

Methylenedioxymethamphetamine (MDMA; Ecstasy) suppresses IL-1 β and TNF- α secretion following an *in vivo* lipopolysaccharide challenge

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Abstract

In this study we examined the effects of methylenedioxymethamphetamine (MDMA) administration on responsiveness to an *in vivo* immune challenge with lipopolysaccharide (LPS; 100 μ g/kg; i.p.). LPS produced an increase in circulating IL-1 β and TNF- α in control animals. MDMA (20 mg/kg; i.p.) significantly impaired LPS-induced IL-1 β and TNF- α secretion. The suppressive effect of MDMA on IL-1 β secretion was transient and returned to control levels within 3 hours of administration. In contrast, the MDMA-induced suppression of TNF- α secretion was evident for up to 12 hours following administration. In a second study we examined the effect of co-administration of MDMA (5, 10 and 20 mg/kg; i.p.) on LPS-induced IL-1 β and TNF- α secretion, and demonstrated that all three doses potently suppressed LPS-induced TNF- α secretion, but only MDMA 10 and 20 mg/kg suppressed LPS-induced IL-1 β secretion. In addition, serum MDMA concentrations displayed a dose-dependent increase, with the concentrations achieved following administration of 5 and 10 mg/kg being in the range reported in human MDMA abusers. In order to examine the possibility that the suppressive effect of MDMA on IL-1 β and TNF- α could be due to a direct effect of the drug on immune cells, the effect of *in vitro* exposure to MDMA on IL-1 β and TNF- α production in LPS-stimulated diluted whole blood was evaluated. However IL-1 β or TNF- α production were not altered by *in vitro* exposure to MDMA. In conclusion, these data demonstrate that acute MDMA administration impairs IL-1 β and TNF- α secretion following an *in vivo* LPS challenge, and that TNF- α is more sensitive to the suppressive effects of MDMA than is IL-1 β . However the suppressive effect of MDMA on IL-1 β and TNF- α could not be attributed to a direct effect on immune cells. The relevance of these findings to MDMA-induced immunomodulation is discussed. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Cytokine; Immunity; In vivo; Immune challenge; IL-1 β ; LPS; Macrophage; MDMA; TNF- α

Introduction

Methylenedioxymethamphetamine (MDMA; “Ecstasy”) is a ring substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. Due to its

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unique behavioural activating properties, MDMA is currently a popular drug of abuse in humans [1]. The subjective effects of MDMA include euphoria, increased self-esteem and enhanced empathy; other effects include hyperactivity, jaw-clenching and teeth grinding [see 1]. More serious side effects such as cardiac arrhythmias, hyperthermia, renal failure, seizures and intracranial haemorrhage have also been reported following MDMA abuse [1–3]. In addition, it has been suggested that the long term neurotoxic effects of MDMA on serotonergic neurons within the central nervous system may be a predisposing factor to psychological disturbances/psychiatric disorders such as depression, anxiety and psychosis [4–6].

In addition to these well described effects of MDMA, an additional problem with any drug of abuse is its potential to disrupt the immune system [see 7]. In this regard we have recently examined the effect of MDMA on aspects of cellular immunity in the rat, and demonstrated that acute MDMA administration produced a suppression of mitogen-stimulated lymphocyte proliferation and a reduction in circulating lymphocyte numbers [8,9]. In addition, we demonstrated that MDMA alters concanavalin A-induced Th₁ and Th₂ type cytokine secretion, and also suppresses LPS-induced secretion of the proinflammatory cytokine TNF- α , but not IL-1 β , from diluted whole blood cultures [10]. Such data give rise to the possibility that, a single administration of this widely abused drug may have immunotoxic potential which could lead to increased disease susceptibility.

