

Role of Hypothermia in the Mechanism of Protection Against Serotonergic Toxicity. I. Experiments using 3,4-Methylenedioxymethamphetamine, Dizocilpine, CGS 19755 and NBQX

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ABSTRACT

High doses of 3,4-methylenedioxymethamphetamine (MDMA) have been shown to cause long-lasting depletions of central serotonin (5-HT) which are indicative of neuronal toxicity. The noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (DZ) attenuates depletions of 5-HT induced by MDMA. Because DZ has been shown to induce hypothermia in rat models of ischemia, the purpose of this study was to assess whether DZ and two other glutamate antagonists, CGS 19755 (CGS) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), protect against MDMA-induced 5-HT depletions by induction of hypothermia. Male Sprague-Dawley rats were injected with either saline (SAL), DZ (2.5 mg/kg), CGS (25.0 or 50.0 mg/kg $\times$ 2 injections) or NBQX (30.0 mg/kg $\times$ 2 injections) or 55.0 mg/kg $\times$ 3 injections) followed by either MDMA (40.0 mg/kg) or SAL. Core body temperature (TEMP) was monitored for 4 h or longer using radiotelemetry. Base-line TEMP was between 37.0 and 37.6°C. Administration of DZ with MDMA decreased TEMP to $34.0 \pm 0.39^\circ\text{C}$ within 2 h of the

MDMA injection, and also protected against serotonergic toxicity. Neither SAL/MDMA nor DZ/SAL had an effect on TEMP over the same period. When rats were treated with DZ/MDMA and TEMP was maintained between 38.4°C and 40.4°C for 4 h, protection against 5-HT depletion was abolished. Coadministration of the competitive NMDA antagonist CGS with MDMA resulted in a decrease in TEMP to $34.5 \pm 0.27^\circ\text{C}$, and provided partial protection against 5-HT depletions. When the AMPA receptor antagonist NBQX was administered with MDMA, TEMP did not differ from rats treated with SAL/MDMA, nor did NBQX protect against 5-HT depletions. The data from this study show that coadministration of NMDA antagonists with MDMA induces hypothermia in dose combinations which protect against serotonergic toxicity, and neuroprotection by DZ is abolished when TEMP is maintained above 38.4°C . These data indicate that hypothermia induced by NMDA receptor antagonism plays a role in protection against serotonergic toxicity.

During the last two decades, epidemic use of substituted amphetamines in Japan (Yukitake 1983), Sweden (Inghe 1969), Great Britain (Lancet, 1968) and the United States (Kramer *et al.*, 1967) has prompted investigation into the long-lasting effects of high-dose psychostimulant abuse. Although cocaine use has surpassed abuse of amphetamines in recent years, the popularity of all-night "rave" parties has risen in Great Britain and the United States, and at these parties 3,4-methylenedioxymethamphetamine (MDMA) is the drug of choice (Randall 1992a, 1992b). Evidence compiled from rats, mice, guinea pigs and rhesus monkeys shows that MDMA is toxic to serotonin (5-HT) neurons with repeated administration of 2 to 4 times the behaviorally effective dose

(Commins *et al.*, 1987b; Schmidt 1987). Although the functional consequences of MDMA-induced toxicity to 5-HT neurons is not well understood, it is important to investigate the mechanism of this toxicity and to look for ways to protect against the damage, because of the renewed popularity of MDMA.

Evidence gathered with multiple neuronal markers shows that MDMA is toxic to 5-HT neurons. High-dose regimens of MDMA produce long-lasting decreases in 5-HT concentration (Commins *et al.*, 1987b; Stone *et al.*, 1986) and in the activity of its rate-limiting synthetic enzyme, tryptophan hydroxylase (TPH) (Schmidt and Taylor 1988). Also, MDMA has been shown to decrease both high-affinity 5-HT uptake sites (Battaglia *et al.*, 1988; Wagner and Peroutka 1990) and uptake of [^3H]5-HT (Schmidt 1987). Morphological degeneration of 5-HT neurons has been documented with the Fink-Heimer

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ABBREVIATIONS: NMDA, N-methyl-D-aspartate; DZ, dizocilpine (MK-801); CGS, CGS 19755; MDMA, 3,4-methylenedioxymethamphetamine; TEMP, core body temperature; 5-HT, serotonin; 5-HIAA, 5-hydroxyindole acetic acid; 5,6-DHT, 5,6-dihydroxytryptamine; DA, dopamine; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; SAL, saline.

stain (Commins *et al.*, 1987b; Ricaurte *et al.*, 1985) and immunocytochemistry techniques (O'Hearn *et al.*, 1988).

One current theory of the mechanism of serotonergic toxicity is the oxidative stress model, which hypothesizes that 5-HT, released by a compound such as MDMA, is converted to the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) via a Fenton reaction (Commins *et al.*, 1987a; Seiden and Vosmer 1984). 5,6-DHT is then taken back into the nerve terminal via the high-affinity uptake transporter where it causes neuronal degeneration. In support of this hypothesis, 5,6-DHT has been detected in rat brain after high doses of *p*-chloroamphetamine (Commins *et al.*, 1987a). Also, 5-HT uptake inhibitors prevent serotonergic toxicity induced by MDMA and by other substituted amphetamines such as *p*-chloroamphetamine, methamphetamine and fenfluramine (Berger *et al.*, 1992; Fuller 1980; Henderson and Fuller 1992; Hotchkiss and Gibb 1980; Schmidt *et al.*, 1987).

