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Short communication

Methylenedioxymethamphetamine-induced suppression of interleukin-1β and tumour necrosis factor-α is not mediated by serotonin

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Abstract

The purpose of the present study was to examine the role of serotonin release in methylenedioxymethamphetamine (MDMA)-induced immunosuppression in rats. We examined the effect of pretreatment with the selective serotonin reuptake inhibitor paroxetine, and the tryptophan hydroxylase inhibitor *para*-chlorophenylalanine on MDMA-induced suppression of interleukin-1β and tumour necrosis factor (TNF)-α secretion following an in vivo lipopolysaccharide challenge. Although paroxetine blocked MDMA-induced serotonin depletion in the cortex and hypothalamus, it failed to alter the suppressive effect of MDMA on lipopolysaccharide-induced TNF-α secretion. Similarly, although *para*-chlorophenylalanine caused a 90% depletion in cortical and hypothalamic serotonin content, it failed to alter the suppressive effect of MDMA on lipopolysaccharide-induced interleukin-1β or TNF-α secretion. In conclusion, although MDMA is a potent releaser of serotonin, the suppressive effects of MDMA on lipopolysaccharide-induced proinflammatory cytokine secretion cannot be attributed to its serotonin-releasing properties.

Keywords: Cytokine; Interleukin-1β; Immunity; MDMA (methylenedioxymethamphetamine); 5-HT (5-hyperoxytryptamine, serotonin); TNF-α (tumour necrosis factor-α)

1. Introduction

Methylenedioxymethamphetamine (MDMA; "Ecstasy") is a ring-substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. Due to its unique behavioural activating properties, MDMA is currently a popular drug of abuse in humans (Hegadoren et al., 1999). We recently reported that administration of MDMA (at doses that yield circulating drug concentrations equivalent to those observed in humans), suppresses a number of aspects of immunity in rats (Connor et al., 1999a, 2000a,b). Moreover, a recent clinical study has demonstrated that MDMA administration produces immunosuppressive effects in humans (Pacifici et al., 2001).

The immunological effects of MDMA administration include a reduction in the number of circulating lymphocytes, a suppression of T-lymphocyte proliferation and changes in Th_1 and Th_2 type cytokine production (Connor et al., 1999a, 2000a). In addition, MDMA impairs the ability of rats to respond to an in vivo challenge with bacterial lipopolysaccharide as indicated by a reduction in interleukin-1β and tumour necrosis factor (TNF)-α production (Connor et al., 2000b). This challenge is a useful way of assessing immunocompetence in laboratory animals as it essentially mimics the initial phase of a bacterial infection. When lipopolysaccharide is injected systemically, it binds to CD 14 on monocyte membranes and stimulates the production of proinflammatory cytokines such as interleukin-1 and TNF-α, which are important signalling molecules in initiating and coordinating a large range of immune responses against invading pathogens (Hamblin, 1994; Henderson, 1994).

Recently, we demonstrated that in vitro incubation of MDMA with lipopolysaccharide-stimulated diluted whole blood cultures did not suppress interleukin-1β or TNF-α production (Connor et al., 2000b). This suggests that the suppressive effect of MDMA on interleukin-1β and TNF-α following an in vivo lipopolysaccharide challenge could not be accounted for by a direct effect of the drug on immune cell function.

In addition to having a direct effect on immune cells, it is possible that a drug such as MDMA, which alters neurotransmission within the central nervous system (CNS) could alter immunocompetence via a CNS-mediated mechanism. Specifically, it is well established that changes in

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CNS neurotransmitter function can alter immunity via changes in endocrine output and sympathetic nervous system activity (see Dantzer and Kelley, 1989). The main neurochemical action of MDMA is that it is a potent releaser of serotonin within the CNS (Hegadoren et al., 1999). Consequently, the objective of the present study was to evaluate the role of serotonin release in MDMA-induced immunosuppression.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing approximately 250–350 g were obtained from a Departmental breeding colony and housed four per cage. The rats were maintained on a 12:12-h light:dark cycle (lights on at 0800 h) in a temperature-controlled room (22–24°C) and food and water were available ad libitum at all times. The experimental protocols were in compliance with the European Communities Council directive (86/609/EEC).

