

3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) Administration Produces Dose-Dependent Neurochemical, Endocrine and Immune Changes in the Rat

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3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) is a widely abused drug that is structurally related to both amphetamines and hallucinogens. In addition to the behavioural and neurochemical effects of MDMA, we recently reported that an acute administration of this drug produces a profound suppression of mitogen-stimulated lymphocyte proliferation and reduction in the number of circulating white blood cells, which was accompanied by elevated circulating corticosterone concentrations. In the present study, the effect of acute MDMA administration on PHA-induced lymphocyte proliferation, leucocyte subpopulations, HPA-axis activity and cortical serotonin utilization were examined in a dose-dependent manner in female Sprague-Dawley rats. The results of this study demonstrate that MDMA induces a suppression of lymphocyte function even at doses that fail to provoke any significant alteration in central 5-HT utilization and plasma corticosterone concentrations, thereby suggesting that the reduced functional responsiveness of lymphocytes to mitogenic stimulation after MDMA administration may be mediated by glucocorticoid independent mechanisms. In contrast, the MDMA-induced reduction in the number of circulating blood lymphocytes was evident only at doses of MDMA which elevated circulating corticosterone concentrations, suggesting that the observed reduction in circulating lymphocytes may be at least partly a glucocorticoid-mediated event. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS — 3,4-methylenedioxymethamphetamine; MDMA; Ecstasy; corticosterone; immunity; lymphocyte proliferation; lymphocytes; psychoneuroimmunology

INTRODUCTION

3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) is a ring-substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. The unique behavioural activating properties of MDMA have led to its widespread abuse in humans. In laboratory animals, acute MDMA administration causes an increase in central serotonin release (Gudelsky and Nash, 1996), increased locomotor activity (McGarvey *et al.*, 1995), changes in body temperature (McNamara *et al.*, 1995a) and activation of both the hypothalamic pituitary adrenal (HPA) axis (McGarvey *et al.*, 1995; McNamara

et al., 1995b) and sympathetic nervous system (SNS) (Grob *et al.*, 1996).

Many of these MDMA-induced neurochemical, behavioural and endocrine alterations closely resemble those elicited by exposure to acute stress, suggesting that MDMA could be regarded as a 'chemical stressor'. In addition to the neurochemical, behavioural and endocrine effects of stressor exposure, it has been reported that a variety of stressors provoke alterations in many aspects of immune function (see Anisman *et al.*, 1993). In this regard, it is well established that a dynamic equilibrium exists between the neuroendocrine and immune systems, inasmuch as altered central nervous system (CNS) function can affect peripheral immunocompetence via the neuroendocrine and sympathetic nervous systems (Black, 1994a; Dantzer and Kelley, 1989; Dunn, 1995), and products of the

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immune system such as cytokines can modulate CNS function (Anisman *et al.*, 1993; Black, 1994b; Plata-Salman, 1991). Therefore, we postulated that an agent such as MDMA, which provokes a profound release in serotonin (Gudelsky and Nash, 1996) and also activates the HPA-axis and SNS, would provoke alterations in immune function. In this regard, we recently demonstrated that acute MDMA administration produced a suppression of mitogen-stimulated lymphocyte proliferation and reduction in total white blood cell count in female rats within 30 min of drug administration, and these effects persisted for at least 6 h following a single dose of MDMA (Connor *et al.*, 1998a). To our knowledge, this was the first study to demonstrate that *in vivo* MDMA administration alters immunoreactivity. Such data gives rise to the possibility that, in addition to the well established toxic effects of MDMA on the central nervous system, a single administration of this widely abused drug may induce a suppression of immune function.

In our first study (Connor *et al.*, 1998a), we only examined the effect of a single dose of MDMA (20 mg/kg; i.p.) on immune, endocrine and central neurotransmitter activity in rats. Other investigators have examined the effect of another serotonin releaser, namely d-fenfluramine, on various aspects of immune function in rats and have observed differential effects dependent on the dose employed. For example, Clancy and Lorens (1996) reported that subchronic treatment with d-fenfluramine in rats caused an enhancement of cellular immune function when administered at low doses (0.6–1.8 mg/kg). In contrast, a suppression of immune function was evident after treatment with high doses (3.0–9.0 mg/kg) of d-fenfluramine (Clancy and Lorens, 1996).