Several studies have demonstrated that N-methyl-D-aspartate (NMDA) receptor antagonists protect against serotonergic neurotoxicity induced by substituted amphetamines. DZ has been shown to attenuate MDMA-, methamphetamine- and *p*-chloroamphetamine-induced decreases in 5-HT levels and methamphetamine-induced decreases in TPH activity (Farfel *et al.*, 1992; Green *et al.*, 1992; Johnson *et al.*, 1989). Dextrorphan and dextromethorphan, morphine derivatives that block NMDA ligand-gated ion channels as well as voltage-gated ion channels, have also been shown to attenuate decreases in 5-HT due to MDMA and *p*-chloroamphetamine, respectively (Finnegan *et al.*, 1991, 1990). Furthermore, NMDA receptor antagonists protect against neuronal injury due to ischemia, hypoglycemia and seizures (Croucher *et al.*, 1982; Leander *et al.*, 1988; Simon *et al.*, 1984; Wieloch 1985) in addition to neurotoxicity induced by MDMA and related amphetamines.

NMDA receptor antagonists induce hypothermia under certain conditions (Buchan and Pulsinelli 1990; Corbett *et al.*, 1990). Conversely, both MDMA and methamphetamine have been shown to induce hyperthermia in rats or mice (Gordon *et al.*, 1991; Itoh *et al.*, 1986; Schmidt *et al.*, 1990a), and this hyperthermia correlates with the severity of neuronal toxicity (Bowyer *et al.*, 1992; Gordon *et al.*, 1991). Hypothermia may contribute to the mechanism of neuroprotection, because it could slow the rate of *in vivo* reactions such as the conversion of 5-HT to 5,6-DHT, or slow transporter-mediated release of 5-HT or uptake of 5,6-DHT into the nerve terminal. The purpose of the present study was to investigate whether administration of MDMA with an NMDA receptor antagonist induces hypothermia, and whether this hypothermia provides protection against serotonergic toxicity. The results show that combination of MDMA with the noncompetitive NMDA receptor antagonist, DZ, or the competitive antagonist CGS, induced hypothermia and provided protection against serotonergic toxicity. Furthermore, maintaining TEMP above 38.4°C after administration of DZ plus MDMA abolished neuroprotection. Coadministration of MDMA with an AMPA antagonist, NBQX, did not induce hypothermia nor did it protect against serotonergic toxicity.

Methods

Experiments in this study fall under two general protocols. (1) "Temperature" experiments measured changes in core body temper-

ature (TEMP) using radiotelemetry with group sizes ($n = 3-6$) that were appropriate for statistical comparison because the temperature data had a low coefficient of variability. (2) "Neurochemistry" experiments using group sizes ($n \geq 8$) which were appropriate for the statistical analysis of monoamine and metabolite levels.

Temperature Experiments

Subjects. Male Sprague-Dawley rats (225–250 g; Harlan, Indianapolis, IN) were housed individually in plexiglass cages (21 × 42 × 20 cm) in a room maintained at 20–22°C (unless otherwise specified) on a 12-h light-dark cycle (lights on from 0700 to 1900 h). Food (Teklad Rat and Mouse Diet, Teklad, Winfield, IA) and water were available *ad libitum* throughout the experiment, except during injection regimens. Rats were tested in groups of 8 or 12, and whenever possible, subjects in a particular dosing group (*e.g.*, SAL/MDMA) were not all tested the same day.

Transmitter implantation. Rats were anesthetized with Nembutal (pentobarbital sodium, 30–40 mg/kg i.p.; Abbott Laboratories, Chicago, IL) 30 min before surgery and supplemented with Metofane inhalation anesthetic (methoxyflurane, Pitman-Moore, Inc., Washington Crossing, NJ) as necessary. The abdominal area was scrubbed with E-Z Scrub (povidine iodine solution, Becton Dickinson, Franklin Lakes, NJ), shaved and wiped with 70% ethanol solution. A ventral midline incision, approximately 1.5 cm in length, was made through the skin and body wall and a sterilized temperature-sensitive radio-transmitter (model VM-FH; Minimitter Co., Inc., Sunriver, OR) was inserted through the incision. The body wall was then sutured and the skin closed with surgical staples. Rats were returned to the home cage after surgery and monitored until they woke up from the anesthesia.

Injections and temperature recordings. Rats were allowed a minimum of 16 h (overnight) to recover from surgery. The day after surgery, base-line measures of core body temperature (TEMP) were recorded at 1000, 1200, 1400, 1600 and 1800 h. TEMP recordings were made from outside the cage using a hand-held receiver (model RLA 3000, Minimitter Co., Inc.) connected to a frequency counter (Model SM-2372, Heath Co., Benton Harbor, MI) which picked up temperature-sensitive radio frequency signals emitted by the implanted transmitters. Frequencies were later converted to TEMP (°C) by use of conversion charts generated from calibration of each transmitter.

