a slight trend toward suppression with increasing concentrations of MDMA, was likewise not significantly affected by drug exposure. Since macrophage function was not specifically addressed in our previous work with methamphetamine, no comparison is possible between the effects of MDMA and methamphetamine on macrophage function. Apparently, however, in vitro MDMA exposure does not adversely affect macrophage function.

In summary, in vitro exposure to MDMA modulates several immune functional parameters in a manner very similar, although not identical, to that of the related compound, methamphetamine. This particular pattern of immunomodulation has not been observed with any other drug examined to date in this laboratory, suggesting a common mechanism of action by these two drugs.

Acknowledgments

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