The aim of the present study was to examine the effect of acute administration of a range of doses (1.25–40 mg/kg; i.p.) of MDMA on both circulating leucocyte numbers, and the functional responsiveness of lymphocytes to mitogenic stimulation with the lectin phytohaemagglutinin (PHA). In addition, both plasma corticosterone and adrenal ascorbic acid concentrations were examined as peripheral markers of HPA-axis activity, while serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) concentrations were measured in the frontal cortex as a measure of central serotonergic activity. In the present study, all measures were taken 1 h following MDMA administration in order to detect neurochemical, endocrine and immunological changes at a single timepoint.

METHODS

Subjects and procedures

Seventy-two female Sprague-Dawley rats weighing approximately 220–250 g were obtained from a Departmental breeding colony and housed four per cage. The rats were maintained on a 12 h:12 h light:dark cycle (lights on at 08.00 h) in a temperature controlled room (22–24°C) and food and water were available *ad libitum* at all times. Female rats were used in the present study, as our previous work concerning the immunological effects of MDMA administration was carried out with female rats (Connor *et al.*, 1998a). In addition, it was previously found that MDMA provoked similar changes in central serotonergic activity and HPA-axis activity in both male and female rats (McNamara *et al.*, 1995b).

Drug administration

MDMA (Plaistow, Cork, Ireland) was dissolved in 0.89 per cent NaCl to give concentrations of 1.25, 2.5, 5, 7.5, 10, 15, 20 and 40 mg/ml. 0.89 per cent saline was administered alone as a vehicle to the control group. Both MDMA and vehicle were administered in an injection volume of 1 ml/kg using the intraperitoneal (i.p.) route, and administered in the following doses: 0, 1.25, 2.5, 5, 7.5, 10, 15, 20 and 40 mg/kg MDMA.

Determination of brain biogenic amine concentrations

The rats were sacrificed by decapitation while under ether anaesthesia 1 h after injection. After sacrifice, the brain was rapidly removed and the left frontal cortex was dissected on an ice cold plate (Heffner *et al.*, 1980). Concentrations of 5-HT and its metabolite 5-HIAA were measured by high performance liquid chromatography (HPLC) coupled with electrochemical detection (Seyfried *et al.*, 1986). The frontal cortex was homogenized by sonication in 1.0 ml of mobile phase (pH 2.8) that was spiked with 20 ng/50 µl of *N*-methyl dopamine (Sigma Chemical Co., Dorset, U.K.) as an internal standard. The mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 0.1 mM EDTA (BDH Chemicals Ltd, Dorset, U.K.), 1.4 mM octane-1-sulphonic acid (Sigma), and 10 per cent (v/v) methanol (Lab-Scan, Dublin, Ireland) and was adjusted to pH 2.8 using

4 N NaOH. Homogenates were centrifuged at 12 000 rpm in a Hettich Mikro/K refrigerated centrifuge for 15 min. A 20 µl sample of the supernatant was injected directly into a reverse phase column (LI Chrosorb RP-18, 25 cm × 4 mm internal diameter, particle size 5 µm) for separation of indoles and catecholamines (flow rate 1 ml/min). An electrochemical detector (Shimadzu) was coupled to the HPLC system and was set at a potential of +0.8 V for the detection of monoamine neurotransmitters and metabolites. The neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator. Neurotransmitter concentrations were expressed as ng of neurotransmitter per g wet weight of brain tissue.

Serum corticosterone concentrations

Prior to sacrifice, the animals were anaesthetized with diethyl ether (Lab-Scan) and a blood sample was obtained by cardiac puncture into an anticoagulant-free syringe. The blood was allowed to clot at room temperature. Serum corticosterone concentrations were measured using a previously described method (Grealy and O'Donnell, 1991). A corticosterone (Sigma) stock solution (100 µg/dl) was prepared and diluted to produce a range of concentrations (10–80 µg/dl). Serum samples and corticosterone standards were then mixed in 600 µl of dichloromethane (Lab-Scan) for 15 s. 500 µl of the resulting dichloromethane extract phase was then transferred into a tube containing 400 µl of concentrated sulphuric acid (BDH): absolute ethanol (Lab-Scan) (65:35) and the tubes were mixed for 15 s using a vortex mixer. Samples were then placed in the dark for 45 min and a 300 µl aliquot of the lower phase was removed and the fluorescence measured at excitation 474 nm and emission 518 nm (Perkin Elmer LS-5 spectrofluorimeter). The results were expressed as µg corticosterone per dl of serum.