2.2. The effect of pretreatment with paroxetine on MDMA-induced immunosuppression

Paroxetine (SmithKline Beecham, UK) (7.5 mg/kg) or vehicle (20% dimethylsulphoxide) were administered subcutaneously 30 min prior to MDMA (10 mg/kg) intraperitoneally (i.p.). Lipopolysaccharide (100 μg/kg; i.p.) was co-administered with MDMA and animals were sacrificed by decapitation 2 h following lipopolysaccharide administration. The dose of paroxetine used in the present study was chosen based on pilot data indicating that paroxetine 7.5 mg/kg (s.c.) administered 30 min prior to MDMA blocked MDMA-induced serotonin depletion in a number of brain regions. The dose of MDMA used in the present study was chosen as it is on the ascending limb of the MDMA dose–response curve for serotonin depletion and also yields circulating serum concentrations, which are in the same range as those achieved in human abusers of the drug (Connor et al., 2000b). Brain and serum samples were collected for neurotransmitter and cytokine measurements, respectively.

2.3. The effect of pretreatment with p-chlorophenylalanine on MDMA-induced immunosuppression

Para-chlorophenylalanine methyl ester HCl (Sigma, UK) (150 mg/kg; i.p.) or vehicle (0.89% NaCl) were administered once daily for three consecutive days by the i.p. route. The animals were administered MDMA (10 mg/kg; i.p.) or vehicle (0.89% NaCl) 72 h after the last injection. We have previously demonstrated that this treatment regimen of *p*-chlorophenylalanine produces a 90% depletion of brain serotonin concentrations in the rat (Cryan et al., 2000). Lipopolysaccharide (100 μg/kg; i.p.) was administered 1 h following MDMA and animals were sacrificed by decapitation 2 h following lipopolysaccharide administration. Brain and serum samples were collected for neurotransmitter and cytokine measurements, respectively.

2.4. Lipopolysaccharide challenge and serum preparation

All animals were challenged with lipopolysaccharide from *Escherichia coli* (serotype 0111:B4) (Sigma) (100 μg/kg) administered in a 1 ml/kg injection volume by the intraperitoneal (i.p.) route. Two hours following the lipopolysaccharide challenge, animals were sacrificed by decapitation and trunk blood was collected. We have previously found that this dose and route of administration of lipopolysaccharide produces quantifiable increases in circulating interleukin-1β and TNF-α concentrations and is optimal for simultaneous sampling of these two cytokines (Connor et al., 2000b). Following collection, blood samples were centrifuged ($800 \times g$ at 4°C for 15 min) and aliquots of serum were removed for determination of interleukin-1β and TNF-α. Serum samples were stored at −20°C until the assays were performed.

2.5. Serum interleukin-1β and TNF-α measurements

Serum interleukin-1β and TNF-α concentrations were determined using specific rat ELISA sandwich assays performed using antibodies and standards obtained from Dr. S. Poole (NIBSC, UK) as previously described (Connor et al., 2000b). Absorbance was read at 450 nm on a microtitre plate reader (Elx 800, Bio-Tek instruments) and results were expressed as pg interleukin-1β/ml of serum or ng TNF-α/ml of serum.

2.6. Determination of hypothalamic and cortical serotonin concentrations

The rats were sacrificed by decapitation. After sacrifice, the brains were rapidly removed and the left frontal cortex and hypothalamus were dissected on an ice-cold plate. Serotonin concentrations were measured by high performance liquid chromatography coupled with electrochemical detection as previously described (Connor et al., 1999b). Brain tissue was sonicated in 1 ml of mobile phase that was spiked with 20 ng/50 μl of *N*-methyl dopamine (Sigma) 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, UK), 1.4 mM octane-1-sulphonic acid (Sigma) and 10% (v/v) methanol (Lab-Scan, Ireland) and was adjusted to pH 2.8 using 4N NaOH (BDH Chemicals). Homogenates were centrifuged at 12,000 rpm in a Hettich Mikro/K refrigerated centrifuge for 15 min. A 20-μl sample of each supernatant was injected onto a reverse phase column (LI Chrosorb

RP-18, 25 cm × 4 mm internal diameter, particle size 5 mm) for separation of serotonin (flow rate 1 ml/min). Serotonin concentrations were quantified by electrochemical detection (Shimadzu) and chromatograms were generated using a Merck-Hitachi D-2000 integrator. Results were expressed as ng of serotonin per g fresh weight of brain tissue.