As outlined above, in our previous studies we examined *ex vivo* measures of immune function. Whilst *ex vivo* determinations of immune function are useful, and yield important information concerning the status of the immune system, there is the concern that *ex vivo* stimulation of immune cells may not be naturalistic and consequently be of limited value when assessing immunocompetence in a whole organism. Thus another approach which has been used by to assess immunological status in laboratory animals, is to examine their ability to respond to an *in vivo* challenge with bacterial lipopolysaccharide (LPS) [11–16]. When LPS is injected systemically it binds to CD 14 on monocyte membranes and stimulates the production of proinflammatory cytokines such as IL-1 and TNF- α which are important signalling molecules in initiating and co-ordinating a large range of immune responses against invading pathogens [17,18]. Essentially an *in vivo* LPS challenge mimics the initial phase of a bacterial infection.

IL-1 is a proinflammatory cytokine released from activated macrophages. During inflammation, injury, immunological challenge or infection IL-1 is produced and because of its multiple biological properties this cytokine is of primary and strategic importance to the outcome of disease, particularly inflammatory and infectious disease [see 19]. TNF- α , like IL-1, is also a proinflammatory cytokine released from activated macrophages. TNF- α is a vital component of the cellular immune response [see 20] and is a key mediator of inflammation and the mammalian host response to neoplasia, injury or invasion by bacteria, viruses and parasites [21,22].

In the present study we sought to examine the effects of acute MDMA administration on circulating IL-1 β and TNF- α concentrations following an *in vivo* challenge with bacterial LPS. As MDMA-induced immunological changes may demonstrate differential times of onset, as well as differential response duration, LPS-induced IL-1 β and TNF- α secretion was examined at different timepoints (up to 24 hr) following acute MDMA administration. In addition, dose related effects of MDMA on LPS-induced IL-1 β and TNF- α secretion were ex-

aminated. Also serum MDMA concentrations were measured following administration of different doses of MDMA, in order to compare the MDMA concentrations in this study with the circulating concentrations reported in humans who abuse the drug. In order to examine the possibility that the suppressive effect of MDMA on IL-1 β and TNF- α secretion could be due to a direct effect of the drug on immune cells, the effect of *in vitro* MDMA exposure on IL-1 β and TNF- α production in LPS-stimulated diluted whole blood cultures was evaluated.

Materials and methods

Subjects and procedures

Female Sprague-Dawley rats (220–250g; 12–14 weeks old) were obtained from a Departmental breeding colony and housed four per cage. The rats were maintained on a 12hr:12hr light:dark cycle (lights on at 08.00 hr) in a temperature controlled room (22–24°C) and food and water were available *ad libitum* at all times. Female rats were used as our previous studies concerning the immunological effects of MDMA were conducted in female rats [8,9]. In addition, previous data generated within our laboratory demonstrated that male and female rats respond in a similar fashion to MDMA [23,24]. Each experiment was conducted once (n=7–8 rats per group). The experimental protocol was in compliance with the European Communities Council directive (86/609/EEC).

MDMA administration

MDMA (Plaistow, Cork, Ireland) was dissolved in 0.89% NaCl to give concentrations of 5, 10 or 20 mg/ml. 0.89% NaCl 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. In study I MDMA was either co-administered with LPS or given 3, 6, 12 or 24 hr prior to LPS challenge. In study II MDMA was co-administered with LPS. In both experiments the animals were sacrificed at the same time of the day between 12:00 and 14:30. In the timecourse experiment the MDMA injections were given at various intervals prior to LPS administration and sacrifice.

In vivo LPS challenge

Animals received a challenge with either saline or LPS at various timepoints following MDMA administration. LPS from *E. coli* serotype 0111:B4 (Sigma Chemical Co., Poole, Dorset, U.K.) was dissolved in sterile 0.89% NaCl at a concentration of 100 μ g/ml. All animals were challenged with either sterile 0.89% NaCl or LPS 100 μ g/kg administered in a 1 ml/kg injection volume by the intraperitoneal route. Two hours following the challenge with either saline or LPS animals were sacrificed by decapitation and trunk blood was collected. We have previously found that this dose and route of administration of LPS produces quantifiable increases in circulating IL-1 β and TNF- α concentrations and is an optimal time-point for simultaneous measurement of IL-1 β and TNF- α in rat serum [12,13,16]. Following collection, blood samples were centrifuged (800 $\times$ g at 4°C for 15 min) and aliquots of serum were removed for determination of TNF- α , IL-1 β and MDMA concentrations. Serum samples were frozen immediately on dry ice and then stored at –80°C until the assays were performed.