On the second day after surgery, base-line TEMP measurements were again recorded at 1000 and 1200 h. Immediately after the second base-line measurement, rats were injected with a glutamate antagonist and MDMA, or control injections. See figure and table legends for exact dose regimens and group *n* values. TEMP was recorded every 15 min during the injection schedule and for at least 4 h after the last drug injection.

Drugs were administered in a volume of 1.0 ml/kg in 0.9% saline vehicle (SAL) with two exceptions: DZ was dissolved in water, and NBQX was dissolved in a solution of 5.5% (w/v) glucose, sodium hydroxide and hydrochloric acid (0.1 or 0.01 M, depending on final volume). NBQX and its vehicle (VEH) control injections were administered in a volume of 6.5 ml/kg. All drug injections were given i.p., with the exception of MDMA and its control injections, which were given s.c. All doses of drugs were calculated for the salt forms. Dizocilpine ((+) MK-801 hydrogen maleate) was purchased from Research Biochemicals, Inc. (Natick, MA). 3,4-Methylenedioxymethamphetamine HCl, CGS 19755 (*cis*-4-(phosphonomethyl)-2-piperidine carboxylic acid) and NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoline) were generously provided by the National Institute on Drug Abuse, the Ciba-Geigy Corporation (Summit, NJ) and Novo Nordisk (Malov, Denmark), respectively.

In some experiments, rats' TEMP was maintained between 38.4 and 40.4°C throughout the injection regimen and the 4-h observation period in an attempt to reverse the protective effect of DZ. To keep TEMP elevated, room temperature was gradually increased to between 23.5 and 26.5°C after the 10:00 A.M. base-line TEMP measure-

ment. After the first injection, rats were transferred to group cages containing heating pads under bedding and towels. TEMP was monitored at least every 5 min, and rats were transferred to cages without heating pads as needed to prevent overheating ($TEMP > 40^{\circ}C$). After the 4-h observation period, rats were returned to their home cages and room temperature was gradually lowered to $20-22^{\circ}C$ over the next several hours.

In all temperature experiments, TEMP was recorded at 1000, 1200, 1400, 1600 and 1800 h on the day after injections. Approximately 72 h after the last drug injection, rats were sacrificed by decapitation and their brains dissected over ice according to the method of Heffner *et al.* (1980). Regions comprising striatum and hippocampus were stored in liquid nitrogen until assayed for 5-HT, 5-HIAA and DA using HPLC with electrochemical detection (Farfel *et al.*, 1992; Kotake *et al.*, 1985). Briefly, samples were eluted on stainless steel RSIL 5 μ m C18-HL ODS reverse-phase columns at a flow rate of 1 ml/min. The aqueous mobile phase contained 0.27 mM EDTA, 0.43 mM octylsodium sulfate, 7.9 mM Na_2HPO_4 , 62.5 mM citric acid and 19.5% methanol. Compounds were detected electrochemically by oxidation over a glassy carbon working electrode at a potential of +0.8 V relative to the Ag/AgCl reference electrode. Output from the detector (ESA Coulochem 5200, ESA Inc., or EG&G Model 400, Princeton Applied Research) was channeled to an integrator (Shimadzu CR5-A) which monitored peak retention times and calculated peak areas and values based on a two-point calibration of standards.

Between-group differences in TEMP over the entire test period were compared using two-way analysis of variance (ANOVA) for repeated measures. ANOVA tests were usually performed with the four treatment conditions as in the neurochemistry experiments; for example, SAL/SAL, DZ/SAL, SAL/MDMA, DZ/MDMA. Graphs of temperature data all have the same ordinate range for ease of comparison, $32-40^{\circ}C$. Other methods which were used to report TEMP data are: (1) numerical changes *vs.* a group's own base line, and (2) single-point comparisons between groups tested by one-way ANOVA. Differences in monoamine or metabolite concentrations between treatment groups were determined by one-way ANOVA followed by Student-Newman-Keuls *post hoc* comparisons. Statistics were calculated using SuperANOVA software (Abacus Concepts, Berkeley, CA) for Apple Macintosh computers. Statistical significance was considered to be $P < .05$.

Neurochemistry Experiments

Subjects. Male Long-Evans rats (200–225 g; Harlan, Indianapolis, IN) were housed individually in plexiglass cages ($21 \times 42 \times 20$ cm) in a room maintained at $20-22^{\circ}C$ on a 12-h light-dark cycle (lights on from 0700 to 1900 h). Food (Teklad Rat and Mouse Diet, Teklad, Winfield, IA) and water were available *ad libitum*, except during the injection regimen.

Procedure. On each test day, approximately 32 rats were randomly assigned to one of four treatment groups, receiving a glutamate receptor antagonist (*e.g.*, DZ) and MDMA or control injections in some combination. (See table legends for exact dose combinations and group *n* values.) All experiments began between 1000 and 1200 h so that the last injections were given at 1300 h.