Adrenal ascorbic acid concentrations

The method used for adrenal ascorbic acid determination was a spectrophotometric method as previously described (Demetriou, 1974). The adrenal glands were dissected free of fat, weighed and placed in 3 ml 7.5 per cent (w/v) trichloroacetic acid (TCA) (BDH). The tissue was homogenized and stored at 20°C until the assay was performed. On the day prior to assay, the samples were thawed and rinsed with 1 ml 7.5 per cent TCA. After centrifugation at 800 g for 20 min using an MSE

benchtop centrifuge, 3 ml of the supernatant was removed and added to 3 ml 7.5 per cent TCA and 250 mg activated charcoal (Sigma). After vigorous mixing the supernatant was filtered through 11 cm filters (Whatman No. 2). The resultant protein free filtrate was stored at 4°C overnight and assayed for ascorbic acid the following morning. The ascorbic acid standards were also activated using activated charcoal. A 2,4-dinitrophenylhydrazine (2,4-DNP) solution was prepared by dissolving 2 g of 2,4-DNP in 100 ml of 9 N H₂SO₄, then adding 4 g of thiourea (Sigma). This solution was filtered through an 11 cm filter (Whatman, UK). A 250 µg/ml ascorbic acid (BDH) stock was diluted using 7.5 per cent TCA to produce a range of concentrations for the standard curve (0, 2.5, 5, 10, 20 and 40 µg/ml). To 1 ml of samples and standards was added 250 µl of 2,4-DNP solution, the tubes were mixed and incubated in a water bath at 37°C for 4.5 h. At the end of the incubation period the tubes were cooled in an ice bath for 5 min, and 1.5 ml of 85 per cent (v/v) H₂SO₄ (BDH) was added to each tube. The tubes were mixed and allowed to stand for 40 min at room temperature, after which time the absorbance of the orange coloured reaction product was read at 515 nm using a spectrophotometer (Shimadzu UV-160). Results were expressed at µg ascorbic acid/mg tissue.

Total and differential leucocyte counts

Prior to sacrifice the animals were anaesthetized with diethyl ether (Lab-Scan) and a blood sample for the immune assays was obtained by cardiac puncture into a heparinized syringe. The total leucocyte counts were performed on heparinized whole blood samples using a haematology counter (Baker Diagnostics, Serono 9000). Blood smeared slides were stained with Wright's stain using an automated slide stainer (Ames, HEMA-TEK, Japan). The relative leucocyte percentages were counted on each slide using standard hospital procedures, under a microscope. The absolute numbers of lymphocytes, neutrophils and monocytes were calculated by multiplying the total leucocyte count by the relative percentages and dividing by 100 (Connor *et al.*, 1997).

PHA-induced lymphocyte proliferation

Heparinized blood was mixed with RPMI 1640 medium (Gibco Life Technologies, Scotland, UK)

(1:1 dilution) and layered onto 4 ml of Nycoprep (Nycoprep AS, Oslo, Norway) gradient. Following centrifugation (Beckman refrigerated benchtop centrifuge) at 600 *g* for 25 min at 20°C, the lymphocyte layer was removed and washed three times in RPMI 1640 medium. The cells were finally resuspended in complete RPMI 1640 medium (RPMI 1640 + 10 per cent (v/v) heat inactivated fetal calf serum + 2 per cent (v/v) penicillin/streptomycin) (Gibco Life Technologies) and the number of lymphocytes was adjusted to 2×10^6 /ml.

Lymphocyte proliferation was performed in triplicate as previously described (Connor *et al.*, 1998a; Luo *et al.*, 1993; Mosmann, 1983). Briefly, 100 µl aliquots of the lymphocyte preparation were pipetted into wells of a 96 well microtitre plate. To each well was added either no mitogen for background transformation or phytohaemagglutinin (PHA) (Sigma) at a working concentration of 2.5, 5.0 or 10.0 µg/ml. Cultures were incubated for 64 h at 37°C in a 5 per cent CO₂ atmosphere prior to addition of 15 µl MTT (Promega, UK) per well. The cultures were then incubated for a further 8 h to allow the dye to be metabolized by actively proliferating cells. At the end of the culture period, 100 µl of the solubilization reagent (Promega) was added to each well of the 96 well microtitre plate and the plates were incubated overnight at 37°C in a 5 per cent CO₂ atmosphere to facilitate complete solubilization of the MTT-formazan product. Microtitre plates were then read using an ELISA plate reader (Dynatech MR5000), at a wavelength of 550 nm, with a reference wavelength of 650 nm. The plates were shaken for 10 s prior to reading to ensure uniform colour distribution within the wells. Mean absorbance readings were calculated for each concentration of PHA and the background absorbance of unstimulated lymphocytes was subtracted from the mean absorbance of stimulated lymphocytes.