2.7. Statistical analysis of data

The cytokine and neurotransmitter data were analysed using a two-way analysis of variance. If any statistical significant change was found, post hoc comparisons were performed using Fishers least significant difference (LSD) multiple comparisons. Data were deemed significant when $P < 0.05$. Data are expressed as group mean with standard errors.

3. Results

3.1. Paroxetine study

3.1.1. Cortical and hypothalamic serotonin concentrations

There was a significant MDMA × paroxetine interaction on serotonin concentrations in the frontal cortex [$F(1,28) = 12.67$, $P < 0.01$] and hypothalamus [$F(1,28) = 10.09$, $P < 0.01$]. Post hoc analysis revealed a reduction ($P < 0.01$) in serotonin concentrations in response to MDMA administration in both the frontal cortex and hypothalamus. This effect of MDMA on cortical and hypothalamic serotonin concentrations was blocked by pretreatment with paroxetine (Table 1).

3.1.2. Interleukin-1β

There was a significant effect of MDMA administration on serum interleukin-1β concentrations [$F(1,24) = 10.80$, $P < 0.01$]. Post hoc analysis revealed that MDMA treatment suppressed ($P < 0.01$) lipopolysaccharide-induced interleukin-1β production. Acute paroxetine pretreatment also produced a modest but significant ($P < 0.05$) reduction in lipopolysaccharide-induced interleukin-1β secretion. Consequently, the animals that received paroxetine alone were not significantly different from those pretreated with paroxetine prior to MDMA administration (Fig. 1A).

3.1.3. Tumour necrosis factor-α

There was a significant MDMA × paroxetine interaction [$F(1,24) = 6.08$, $P < 0.05$] on serum TNF-α concentrations. Post hoc analysis revealed that MDMA treatment provoked a suppression ($P < 0.01$) of lipopolysaccharide-induced TNF-α secretion. Paroxetine pretreatment alone also suppressed ($P < 0.01$) lipopolysaccharide-induced TNF-α secretion; however, pretreatment with paroxetine did not block the MDMA-induced suppression of TNF-α (Fig. 1B).

3.2. p-chlorophenylalanine study

3.2.1. Cortical and hypothalamic serotonin concentrations

There was a significant MDMA × *p*-chlorophenylalanine interaction on serotonin concentrations in the frontal cortex [$F(1,32) = 35.91$, $P < 0.0001$] and hypothalamus [$F(1,32) = 47.37$, $P < 0.0001$]. Post hoc analysis revealed a reduction ($P < 0.01$) in serotonin concentrations in both the frontal cortex (96%) and hypothalamus (91%) in *p*-

Table 1
Cortical and hypothalamic serotonin concentrations following MDMA administration: effect of pretreatment with paroxetine or *p*-chlorophenylalanine
Paroxetine study: rats were challenged with MDMA (10 mg/kg; i.p.) 30 min following paroxetine treatment (7.5 mg/kg; s.c.) and sacrificed 2 h post-MDMA administration.
p-Chlorophenylalanine study: rats were challenged with MDMA (10 mg/kg; i.p.) 72 h following *p*-chlorophenylalanine treatment (150 mg/kg per day for 3 days; i.p.). Rats were sacrificed 3 h post-MDMA administration.
Data expressed as means with standard errors ($n = 8$–10).

	Frontal cortex (serotonin, ng/g of tissue)	Hypothalamus (serotonin, ng/g of tissue)
Paroxetine study		
Saline + Vehicle	529 ± 33	1199 ± 29
Saline + MDMA	222 ± 23[a]	758 ± 47[a]
Paroxetine + Vehicle	552 ± 44	1355 ± 46
Paroxetine + MDMA	509 ± 44	1237 ± 72
p-chlorophenylalanine study		
Saline + Vehicle	602 ± 30	1247 ± 38
Saline + MDMA	292 ± 38[a]	843 ± 35[a]
p-chlorophenylalanine + Vehicle	25 ± 5[a]	118 ± 10[a]
p-chlorophenylalanine + MDMA	3 ± 3[b]	88 ± 8[b]

[a] $P < 0.01$ vs. Saline + Vehicle.
[b] $P < 0.05$ vs. *p*-chlorophenylalanine + Vehicle (Fishers LSD).