In vitro incubation of MDMA with LPS-stimulated diluted whole blood

In the present study a diluted whole blood method was used for the assessment of cytokine production. In diluted whole blood the natural cell-cell interactions are preserved, whereas the methods used to isolate peripheral blood mononuclear cells (PBMCs) modify the lymphocyte/monocyte ratio and eliminate endogenous immunomodulatory agents. Thus *in vivo* conditions are better represented using whole blood culture methods, whilst the variability in cytokine production by isolated PBMC cultures is much larger than in diluted whole blood cultures [see 10]. Blood samples were obtained via cardiac puncture from five female Sprague-Dawley rats (220–250 g) whilst under ether anaesthesia into a sterile heparinized syringe. Heparinized blood was mixed with complete RPMI 1640 medium [RPMI 1640 + 10% (v/v) heat inactivated foetal calf serum + 2% (v/v) penicillin/streptomycin] (Gibco Life Technologies, Scotland) (1:10 dilution) as previously described [10]. Briefly, 1 ml aliquots of diluted whole blood were pipetted into wells of a sterile flat bottomed 24 well plate (Starstedt, Ireland). To each well was added 100 μ l lipopolysaccharide (LPS) (Sigma Chemical Co., Poole, Dorset, U.K.) at a working concentration of 50 μ g/ml. MDMA was dissolved in RPMI 1640 culture medium to yield a variety of concentrations. To each well was added either 100 μ l of RPMI 1640 culture medium alone (control) or 100 μ l of MDMA at a concentration of either 10, 50, 100, 250, 500, 1000, 2500, 5000 or 10,000 ng/ml. Cultures were incubated for 48 hr at 37°C in a 5% CO₂ atmosphere. We have previously found these conditions to be optimal for the production of IL-1 β and TNF- α in diluted whole blood cultures (Unpublished data). At the end of the culture period the contents of each well was transferred into eppendorf tubes and centrifuged at 12,000 RPM for 15 min at 4°C. Following centrifugation the supernatants were stored at –80°C until cytokine assays were performed.

IL-1 β and TNF- α measurements

Serum and culture supernatant TNF- α concentrations were measured using a commercially available rat TNF- α ELISA kit (Genzyme Diagnostics, Cambridge, U.S.A.). By convention where TNF- α was undetectable the detection limit of the assay (10 pg/ml) was used for analysis. Results were expressed as ng TNF- α /ml of serum or as pg TNF- α /ml culture supernatant.

IL-1 β concentrations were determined using a specific rat IL-1 β ELISA sandwich assay performed using antibodies and standards obtained from Dr. S. Poole (NIBSC, Potters Bar, Herts, U.K.) which was adapted from the original immunoradiometric assay of Bristow *et al.* [25]. Briefly, 96 well Maxisorp microtitre plates (Nunc) were coated with sheep anti-rat IL-1 β polyclonal antibodies (2 μ g/ml in bicarbonate coating buffer; 0.1 M NaHCO₃, 0.1 M NaCl, pH 8.2, for 20 hr at 4°C), then washed three times with wash buffer (0.5 M NaCl, 2.5 mM NaH₂PO₄, 7.5 mM Na₂HPO₄, 0.1% Tween 20, pH 7.2). 100 μ l of a 1% (w/v) ovalbumin (Sigma Chemical Co., Poole, Dorset, UK) solution in bicarbonate coating buffer was added to each well and incubated at 37°C for 1 hr. Following three washes, 100 μ l of samples and standards were added and plates were incubated at 4°C for 20 hr. After three washes, 100 μ l of the biotinylated sheep anti-rat IL-1 β antibody (1:1000 dilution in wash buffer containing 1% sheep serum, Sigma Chemical Co., Poole, Dorset, UK) was added to each well. A further incubation was carried out for 1 hr at room temperature. After three washes, 100 μ l avidin-HRP (Dako Ltd, UK) (1:5000 dilution in wash buffer) was added per well and plates were in-

cubated at room temperature for 15 min. Following three washes, 100 μ l of TMB substrate solution (Dako Ltd., UK) was added per well and the plates were incubated for 10 min at room temperature. At the end of the incubation period 100 μ l of 1 M H_2SO_4 was added per well to stop the reaction and to facilitate colour development. Absorbance was read at 450 nm on a microtitre plate reader. The detection limit of the assay was determined to be 4.3 pg/ml. Results were expressed as pg IL-1 β /ml.