Statistical analysis of data

All data with the exception of the lymphocyte proliferation were analyzed by a one-way analysis of variance. Lymphocyte proliferation data were analyzed using a two-way analysis of variance with repeated measures for mitogen concentration. If any statistically significant change was found, *post hoc* comparisons were performed using Student Newman-Keuls multiple range test. Data were deemed significant when $p \leq 0.05$. Results are expressed as group mean with standard errors.

RESULTS

5-HT and 5-HIAA concentrations in the frontal cortex

There was a significant effect of MDMA administration of 5-HT [$F_{(8,63)} = 22.46$, $p < 0.001$] and 5-HIAA [$F_{(8,63)} = 2.74$, $p < 0.05$] concentrations in the frontal cortex. *Post hoc* analysis revealed a significant ($p < 0.01$) reduction in cortical 5-HT concentrations in response to doses of 5 mg/kg MDMA and greater. In addition, there was a significant ($p < 0.05$) increase in cortical 5-HT in response to the 1.25 mg/kg dose of MDMA. *Post hoc* analysis did not detect any significant change in 5-HIAA concentrations between the control and MDMA treated groups (Table 1). When the 5-HIAA/5-HT ratio was used as an index of 5-HT turnover, there was a significant effect of MDMA administration [$F_{(8,63)} = 21.20$, $p < 0.0001$]. *Post hoc* analysis revealed a significant increase in the cortical 5-HIAA/5-HT ratio in response to doses of 2.5 mg/kg MDMA and above (Figure 1).

Serum corticosterone concentrations

There was a significant effect of MDMA administration on serum corticosterone concentrations [$F_{(8,63)} = 6.01$, $p < 0.0001$]. *Post hoc* analysis revealed a significant increase in serum corticosterone in response to doses of 10 mg/kg MDMA and above (Figure 2a).

Table 1. Effect of acute MDMA administration on 5-HT and 5-HIAA concentrations in the frontal cortex

Group	5-HT	5-HIAA
Control	468 ± 17	228 ± 16
MDMA (1.25 mg/kg)	534 ± 18*	260 ± 8
MDMA (2.5 mg/kg)	429 ± 39	224 ± 6
MDMA (5 mg/kg)	356 ± 17**	239 ± 15
MDMA (7.5 mg/kg)	342 ± 24**	271 ± 11
MDMA (10 mg/kg)	272 ± 21**	258 ± 13
MDMA (15 mg/kg)	239 ± 13**	252 ± 7
MDMA (20 mg/kg)	242 ± 20**	279 ± 8
MDMA (40 mg/kg)	295 ± 19**	272 ± 16

Data expressed as means with standard errors. * $p < 0.05$, ** $p < 0.01$ versus control (Student Newman-Keuls multiple range test) ($n = 8$). Neurotransmitter concentrations are expressed as ng/g wet weight of tissue. 5-HT: Serotonin; 5-HIAA: 5-hydroxyindoleacetic acid.

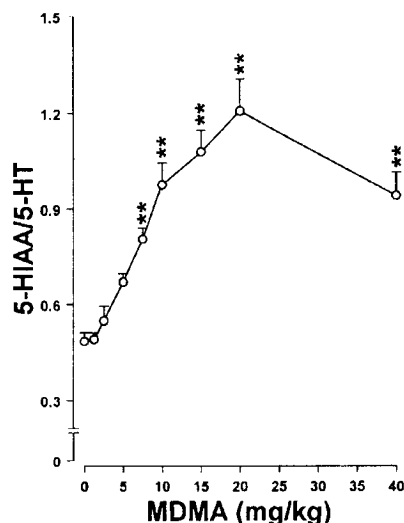


Figure 1. Effect of acute MDMA administration on the 5-HIAA/5-HT ratio. Data expressed as means with standard errors. ** $p < 0.01$ versus control (Student Newman-Keuls multiple range test) ($n = 8$)

Adrenal ascorbic acid concentrations

There was a significant effect of MDMA administration on adrenal ascorbic acid concentrations [$F_{(8,63)} = 14.77$, $p < 0.0001$]. *Post hoc* analysis revealed a significant decrease in adrenal ascorbic acid in response to doses of 2.5 mg/kg MDMA and above (Figure 2b).