Approximately 72 h after the last injection, rats were sacrificed by decapitation and their brains dissected as described under "Temperature Experiments." Differences in monoamine or metabolite concentrations between treatment groups in a single experiment were determined by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls *post hoc* comparisons with SuperANOVA software (Abacus Concepts, Berkeley, CA) for Apple Macintosh computers. Statistical significance was considered to be $P < .05$.

Results

3,4-Methylenedioxymethamphetamine and dizocilpine. Coadministration of DZ (2.5 mg/kg) with MDMA

(40.0 mg/kg) decreased TEMP ($F = 22.1$, $P < .0001$). TEMP reached $3.3^{\circ}C$ below base-line at 135 min (fig. 1) and returned to base-line level by the next day. Injection of MDMA with SAL did not affect TEMP significantly compared with SAL/SAL when comparison was made over the entire 4-h period using ANOVA for repeated measures. There was a decrease in TEMP in the SAL/MDMA group at 30 min which was statistically significant ($F = 44.4$, $P < .001$) when compared with base-line by one-way ANOVA.

In temperature rats, SAL/MDMA (40.0 mg/kg) depleted 5-HT and 5-HIAA to 63% and 65% of control, respectively, in hippocampus ($F = 6.92$, $P < .01$; $F = 15.9$, $P < .0001$). In striatum, 5-HT and 5-HIAA were 58% and 61% of control ($F = 3.32$, $P < .05$; $F = 5.06$, $P < .01$). Coadministration of DZ (2.5 mg/kg) protected against the depletions. These data agree with results from a neurochemistry experiment which were published previously (Farfel *et al.*, 1992).

Reversing protection by dizocilpine. Table 1 shows the 5-HT and 5-HIAA levels from temperature experiments in which TEMP was maintained between 38.4 and $40.4^{\circ}C$ during and after the injection regimen. In hippocampus, SAL/MDMA depleted 5-HT to 60% of the SAL/SAL group level ($F = 13.23$, $P \leq .001$), whereas DZ/MDMA depleted 5-HT to 35% of the SAL/SAL level. In striatum, SAL/MDMA depleted 5-HT to 44% of control level ($F = 11.53$, $P \leq .001$) whereas DZ/MDMA depleted 5-HT to 39% of control. SAL/MDMA depleted 5-HIAA in hippocampus as well ($F = 4.34$, $P \leq .05$). In striatum, no 5-HIAA changes were significant.

3,4-Methylenedioxymethamphetamine and CGS 19755. Administration of the competitive NMDA-receptor antagonist CGS (25.0 mg/kg $\times$ 2 injections) with MDMA caused a decrease ($F = 10.85$, $P < .001$) in TEMP of $2.9^{\circ}C$ at 160 min (fig. 2). Administration of CGS (50.0 mg/kg $\times$ 2 injections) with MDMA decreased TEMP in a similar manner, reaching $2.7^{\circ}C$ below base line at 240 min. There was no statistical difference between the two groups receiving CGS/MDMA. When CGS (25.0 mg/kg $\times$ 2 injections) was given with SAL, TEMP increased $1.4^{\circ}C$ above base line at 360 min. SAL/SAL data were not measured at this time point, precluding statistical comparison.

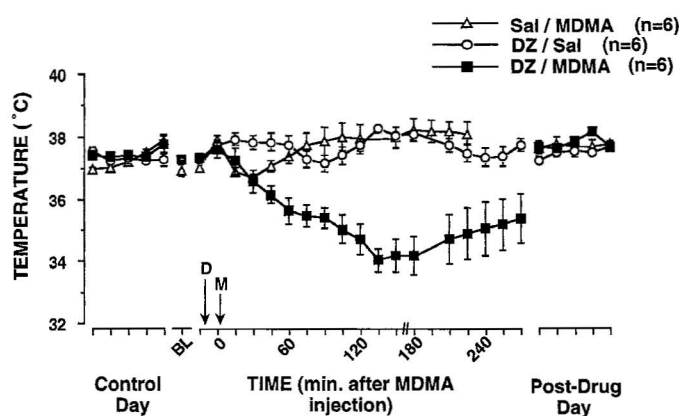


Fig. 1. Effects of dizocilpine and 3,4-methylenedioxymethamphetamine on TEMP in rats. Rats were administered either DZ (2.5 mg/kg) or SAL (1.0 ml/kg) followed 15 min later by MDMA (40.0 mg/kg) or SAL (1.0 ml/kg). D arrow represents DZ injection; M arrow represents MDMA injection; BL represents base-line on test day. DZ plus MDMA caused a significant, prolonged decrease in TEMP not seen with either drug alone.

TABLE 1

Levels of 5-HT and 5-HIAA in hippocampus and striatum after 3,4-methylenedioxymethamphetamine and dizocilpine, with body temperature maintained between 38.4 and 40.4°C

DZ (2.5 mg/kg) or SAL (1.0 ml/kg) was administered 15 min before MDMA (40.0 mg/kg) or SAL. 5-HT and 5-HIAA levels were measured 72 h after injections. Rats' TEMP was maintained between 38.4 and 40.4°C by elevating room temperature and housing rats in cages equipped with heating pads. Values represent means $\pm$ S.E.M. expressed in ng/mg wet weight tissue. Numbers in parentheses are group *n* values.