Serum drug determinations

MDMA concentrations were measured by HPLC coupled with fluorescence detection as previously described [26]. Serum samples were subjected to a clean-up procedure prior to HPLC analysis. Briefly, 1 ml 7.5% (v/v) ice-cold, perchloric acid (BDH Chemicals, Poole, U.K.) was added to 1 ml of serum on ice and was sonicated for 5 sec until the sample became a homogeneous cloudy suspension. Following sonication, a further 1 ml 7.5% (v/v) perchloric acid was added to the sample and the tube was mixed gently. Samples were incubated on ice for 20 min and then centrifuged at 3,900 RPM for 15 min at 4°C in a refrigerated bench top centrifuge (Sorvall RT7). Following centrifugation, 0.5 ml of an ice-cold 20 mM EDTA solution (BDH Chemicals, Poole, U.K.) was added to the supernatant. Following a 20 min incubation on ice the samples were centrifuged again at 3,900 RPM for 15 min at 4°C and the supernatant was removed. This sample clean up procedure yielded in excess of 80% recovery. 120 μ l of the supernatant was removed and to it added 40 μ l of internal standard; N-methyldopamine, 250 ng/40 μ l (Sigma-Aldrich, Poole, Dorset, U.K.) and the tubes were mixed. 20 μ l of samples and standards were injected onto a reverse phase column (Lichrosorb RP-18, 250 $\times$ 4 mm internal diameter, particle size 5 μ m). The mobile phase consisted of a 20 mM potassium dihydrogen phosphate (BDH Chemicals, Poole, U.K.):acetonitrile buffer, (85:15 (v/v), pH 3) and was delivered at a flow rate of 0.7 ml per minute. Detection of MDMA in the column effluent was carried out using a fluorescence detector (Excitation λ 286 nm and Emission λ 322 nm). Quantitation of MDMA was accomplished by measuring the area under the curve and comparing these values to that of a known external standard and the internal standard, using the computerised Class-VP chromatography laboratory software system (Shimadzu Europa GMBH, Germany). Results were expressed as ng MDMA/ml of serum.

Statistical analysis of data

Serum IL-1 β and TNF- α were analysed using a two-way analysis of variance and culture supernatant IL-1 β and TNF- α were analysed using a one-way analysis of variance. If any significant change was found, *post hoc* comparisons were performed using Fishers LSD. Data was deemed significant when $P < 0.05$ and data are expressed as mean $\pm$ SEM.

Results

Study I: timecourse experiment

Interleukin-1 β

There was a significant effect of the LPS challenge [$F(1,84) = 109.44$, $P < 0.0001$], of MDMA treatment [$F(5,84) = 3.12$, $P < 0.05$] and a significant LPS $\times$ MDMA interaction

[$F(5,84) = 2.76$, $P < 0.05$] on serum IL-1 β concentrations. *Post hoc* analysis revealed that LPS challenge provoked a large increase in circulating IL-1 β ($P < 0.01$) in vehicle treated rats. MDMA treatment produced a transient suppression of LPS-induced IL-1 β production, which had dissipated by 3 hr following MDMA administration (Fig. 1a).

Tumour necrosis factor- α

There was a significant effect of the LPS challenge [$F(1,83) = 28.84$, $P < 0.0001$], of MDMA treatment [$F(5,83) = 5.26$, $P < 0.001$] and a significant LPS $\times$ MDMA interaction [$F(5,83) = 5.26$, $P < 0.001$] on serum TNF- α concentrations. *Post hoc* analysis revealed that LPS challenge provoked a large increase in circulating TNF- α ($P < 0.01$) in vehicle treated rats, which was suppressed for up to 12 hr following MDMA administration (Fig. 1b).