Total and differential leucocyte counts

There was a significant effect of MDMA on total leucocyte count [$F_{(8,63)} = 3.47$, $p < 0.01$]. *Post hoc* analysis revealed a significant reduction in the number of circulating leucocytes after administration of a dose of 7.5 mg/kg MDMA or greater (Figure 3). There was a significant effect of MDMA on the absolute lymphocyte count [$F_{(8,63)} = 3.51$, $p < 0.01$]. *Post hoc* analysis revealed a significant reduction in circulating lymphocyte numbers in response to 7.5 mg/kg MDMA and greater (Figure 4). MDMA administration failed to significantly alter absolute neutrophil or monocyte counts (data not shown).

PHA-induced lymphocyte proliferation

There was a significant effect of MDMA administration [$F_{(8,60)} = 2.64$, $p < 0.01$] on PHA-induced

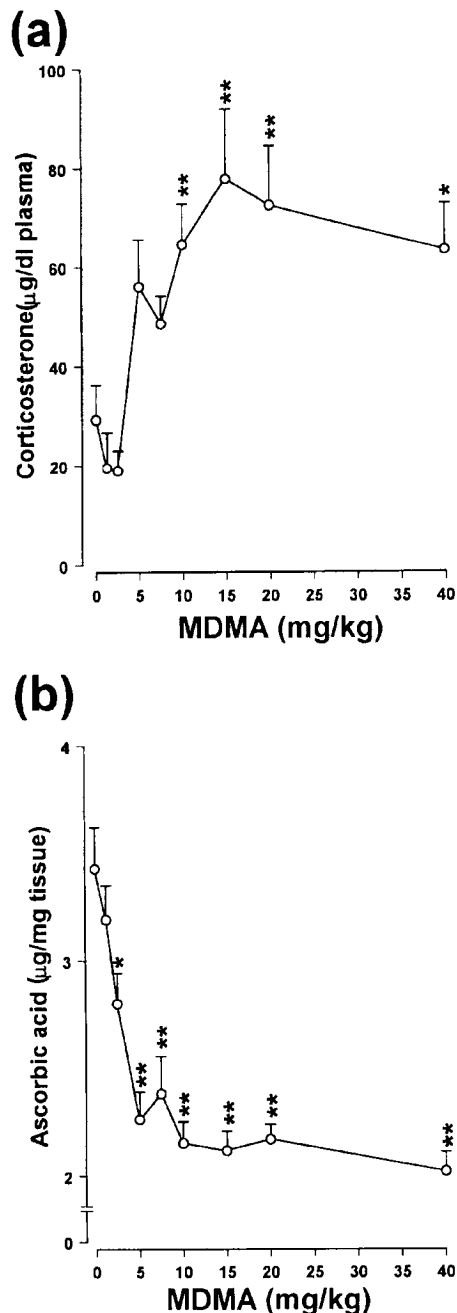


Figure 2. Effect of acute MDMA administration on (a) plasma corticosterone and (b) adrenal ascorbic acid concentrations. Data expressed as means with standard errors. * $p < 0.05$, ** $p < 0.01$ versus control (Student Newman-Keuls multiple range test) ($n = 8$)

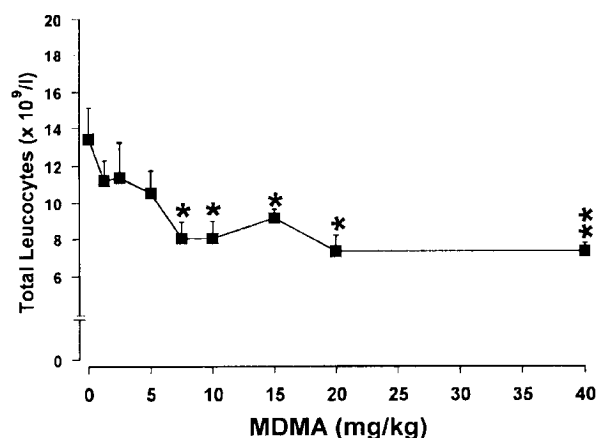


Figure 3. Effect of acute MDMA administration on circulating lymphocyte numbers. Data expressed as means with standard errors. * $p < 0.05$, ** $p < 0.01$ versus control (Student Newman-Keuls multiple range test) ($n = 8$)