Dose (mg/kg)	Hippocampus		Striatum	
	5-HT	5-HIAA	5-HT	5-HIAA
SAL/SAL (5)	0.20 $\pm$ 0.01	0.16 $\pm$ 0.01	0.18 $\pm$ 0.01	0.20 $\pm$ 0.02
DZ/SAL (4)	0.21 $\pm$ 0.01	0.17 $\pm$ 0.01	0.16 $\pm$ 0.01	0.28 $\pm$ 0.08
SAL/MDMA (5)	0.12 $\pm$ 0.03*	0.11 $\pm$ 0.02*	0.08 $\pm$ 0.03*	0.15 $\pm$ 0.00
DZ/MDMA (6)	0.07 $\pm$ 0.01*	0.11 $\pm$ 0.01*	0.07 $\pm$ 0.02*	0.20 $\pm$ 0.03

* Different from SAL/SAL ($P < .05$).

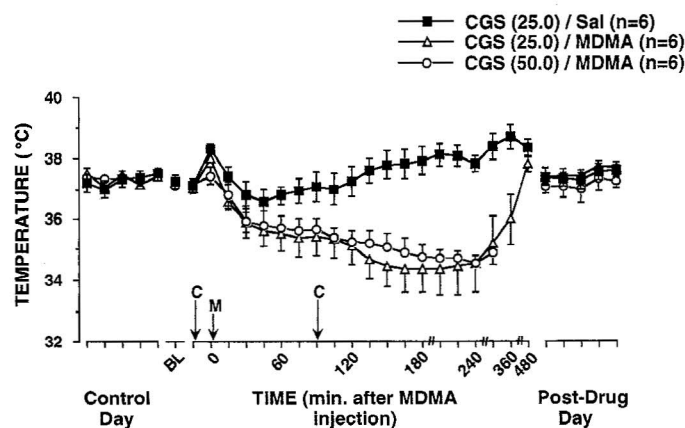


Fig. 2. Effects of CGS 19755 and 3,4-methylenedioxymethamphetamine on TEMP in rats. Rats were administered CGS (25.0 or 50.0 mg/kg) followed 15 min later by MDMA (40.0 mg/kg) or SAL (1.0 ml/kg). A second injection of CGS was administered 90 min after the MDMA or SAL. C arrows represent CGS injections; M arrow represents MDMA injection. CGS plus MDMA caused a significant decrease in TEMP not seen with CGS alone.

In neurochemistry experiments, SAL/MDMA (40.0 mg/kg) depleted hippocampal 5-HT to 37% ($F = 17.64$, $P < .0001$) of control whereas 5-HT was depleted to 58% of control when CGS (25.0 mg/kg $\times$ 2 injections) was coadministered with MDMA (table 2). In a second experiment, SAL/MDMA (40.0 mg/kg) depleted hippocampal 5-HT to 57% of control ($F = 9.48$, $P < .001$), and administration of the higher dose of CGS (50 mg/kg $\times$ 2 injections) with MDMA depleted 5-HT to only 71% of the control value. Thus, both doses of CGS provided

partial protection against MDMA-induced depletions of 5-HT and 5-HIAA.

In striatum, SAL/MDMA depleted 5-HT to 28% of control ($F = 10.56$, $P < .0001$), and with coadministration of CGS (25.0 mg/kg $\times$ 2 injections), 5-HT was 62% of control value. In the experiment with the 50.0 mg/kg dose of CGS, striatal 5-HT was 66% of control after SAL/MDMA, but this decrease was not significant ($F = 2.38$, $P = .09$). Coadministration of CGS (50.0 mg/kg $\times$ 2 injections) with MDMA resulted in 5-HT levels which were 99% of the SAL/SAL value.

SAL/MDMA decreased 5-HIAA in hippocampus ($F = 9.89$, $P < .001$; $F = 7.44$, $P < .001$) and striatum ($F = 13.24$, $P < .0001$; $F = 5.72$, $P < .01$) in the neurochemistry experiments. Coadministration of CGS (25.0 or 50.0 $\times$ 2 injections) protected against the decreases at both doses in hippocampus. In striatum, the 25.0 mg/kg dose partially attenuated the depletion (45% of control for SAL/MDMA vs. 80% of control for CGS/MDMA). The 50.0 mg/kg dose of CGS did not attenuate 5-HIAA depletion when administered with MDMA.

In the temperature experiments, analysis of 5-HT and 5-HIAA levels showed that both doses of CGS provided protection against MDMA-induced depletions, agreeing with the data from the neurochemistry experiments.