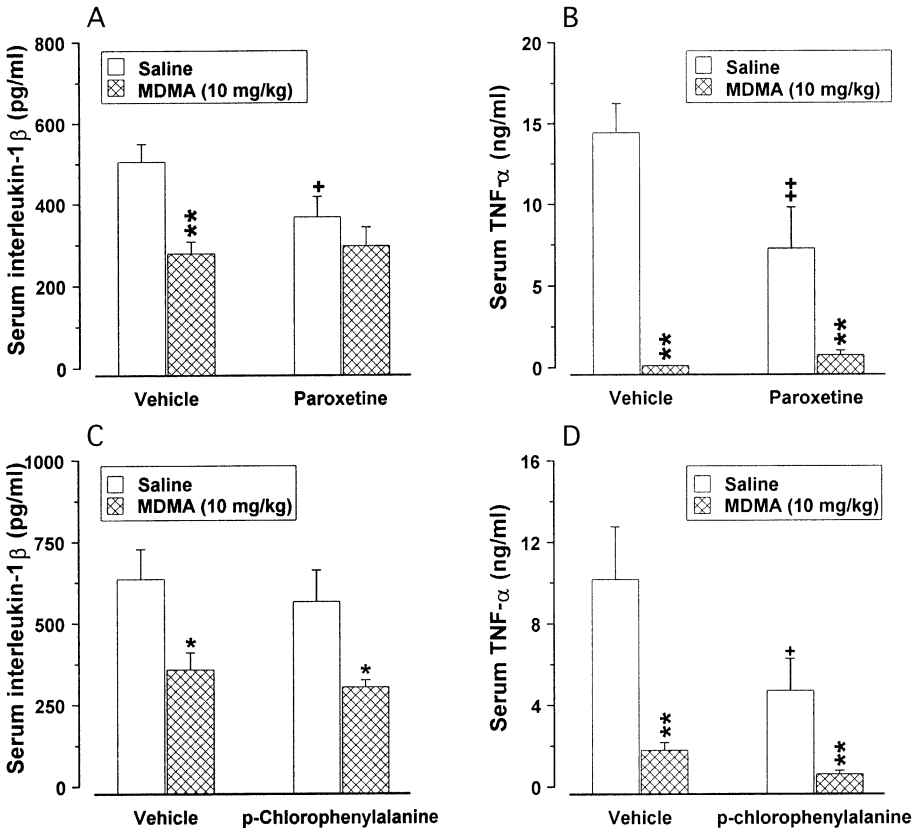

Fig. 1. Effect of paroxetine pretreatment (A,B) and *p*-chlorophenylalanine pretreatment (C,D) on MDMA-induced suppression of interleukin-1β (A,C) and TNF-α (B,D) following an in vivo lipopolysaccharide challenge. Data expressed as means with standard errors ($n = 6$–10). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. saline-treated counterparts. $+P < 0.05$, $++P < 0.01$ vs. Saline + Vehicle (Fishers LSD).

chlorophenylalanine-treated animals. In addition, MDMA administration produced a reduction ($P < 0.01$) in both cortical and hypothalamic serotonin concentrations, and further enhanced the *p*-chlorophenylalanine-induced serotonin depletion in both brain regions (Table 1).

3.2.2. Interleukin-1β

There was a significant effect of MDMA administration on serum interleukin-1β concentrations [$F(1,30) = 10.13$, $P < 0.01$]. Post hoc analysis revealed that MDMA treatment suppressed ($P < 0.05$) lipopolysaccharide-induced interleukin-1β production, which was not significantly altered by pretreatment with *p*-chlorophenylalanine (Fig. 1C).

3.2.3. Tumour necrosis factor-α

There was a significant effect of MDMA administration [$F(1,28) = 19.03$, $P < 0.001$] and *p*-chlorophenylalanine pretreatment [$F(1,28) = 4.83$, $P < 0.05$] on serum TNF-α concentrations. Post hoc analysis revealed that MDMA treatment suppressed ($P < 0.01$) lipopolysaccharide-induced TNF-α production, which was not significantly altered by pretreatment with *p*-chlorophenylalanine. In addition, *p*-chlorophenylalanine pretreatment caused a reduction ($P < 0.05$) in lipopolysaccharide-stimulated TNF-α secretion (Fig. 1D).