Study II: dose-response experiment

Interleukin-1 β

There was a significant effect of the LPS challenge [$F(1,51) = 50.38$, $P < 0.0001$], of MDMA treatment [$F(3,51) = 2.83$, $P < 0.05$] and a significant LPS $\times$ MDMA interaction [$F(3,51) = 3.35$, $P < 0.05$] on serum IL-1 β concentrations. *Post hoc* analysis revealed that LPS challenge provoked a large increase in circulating IL-1 β ($P < 0.01$) in vehicle treated rats. MDMA treatment (10 and 20 mg/kg) produced a suppression of LPS-induced IL-1 β production, whereas the lower dose of MDMA did not alter LPS-stimulated IL-1 β secretion (Fig. 2a).

Tumour necrosis factor- α

There was a significant effect of the LPS challenge [$F(1,55) = 19.47$, $P < 0.0001$], of MDMA treatment [$F(3,55) = 12.50$, $P < 0.0001$] and a significant LPS $\times$ MDMA interaction [$F(3,55) = 12.50$, $P < 0.0001$] on serum TNF- α concentrations. *Post hoc* analysis re-

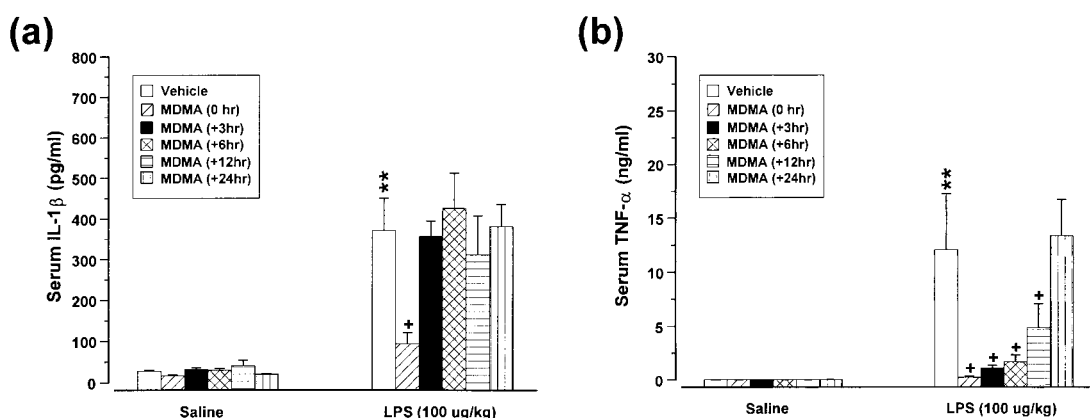


Fig. 1. Effect of acute MDMA (20 mg/kg; i.p.) administration on (a) IL-1 β and (b) TNF- α secretion following an *in vivo* challenge with LPS (100 μ g/kg; i.p.): A timecourse study. MDMA was either co-administered (0 hr) or administered 3 hr, 6 hr, 12 hr or 24 hr prior to the LPS challenge. Rats were sacrificed 2 hr following the LPS challenge. Data expressed as means $\pm$ SEM ($n = 7-8$). ** $P < 0.01$ vs. Saline challenged counterparts, + $P < 0.01$ vs. Vehicle + LPS (Fishers LSD).