3,4-Methylenedioxymethamphetamine and NBQX. Coadministration of the AMPA receptor antagonist NBQX (30.0 mg/kg $\times$ 2 injections) with MDMA (40.0 mg/kg) did not have any significant effect on TEMP (fig. 3) when tested vs. SAL/SAL and SAL/MDMA. The effect of NBQX/MDMA on TEMP was very similar to the effect of SAL/MDMA, including a 1.9°C decrease in TEMP at 30 min, although the NBQX/

TABLE 2

Levels of 5-HT and 5-HIAA in hippocampus and striatum after 3,4-methylenedioxymethamphetamine and CGS 19755

CGS (25.0 or 50.0 mg/kg) or SAL (1.0 ml/kg) was administered 15 min before MDMA (40.0 mg/kg) or SAL, and a second injection of CGS or SAL was administered 90 min later. 5-HT and 5-HIAA levels were measured 72 h after injections. Values represent means $\pm$ S.E.M. expressed in ng/mg wet weight tissue. Numbers in parentheses are group *n* values.

Dose (mg/kg)	Hippocampus		Striatum	
	5-HT	5-HIAA	5-HT	5-HIAA
SAL/SAL (6)	0.19 $\pm$ 0.01	0.16 $\pm$ 0.02	0.25 $\pm$ 0.01	0.20 $\pm$ 0.01
CGS (25.0)/SAL (8)	0.18 $\pm$ 0.01	0.16 $\pm$ 0.01	0.21 $\pm$ 0.02	0.20 $\pm$ 0.01
SAL/MDMA (5)	0.07 $\pm$ 0.02*	0.07 $\pm$ 0.02*	0.08 $\pm$ 0.03*	0.09 $\pm$ 0.02*
CGS (25.0)/MDMA (10)	0.12 $\pm$ 0.01*†	0.13 $\pm$ 0.01	0.15 $\pm$ 0.02*†	0.16 $\pm$ 0.01*†
SAL/SAL (7)	0.21 $\pm$ 0.01	0.17 $\pm$ 0.01	0.18 $\pm$ 0.02	0.23 $\pm$ 0.02
CGS (50.0)/SAL (6)	0.20 $\pm$ 0.02	0.16 $\pm$ 0.02	0.18 $\pm$ 0.03	0.21 $\pm$ 0.02
SAL/MDMA (9)	0.10 $\pm$ 0.02*	0.10 $\pm$ 0.01*	0.12 $\pm$ 0.02	0.15 $\pm$ 0.02*
CGS (50.0)/MDMA (10)	0.15 $\pm$ 0.01*†	0.13 $\pm$ 0.01	0.18 $\pm$ 0.02	0.17 $\pm$ 0.01*

* Different from SAL/SAL ($P < .05$).

† Different from SAL/MDMA ($P < .05$).

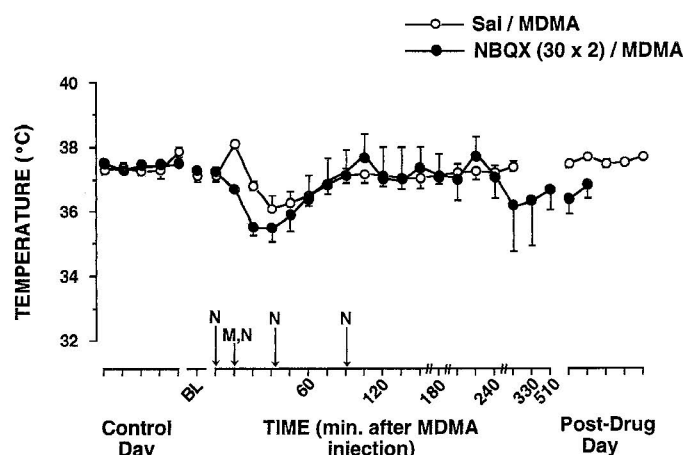


Fig. 3. Effects of NBQX and 3,4-methylenedioxymethamphetamine on TEMP in rats. ○, Rats ($n = 6$) received SAL (1.0 ml/kg) followed 15 min later by MDMA (40.0 mg/kg). ●, Rats ($n = 6$) received NBQX (30.0 mg/kg) followed 15 min later by MDMA (40.0 mg/kg). A second injection of NBQX was administered 90 min after MDMA. N arrows represent NBQX injections; M arrow represents MDMA injection. No differences in TEMP were observed between the two groups.

MDMA group had greater variability. Levels of 5-HT and 5-HIAA in the NBQX/MDMA temperature rats were lower than in the SAL/SAL group yet higher than the SAL/MDMA group, which led us to investigate the protective effect of three injections of 55.0 mg/kg NBQX on neurotransmitter levels.

In the neurochemistry experiment, VEH/MDMA (40.0 mg/kg) depleted 5-HT to 39% of control in hippocampus ($F = 29.14$, $P < .0001$) and 47% of control in striatum ($F = 11.29$, $P < .0001$; table 3). Coadministration of NBQX (55 mg/kg $\times$ 3 injections) with MDMA resulted in 5-HT depletions which were 47% and 58% of control in hippocampus and striatum, respectively, and did not differ from the SAL/MDMA-treated rats.

VEH/MDMA decreased 5-HIAA to 33% of control in hippocampus ($F = 14.54$, $P < .0001$), and coadministration of NBQX (55 mg/kg $\times$ 3 injections) did not attenuate the depletions. In striatum, none of the changes in 5-HIAA concentration reached statistical significance due to variability in three of the four treatment groups.