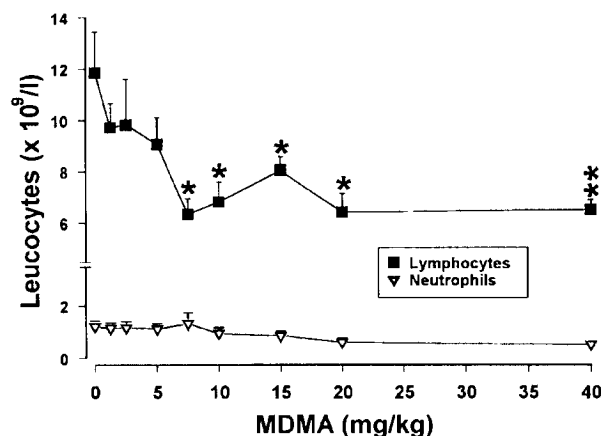


Figure 4. Effect of acute MDMA administration on circulating lymphocyte and neutrophil counts. Data expressed as means with standard errors. * $p < 0.05$, ** $p < 0.01$ versus control (Student Newman-Keuls multiple range test) ($n = 8$)

lymphocyte proliferation, a significant effect of PHA concentration [$F_{(2,120)} = 88.04$, $p < 0.0001$] and a MDMA $\times$ PHA concentration interaction [$F_{(16,120)} = 4.19$, $p < 0.0001$]. *Post hoc* analysis showed that there was a significant reduction in PHA-induced lymphocyte proliferation after MDMA administration at all doses (Figure 5).

DISCUSSION

In this study an increase in serotonin turnover was observed in the frontal cortex as a result of

MDMA administration (7.5–40 mg/kg), which is consistent with previous reports which examined the effects of MDMA on 5-HT concentrations in postmortem rat brain (McGarvey *et al.*, 1995; McNamara *et al.*, 1995a,b) and in freely moving animals using *in vivo* microdialysis (Gudelsky and Nash, 1996). These alterations in cortical 5-HT concentrations reflect increased release of 5-HT accompanied by a reduction in 5-HT synthesis due to the inhibitory action of MDMA on tryptophan hydroxylase activity (Stone *et al.*, 1987).

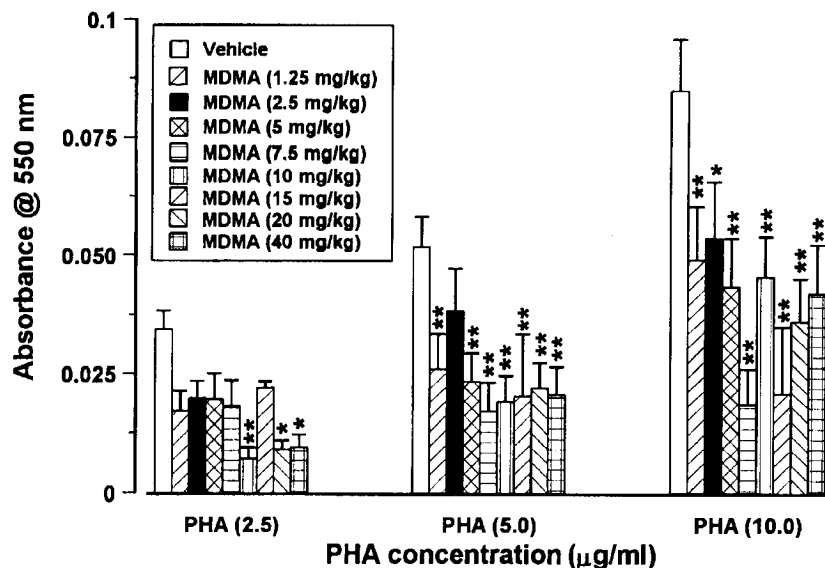


Figure 5. Effect of acute MDMA administration on circulating leucocyte numbers. Data expressed as means with standard errors. * $p < 0.05$, ** $p < 0.01$ versus control (Student Newman-Keuls multiple range test) ($n = 8$)