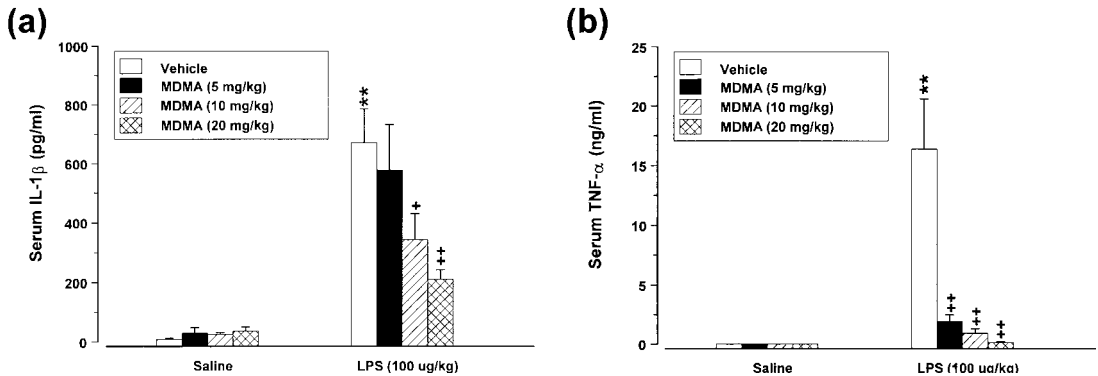


Fig. 2. Effect of acute MDMA administration (5, 10 and 20 mg/kg; i.p.) on (a) IL-1 β and (b) TNF- α secretion following an *in vivo* challenge with LPS (100 μ g/kg; i.p.). MDMA was co-administered with LPS. Rats were sacrificed 2 hr following the LPS challenge. Data expressed as means $\pm$ SEM ($n = 7-8$). ** $P < 0.01$ vs. Saline challenged counterparts, + $P < 0.05$, ++ $P < 0.01$ vs. Vehicle + LPS (Fishers LSD).

vealed that LPS challenge provoked a large increase in circulating TNF- α ($P < 0.01$) in vehicle treated rats. All doses of MDMA produced a profound suppression of LPS-induced TNF- α secretion (Fig. 2b).

Serum drug concentrations

Serum MDMA concentrations were measured 2, 5, 8, 14, and 26 hr following drug administration (20 mg/kg). In addition, serum MDMA concentrations were measured 2 hr following administration of MDMA (5, 10 and 20 mg/kg). MDMA was not detected in serum samples from vehicle treated animals, however MDMA was detected and demonstrated both dose- and time-dependency in the MDMA treated groups (Fig. 3).

Effect of *in vitro* exposure to MDMA on LPS-stimulated IL-1 β and TNF- α production in diluted whole blood

One-way ANOVA indicated that *in vitro* exposure to MDMA at concentrations equivalent to the serum levels achieved following *in vivo* administration did not significantly alter LPS-stimulated IL-1 β or TNF- α production in diluted whole blood (Table 1).

Discussion

In the present series of studies we observed that LPS induced a large increase in circulating concentrations of the proinflammatory cytokines interleukin (IL)-1 β and tumour necrosis factor (TNF)- α in vehicle treated animals as previously described [12,13,27]. MDMA administration provoked a significant impairment of these LPS-induced responses. The effect of MDMA on IL-1 β was transient in that it had returned to control within 3 hr of MDMA administration. In contrast, the MDMA-induced suppression of TNF- α secretion was evident for 12 hr following MDMA administration. Although IL-1 β and TNF- α are both macrophage-derived proinflammatory cytokines, the results demonstrate a differential sensitivity of these