Discussion

The data from this study show that NMDA receptor antagonists provide protection against MDMA-induced serotonergic toxicity by induction of hypothermia. Both the noncompetitive NMDA receptor antagonist DZ and the competitive

antagonist CGS provided protection against depletions of 5-HT and 5-HIAA, and the protective effect of DZ was abolished when TEMP was maintained above 38.4°C for 4 h after drug administration. The AMPA antagonist NBQX did not provide protection against MDMA-induced serotonergic toxicity, nor did it decrease TEMP when combined with MDMA.

The data presented in the study support a previous report (Farfel *et al.*, 1992) that DZ attenuates MDMA-induced serotonergic toxicity. Protection against MDMA-induced toxicity has also been demonstrated with dextrorphan, a noncompetitive antagonist of NMDA receptors which also blocks voltage-sensitive Ca^{++} channels (Carpenter *et al.*, 1988; Finnegan *et al.*, 1990). However, a study by Johnson *et al.* (1989) showed DZ did not provide protection against MDMA-induced decreases in TPH activity. Accepting that both neurotransmitter levels and TPH activity are accurate indicators of neuronal toxicity, there are two differences in methodology which may account for the discrepancy in the data. In the Johnson *et al.* study, TPH activity was measured 20 h after the last injection, whereas this study measured transmitter and metabolite levels 72 h after the last injection. Also, Johnson *et al.* administered MDMA in 4 injections of 10.0 mg/kg, with 2.5 mg/kg DZ preceding each MDMA (10.0 mg/kg) injection. In this study, rats received a single injection of 40.0 mg/kg MDMA with a single pretreatment injection of 2.5 mg/kg DZ. The data from the present study combining MDMA with dextrorphan (Finnegan *et al.*, 1990), indicate that NMDA receptor antagonists do provide protection against MDMA-induced serotonergic toxicity.

Administration of NBQX (55.0 mg/kg $\times$ 3 injections) with MDMA did not protect against depletions of 5-HT or 5-HIAA, in contrast to the effects of the NMDA receptor antagonists. The dose used (55.0 mg/kg $\times$ 3 injections) was higher than what has been shown to protect against ischemic damage in rats and gerbils (20.0–40.0 mg/kg $\times$ 2 or 3 injections or i.v. infusion) (Loschmann *et al.*, 1991; Nellgard and Wieloch 1992; Sheardown *et al.*, 1990; Taylor and Vartanian, 1992). When NBQX (55.0 $\times$ 3 injections) was administered with MDMA (40.0 mg/kg) and TEMP was recorded ($n = 1$), TEMP did not differ from the SAL/MDMA-treated rats. NBQX is a selective inhibitor of the AMPA (quisqualate) type of glutamate receptor, having little or no affinity for NMDA or glycine binding sites (Sheardown *et al.*, 1990). Because coadministration of NBQX with MDMA did not decrease TEMP, nor did it provide protection against serotonergic toxicity, the results support the hypotheses that (1) NMDA receptor antagonism induces hypothermia when the antagonist is com-

TABLE 3

Levels of 5-HT and 5-HIAA in hippocampus and striatum after 3,4-methylenedioxymethamphetamine and NBQX

Three injections of NBQX (55.0 mg/kg) or VEH (6.5 ml/kg) were administered 15 min apart. MDMA (40.0 mg/kg) or SAL (1.0 ml/kg) was administered concurrent with the second injection of NBQX or VEH. 5-HT and 5-HIAA levels were measured 72 h after injections. Values represent means $\pm$ S.E.M. expressed in ng/mg wet weight tissue. Numbers in parentheses are group n values.

Dose (mg/kg)	Hippocampus		Striatum	
	5-HT	5-HIAA	5-HT	5-HIAA
VEH/SAL (9)	0.21 $\pm$ 0.01	0.18 $\pm$ 0.01	0.20 $\pm$ 0.01	0.21 $\pm$ 0.02
NBQX (55.0)/SAL (6)	0.24 $\pm$ 0.02	0.26 $\pm$ 0.04	0.21 $\pm$ 0.03	0.27 $\pm$ 0.03
VEH/MDMA (9)	0.08 $\pm$ 0.01*	0.06 $\pm$ 0.01*	0.10 $\pm$ 0.01*	0.16 $\pm$ 0.03
NBQX (55.0)/MDMA (8)	0.10 $\pm$ 0.02*	0.11 $\pm$ 0.02*	0.12 $\pm$ 0.02*	0.20 $\pm$ 0.03

* Different from VEH/SAL ($P < .05$).

bined with MDMA, and (2) hypothermia underlies protection against MDMA-induced serotonergic toxicity.

The influence of ambient temperature on pharmacologically induced hypothermia and subsequent neuroprotection has been examined in several studies. In studies of toxicity from MDMA or methamphetamine, lowering ambient temperature to 10°C or below lowered TEMP and attenuated serotonergic and dopaminergic toxicity (Bowyer *et al.*, 1992; Schmidt *et al.*, 1990c). In one of these studies (Schmidt *et al.*, 1990c), the 5-HT₂ receptor antagonist MDL 11,939 also protected against MDMA-induced hyperthermia at doses which protected against neurotoxicity. However, at higher doses of MDMA, rats became hyperthermic although MDL 11,939 still provided neuroprotection. The authors concluded that hypothermia might contribute to neuroprotection, but 5-HT₂ receptor antagonism could mediate protection independently of hypothermia. A similar effect was seen in rats given chloral hydrate (400 mg/kg) with MDMA (20 mg/kg) and kept on 37°C isothermal pads (Schmidt *et al.*, 1990b).