4. Discussion

In order to evaluate the role of serotonin release in MDMA-induced immunosuppression, we examined the effect of pretreatment with the selective serotonin reuptake inhibitor paroxetine, and the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine, on MDMA-induced suppression of interleukin-1β and TNF-α secretion following an in vivo lipopolysaccharide challenge in rats. Paroxetine pretreatment prevents MDMA from entering serotonergic neurons thus preventing MDMA-induced serotonin release. This was evident in the present study in that paroxetine pretreatment completely blocked MDMA-induced serotonin depletion in both the frontal cortex and hypothalamus. Nonetheless, paroxetine pretreatment failed to alter the suppressive effects of MDMA on lipopolysaccharide-induced TNF-α secretion. Paroxetine treatment alone suppressed both lipopolysaccharide-induced interleukin-1β and TNF-α secretion by 27% and 50%, respectively, but there was no additive effect of the combination treatment. This finding is consistent with the results of a previous study

conducted in our laboratory where acute paroxetine treatment (7.5 mg/kg; i.p) suppressed interleukin-1β and TNF-α secretion when administered 1 h prior to an in vivo lipopolysaccharide challenge (Nolan, 1999).

It is also possible that MDMA can enter serotonergic neurons independently of the serotonin transporter by passive diffusion and stimulate serotonin release. Therefore, we also examined the effect of pretreatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine on MDMA-induced suppression of interleukin-1β and TNF-α secretion following a lipopolysaccharide challenge. The treatment regimen of *p*-chlorophenylalanine used caused in excess of a 90% depletion of brain serotonin concentrations. However, the suppressive effects of MDMA on lipopolysaccharide-induced interleukin-1β and TNF-α was equivalent in both saline and *p*-chlorophenylalanine-treated groups. These data further imply that the immunosuppressive effects of MDMA occur by a mechanism(s) independent of serotonin release. It is also of interest that *p*-chlorophenylalanine-treated animals displayed a reduced TNF-α response to lipopolysaccharide. This suggests that physiological levels of serotonin may facilitate lipopolysaccharide-induced macrophage TNF-α secretion. In this regard, it has been reported that serotonin is required for optimal accessory function of monocytes and macrophages (see Mossner and Lesch, 1998).

It is of interest that previous studies demonstrated that depletion of serotonin concentrations with *p*-chlorophenylalanine attenuated the locomotor stimulant effect of MDMA (Callaway et al., 1990). In addition, a study from this laboratory reported that paroxetine pretreatment blocked both the serotonin depleting and behaviourally activating properties of MDMA (McGarvey, 1995). This is consistent with the ability of other selective serotonin reuptake inhibitors such as fluoxetine, sertraline and zimelidine to block MDMA-induced locomotor hyperactivity (Callaway et al., 1990). It is also noteworthy that in the present study upon visual examination, *p*-chlorophenylalanine-pretreated rats that received MDMA appeared less hyperactive and sweated less than vehicle-treated animals that received the MDMA challenge. Thus, it is of interest, as although pretreatment with either paroxetine or *p*-chlorophenylalanine appears to attenuate the behavioural effects of MDMA, the immunosuppressive effects were still evident. In this regard, we recently demonstrated that the non-psychostimulant amphetamine derivative fenfluramine has potent immunosuppressive properties following acute administration (Connor et al., 2000a), supporting the view that the psychoactive and immunosuppressive properties of substituted amphetamines are not necessarily linked. In addition to the potent serotonin-releasing properties of MDMA, it is also well established that MDMA releases dopamine within the CNS, although with less potency (Koch and Galloway, 1997). Thus, it is possible that dopamine release may play a role in the immunosuppressive effects of MDMA. In this regard, it was previously demonstrated that d-amphetamine, a psychostimulant that is a potent dopamine releaser, elicits immunosuppressive effects in rodents (Freire-Garabal et al., 1992; Pezzone et al., 1992).

In conclusion, although MDMA is a potent releaser of serotonin, the suppressive effects of MDMA on lipopolysaccharide-induced proinflammatory cytokine secretion could not be attributed to its serotonin-releasing properties.

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