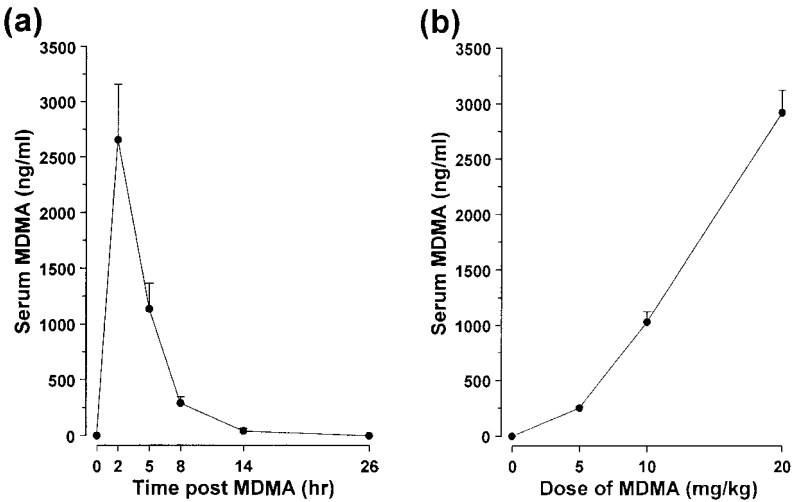


Fig 3. Effect of (a) MDMA (20 mg/kg; i.p.) administered 2, 5, 8, 14 and 26 hr prior to sacrifice and (b) MDMA (5, 10 and 20 mg/kg; i.p.) administered 2 hr prior to sacrifice on serum MDMA concentrations. Data expressed as means $\pm$ SEM (n = 7–8). Drug concentrations were measured by HPLC.

cytokines to the suppressive effects of MDMA, in that the suppression of TNF- α persisted for a much longer duration than that of IL-1 β . The dose of MDMA selected in Study I (20 mg/kg) was chosen based on previous data generated in this laboratory demonstrating that this dose of MDMA provoked a suppression of circulating leucocyte numbers and impaired the ability of lymphocytes to proliferate in response to a mitogenic stimulus [8]. In the second study, we examined the ability of a number of doses of MDMA (5, 10, and 20 mg/kg) to suppress IL-1 β and TNF- α secretion when co-administered with LPS. Co-administration of

Table 1
Effect of *in vitro* exposure to MDMA on LPS-stimulated IL-1 β and TNF- α production in diluted whole blood cultures

MDMA conc. (ng/ml)	IL-1 β (pg/ml)	TNF- α (pg/ml)
0	137 $\pm$ 21	517 $\pm$ 41
10	166 $\pm$ 34	639 $\pm$ 33
50	122 $\pm$ 16	591 $\pm$ 27
100	143 $\pm$ 18	623 $\pm$ 94
250	159 $\pm$ 15	601 $\pm$ 80
500	158 $\pm$ 19	676 $\pm$ 81
1000	161 $\pm$ 21	673 $\pm$ 90
2500	124 $\pm$ 18	567 $\pm$ 52
5000	165 $\pm$ 38	679 $\pm$ 105
10000	142 $\pm$ 25	608 $\pm$ 67

Data expressed as means $\pm$ SEM (n = 5). LPS-stimulated diluted whole blood was incubated for 48 hr in the presence of MDMA.

MDMA and LPS was used in study II as the results of study I demonstrated that a maximal suppression of both IL-1 β and TNF- α was observed when MDMA was co-administered with LPS as opposed to it being administered at timepoints prior to LPS administration. The data generated from study II demonstrate that all three doses of MDMA examined (5, 10 and 20 mg/kg) provoked a profound suppression (88–94%) of LPS-induced TNF- α secretion without any evidence of a dose response relationship. In contrast, MDMA administration provoked a dose-dependent suppression of IL-1 β secretion in response to the LPS challenge (20 mg/kg: 68%; 10 mg/kg: 49%; 5 mg/kg: 14%) with only the two higher doses of MDMA significantly altering LPS-induced IL-1 β secretion. These data demonstrate that LPS-induced secretion of TNF- α is much more susceptible to the suppressive effects of MDMA than is LPS-induced secretion of the related proinflammatory cytokine IL-1 β . Previous studies have also demonstrated differential effects of drug treatments on IL-1 β and TNF- α secretion. For example, one study demonstrated a differential response of IL-1 β and TNF- α following exposure to selegiline, a drug used in the treatment of Parkinson's disease [28]. The *in vitro* study revealed that treatment of human peripheral blood mononuclear cells with the monoamine oxidase-B inhibitor selegiline, increased the synthesis of IL-1 β , but reduced that of TNF- α [28]. Furthermore, in a recent study we found that following an LPS challenge in rats, TNF- α was more sensitive to the suppressive effects of a single administration of a high dose of the tricyclic antidepressant imipramine than IL-1 β [13]. The mechanism by which drugs can differentially modulate IL-1 β and TNF- α is presently unknown. However the results of our *in vitro* studies suggest that the suppressive effect of MDMA on IL-1 β and TNF- α following an *in vivo* LPS challenge could not be attributed to a direct effect on immune cells. This is in accordance with a previous study which reported that *in vitro* administration of MDMA in the same dose range as used in the present study did not significantly alter LPS-stimulated TNF- α production in murine macrophages [29]. In contrast to the *in vitro* situation, *in vivo* studies must take into account the contribution of physiological elements such as glucocorticoids, catecholamines and antiinflammatory cytokines such as IL-10 and TGF- β , which all demonstrate the ability to inhibit LPS-induced secretion of IL-1 β and/or TNF- α [30–35].