Several studies have reported that MDMA itself causes hyperthermia in rats (Gordon *et al.*, 1991; Schmidt *et al.*, 1990a), and this effect is considered to be involved in the mechanism of neurotoxicity. In the present study, MDMA caused a decrease (<1°C) in TEMP at 30 min compared with SAL/SAL-treated rats, returning to base-line during the next 30 min. Gordon *et al.* (1991) have demonstrated that at 10°C ambient temperature, rats given 30 mg/kg MDMA decrease TEMP by 2°C, whereas the same dose at 20°C causes no change in TEMP, and at 30°C causes TEMP to increase by 3°C. Because TEMP decreased in MDMA-treated rats in this study, hyperthermia does not seem to be necessary to induce neurotoxicity, although it may potentiate neurotoxic drug effects.

The mechanism of neurotoxicity induced by amphetamines such as MDMA is not understood entirely. MDMA, *p*-chloroamphetamine and possibly methamphetamine enter serotonergic neurons by diffusion (Zaczek *et al.*, 1990). Research indicates MDMA and *p*-chloroamphetamine can also enter neurons by uptake through the temperature-dependent 5-HT transporter (Chang *et al.*, 1989; Fischer and Cho 1979; Fuller 1992; Rudnick and Wall 1992). Once inside, these phenethylamines are thought to release 5-HT from the cytoplasmic (nonvesicular) pool. There seem to be two different transport proteins involved in this process: the Na⁺, Cl⁻ and K⁺-dependent membrane transporter, and the intracellular vesicle transporter which is coupled to a proton pump and derives its energy from ATP hydrolysis (Rudnick and Wall, 1992). It has been demonstrated that MDMA is a substrate for both types of transporters (Rudnick and Wall, 1992). Once inside the vesicles, MDMA stimulates monoamine efflux into the cytosolic pool in two ways. First, 5-HT can be released by 5-HT-MDMA exchange at the transporter. Also, MDMA decreases the intravesicular pH, which further stimulates efflux (Rudnick and Wall, 1992; Zaczek *et al.*, 1990). 5-HT released from the cytosolic pool may be converted to a neurotoxin such as 5,6-DHT (Commins *et al.*, 1987a) or tryptamine-4,5-dione (Crino *et al.*, 1989) and then taken back up into the cell, possibly *via* the membrane transporter. Alternatively, it has been suggested that MDMA is converted to a toxic metabolite (Paris and Cunningham, 1991) which mediates neurotoxicity when it is taken into the nerve terminal.

There are several points in this process where hypothermia could affect transport or reaction rates and prevent neurotoxicity. A large, prolonged hypothermia could slow the uptake of MDMA into the neuron, or into the vesicle. It could also slow the rate of transporter-mediated exchange of vesicular monoamines into the cytosol, which would decrease the pool available for release. Another point in the process where decreased temperature might prevent neurotoxicity would be to slow the conversion of 5-HT to a toxic metabolite. Although the Fenton reaction (which can convert 5-HT into 5,6-DHT *in vitro*) is nonenzymatic and uses EDTA to chelate ferrous iron, it is possible that an enzyme is used in its place *in vivo*.

Antagonism of NMDA receptors also blocks cellular Ca⁺⁺ influx, and this action could also be responsible for neuroprotection. Intracellular Ca⁺⁺ overload promotes formation of reactive oxygen species within a neuron (Choi, 1988), which in turn can promote release of excitatory amino acids causing a further increase in intracellular Ca⁺⁺. Osmotic stress also results from an increase in intracellular Ca⁺⁺ due to the influx of Na⁺, Cl⁻ and water, and ATP-dependent pumps may deplete cellular energy sources trying to maintain homeostasis (Pazdernik *et al.*, 1992). However, there is no evidence linking MDMA to large influxes of Ca⁺⁺, and MDMA-induced neurotransmitter release has been demonstrated to be calcium-independent (Arnold *et al.*, 1977; Johnson *et al.*, 1986; Zaczek *et al.*, 1990).

Calcium channel antagonists protect against neuronal toxicity due to ischemia, hypoglycemia and excitatory amino acid overdose, as well as MDMA and related substituted amphetamines. Also, a variety of drugs from different classes (*e.g.*, chloral hydrate, MDL 11,939) have been shown to attenuate the toxic effects of methamphetamine, MDMA and *p*-chloroamphetamine. The existence of a common underlying factor in neuroprotection would help explain why such a variety of drugs can protect against neurotoxicity. The data from the present study indicate that hypothermia is involved in protection by NMDA antagonists against serotonergic toxicity due to MDMA, and suggests that some other protective agents might also work through this mechanism. In a clinical setting, induction of hypothermia may be an effective way to protect against neuronal damage from exogenous toxins.

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