Acute MDMA administration also elevated plasma corticosterone concentrations at doses at and above 10 mg/kg. However, a significant depletion in adrenal ascorbic acid was evident at a dose of 2.5 mg/kg MDMA or above. Previously, it has been demonstrated that both neurogenic (Connor *et al.*, 1998b; Kelliher *et al.*, 1997; Nagyova and Ginter, 1993) and metabolic (Nolan *et al.*, 1998) stressors provoke a large and long lasting decrease in adrenal ascorbic acid concentrations, and that such a reduction in adrenal ascorbic acid is a reliable peripheral marker of stress in rodents. In previous studies, decreased adrenal ascorbic acid concentrations have been attributed to both increased synthesis and secretion of corticosterone from the adrenal cortex (Nagyova and Ginter, 1993) and increased release of catecholamines from the adrenal medulla (Grunwald, 1993). Moreover, it has been demonstrated that there is a rapid release of ascorbic acid from the adrenals into the blood stream in response ACTH administration (Eisenstein, 1967). In previous studies in this laboratory, a strong inverse relationship between adrenal ascorbic acid concentrations and plasma corticosterone has been observed (Connor *et al.*, 1998b; Kelliher *et al.*, 1997; Nolan

et al., 1998). However, in the present study there was a clear dissociation between the effect of systemic MDMA administration on corticosterone secretion and on adrenal ascorbic acid concentrations, insofar as the depletion of adrenal ascorbic acid occurs in response to doses of MDMA as low as 2.5 mg/kg, whereas plasma corticosterone concentrations are not significantly altered at doses of MDMA below 10 mg/kg. These results demonstrate that reduced adrenal ascorbic acid concentrations occur in response to doses of MDMA which fail to increase circulating corticosterone concentrations, suggesting that other mechanisms (such as increased sympathetic activity) may be responsible for alterations in adrenal ascorbic acid concentrations observed at doses of MDMA below 10 mg/kg. From these data, it appears that the 5-HT releasing effects of MDMA at doses less than 10 mg/kg are not sufficient to significantly alter HPA-axis activity, but nonetheless provoke significant decreases in adrenal ascorbic acid concentrations.

In a previous study, we demonstrated that acute MDMA administration (20 mg/kg; i.p.) produced a reduction in the total number of circulating leucocytes in female rats (Connor *et al.*, 1998a).

However, it was not clear what leucocyte subsets were altered after MDMA administration. In the present investigation we extend our previous findings by demonstrating that MDMA causes a reduction in circulating lymphocyte numbers without appreciably altering the numbers of neutrophils or monocytes in the peripheral blood. In addition, the reduction in circulating lymphocytes was only observed at doses of MDMA which provoked an increase in plasma corticosterone concentrations, thereby suggesting that MDMA-induced activation of the HPA-axis, and the consequential rise in circulating corticosterone concentrations, may mediate the observed reduction in circulating lymphocytes. This hypothesis is supported by the fact that administration of exogenous corticosterone to rats produces a similar reduction in circulating lymphocyte numbers (Dhabhar *et al.*, 1995). One explanation for the reduction in circulating lymphocytes observed in the present study is that lymphocytes have migrated from the peripheral blood and entered other immune compartments (lymph nodes, spleen, bone marrow, Peyer's patches, for example) as a result of acute MDMA administration. Alternatively, the reduction in lymphocyte numbers may indicate that lymphocyte apoptosis occurred due to the elevated circulating levels of corticosterone. However, further studies are required to examine the exact mechanism(s) that are responsible for MDMA-induced alterations in circulating lymphocytes.

In addition to the effect of MDMA administration on immune cell numbers, we previously reported that acute MDMA administration produced a rapid suppression of mitogen-stimulated lymphocyte activity in rats (Connor *et al.*, 1998a). Clancy and Lorens (1996) examined the effect of the structurally-related serotonin releaser, d-fenfluramine on various aspects of immune function, and observed that subchronic treatment in rats caused an enhancement of cellular immune function when administered at low doses, whilst a suppression of immune function was evident after treatment with high doses of d-fenfluramine. In the present study, a large range of doses of MDMA were used, and all doses of MDMA (even doses that did not alter central serotonergic activity or plasma corticosterone concentrations) produced a suppression of PHA-induced lymphocyte proliferation. These results demonstrate that although both MDMA and d-fenfluramine act as central serotonin releasers, the effects of these drugs on

the immune system vary considerably, in that MDMA does not have any detectable immunoaugmenting properties even at low doses. However, further studies examining the effect of pretreatment with specific serotonin receptor antagonists on MDMA-induced immunological alterations may help to elucidate the exact contribution of central serotonin release to such alterations in immune function.