The differential effect of MDMA on TNF- α and IL-1 β secretion is of interest in so far as the kinetics of secretion of these monokines following an LPS challenge differs greatly [27,35]. For example, whilst LPS induces the transcription and secretion of both IL-1 β and TNF- α , the kinetics of mRNA appearance would suggest differences in the mechanisms of activation [35]. It is noteworthy that TNF- α secretion appears to be more sensitive to the suppressive effects of glucocorticoids than is IL-1 β [see 35]. Thus, it is possible that the well documented ability of MDMA to activate the hypothalamic pituitary adrenal axis and thereby increase circulating glucocorticoid concentrations [8,9,24,36] may have had a greater impact on LPS-induced TNF- α secretion in comparison to LPS-induced IL-1 β secretion. However we must also acknowledge that MDMA activates the sympathetic nervous system (SNS) [37]. Thus it is reasonable to suggest that the suppressive effect of MDMA on IL-1 β and TNF- α may be mediated by this mechanism as previous studies indicate that increased SNS activity can suppress LPS-induced proinflammatory cytokine production [31]. In addition to HPA and SNS activation, it is possible that MDMA could alter the secretion of other negative immunoregulatory substances such as IL-10 or β -endorphin. However as of yet the effect of MDMA on such substances remains to be examined and will be the focus of further studies.

Although it is difficult to equate drug doses in rats and humans due to the different routes of administration used (oral in humans, and i.p. in the present studies), and different rates of metabolism between species, the doses of MDMA used in the present experiments are within the range which are used by humans that abuse the drug. Reports indicate that a wide range of doses are ingested by humans—anything ranging from about 1 to 30 mg/kg [5]. Moreover, the serum concentrations of MDMA in rats treated with 5 mg/kg (253 ng/ml) and 10 mg/kg (1031 ng/ml) are within the range reported following administration of MDMA in humans. For example, a 1.5 mg/kg human oral dose of MDMA yielded a peak plasma concentration of 331 ng/ml MDMA 2 hr following administration [38]. Also, in the context of the dose range reported in humans by Schifano, (1995) [5], circulating MDMA concentrations would be expected to easily reach 1031 ng/ml, as measured following 10 mg/kg MDMA in the present study. It should also be noted from the timecourse study that the serum concentrations of MDMA appear to be more closely related to the suppression of TNF- α as opposed to that of IL-1 β . However as a direct suppressive effect of MDMA on immune cells has been ruled out by our *in vitro* studies, the relevance of this finding is as of yet unclear and requires further study.

In conclusion, these data provide evidence that acute administration of MDMA at doses that yield circulating concentrations similar to those reported in human MDMA abusers, impairs the production of the proinflammatory cytokines IL-1 β and TNF- α following an *in vivo* LPS challenge. Although IL-1 β and TNF- α are both macrophage-derived proinflammatory cytokines, the present study demonstrates that TNF- α is more sensitive to the suppressive effects of MDMA than is IL-1 β . These data also demonstrate that a critical 12 hr period following acute MDMA administration exists where impaired immunocompetence is maximal. It is possible that such a drug-induced suppression of TNF- α could prevent normal T cell proliferation or possibly lead to an abnormal immune response in times of infection or illness. In this regard it was previously demonstrated that administration of d-amphetamine the parent compound of MDMA increased the susceptibility to infection by influenza A virus and the bacteria *Listeria monocytogenes* [39,40]. Moreover, there have been cases where abuse of MDMA in humans closely preceded the development of meningococcal meningitis [41].

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