It is well established that glucocorticoids, including corticosterone, have immunosuppressive properties (Bateman *et al.*, 1989), and many previous studies have implicated corticosterone to be a key mediator in stress-induced suppression of cell-mediated immunity (CMI) (Shu *et al.*, 1993; Song *et al.*, 1994). However, other researchers have demonstrated that stress-induced suppression of CMI occurs even in the absence of corticosterone secretion, suggesting that elevated corticosterone levels are not responsible for stress-induced impairments in CMI (Jain *et al.*, 1991; Jaing *et al.*, 1990; Keller *et al.*, 1983; Shavit *et al.*, 1984). In the present study, the suppression of PHA-induced lymphocyte proliferation was evident even at doses of MDMA that did not increase plasma corticosterone, thereby suggesting that the reduced functional responsiveness of lymphocytes to PHA after MDMA administration may be mediated by a glucocorticoid independent mechanism. It is interesting that in a previous study it was reported that MDMA's parent compound, d-amphetamine, provoked a drastic reduction in PHA-stimulated lymphocyte proliferation which was also independent of corticosterone secretion (Pezzone *et al.*, 1992). MDMA also activates the sympathetic nervous system (SNS) (Grob *et al.*, 1996); it has been recently suggested that SNS activation is an important contributory factor in stress-induced suppression of CMI (Freidman and Irwin, 1997; Irwin, 1993). Furthermore, amphetamine-induced reductions in PHA-induced lymphocyte mitogenesis are completely reversed by antagonists of peripheral β -adrenoceptors (Pezzone *et al.*, 1992). Therefore, it is not unreasonable to suggest that MDMA may suppress the functional activity of lymphocytes by increasing sympathetic activity. However, further studies using pretreatment with β -adrenoceptor antagonists are required to firmly establish any effect of sympathetic activation on lymphocyte function following MDMA administration.

The impaired lymphocyte proliferation response seen after MDMA administration is unlikely to be

due to a reduced number of lymphocytes in the peripheral blood, as the lymphocytes were adjusted to a standard cell density prior to assessment of their functional activity. However, the reduced functional responsiveness seen in the present study may be due to an alteration in lymphocyte subpopulations, such as a reduction in T-lymphocytes. In this respect, it has been previously suggested that stressor-induced alterations in CMI occur as a result of an alteration in leucocyte subpopulations (Fleshner *et al.*, 1992).

House *et al.* (1995) examined the effect of *in vitro* MDMA exposure on various aspects of immune function. *In vitro* exposure to MDMA produced a suppression of IL-2 production at high doses, but an enhancement at lower doses, with intermediate doses failing to significantly alter this response. Moreover, *in vitro* MDMA also produced a suppression of cytotoxic T-lymphocyte induction (House *et al.*, 1995). However, B-lymphocyte function was not significantly altered by any of the MDMA concentrations used (House *et al.*, 1995). Therefore, the altered PHA-induced proliferative response of lymphocytes seen in the present study may be due to a combined effect of both a direct action of MDMA on lymphocytes, and due to activation of the HPA-axis and/or SNS via central mechanisms. However, the exact contribution of direct and indirect mechanisms of MDMA-induced changes in lymphocyte activity remain to be elucidated.

In conclusion, acute MDMA administration produced a variety of dose-dependent neurochemical, endocrine and immune alterations in the rat. The results of this study demonstrate that MDMA provoked a suppression of lymphocyte responsiveness to mitogenic stimulation at doses which did not significantly alter central serotonergic function or HPA-axis activity. In contrast, the reduction in circulating lymphocyte numbers observed after MDMA administration occurred only at doses of MDMA that provoked an increase in central serotonergic activity and elevated plasma corticosterone concentrations. These data imply that the alteration in functional activity of lymphocytes induced by MDMA may occur by a glucocorticoid-independent mechanism, whereas the observed reduction in circulating lymphocytes may be a glucocorticoid-mediated event. However, further studies using pretreatment with both glucocorticoid and β -adrenoceptor antagonists prior to MDMA administration are required to confirm this hypothesis.

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