ELSEVIER

Pharmacology, Biochemistry and Behavior 68 (2001) 565–574

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

The effect of (*R*)-HA966 or ACEA 1021 on dexfenfluramine or (*S*)-MDMA-induced changes in temperature, activity, and neurotoxicity

B.R. Russell, R. Laverty*

Department of Pharmacology, School of Medical Sciences, University of Otago, P.O. Box 913, Dunedin 9001, New Zealand

Received 27 March 2000; received in revised form 10 November 2000; accepted 8 December 2000

Abstract

The glycine site-specific *N*-methyl-D-aspartate (NMDA) antagonist 5-nitro-6,7-dichloro-2,3-quinoxalinedione (ACEA 1021, 4 × 30 mg/kg, ip) given 30 min before dexfenfluramine (4 × 15 mg/kg, ip, every 2 h) was unable to prevent dexfenfluramine-induced depletion of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) content, and 5-HT transporter (5-HTT) density. Another glycine site-specific NMDA antagonist, *R*(+)-3-aminohydroxypyrrolidin-2-one [(*R*)-HA 966] (2 × 30 mg/kg, ip), given 30 min before dexfenfluramine (2 × 10 mg/kg, ip, 2 hourly) was also unable to prevent regional depletion of 5-HT, 5-HIAA, and 5-HTT density. However, ACEA 1021 (4 × 30 mg/kg, ip) given 30 min before (*S*)-3,4-methylenedioxymethamphetamine (MDMA, 4 × 10 mg/kg, 2 hourly, ip) attenuated the regional depletion of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-HIAA content, and 5-HTT density. ACEA 1021 combined with (*S*)-MDMA also prevented (*S*)-MDMA-induced hyperthermia without causing hypothermia or preventing an (*S*)-MDMA-induced increase in locomotor activity. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: ACEA 1021; (*R*)-HA 966; NMDA antagonists; Neurotoxicity; Rats; Temperature; Dexfenfluramine; MDMA

1. Introduction

3,4-methylenedioxymethamphetamine (MDMA), a chemical analogue of dexamphetamine, has been used as a recreational drug of abuse for the last two decades with a perception by the lay press that it is "safe," although this is changing (e.g., see *Time* magazine, June 7). There has been an increasing number of reports of its wide spread use (Lenton et al., 1997; Webb et al., 1996). However, there has also been an increasing number of reports involving severe toxicity and death following its ingestion, including abnormalities of water homeostasis (Henry et al., 1998), and renal or hepatic failure, related to hyperthermia and dehydration (Fallon et al., 1999). MDMA-induced neurotoxicity in rodent studies (Commins et al., 1987) and observations that doses taken by recreational users of MDMA are comparable to those that produce nerve damage in nonhuman primates (Hatzidimitriou et al., 1999; Ricaurte et al., 1988) raised concerns about possible long-term toxic effects in humans. Most recently, MDMA has been shown to reduce the number of 5-hydroxytryptamine transporter (5-HTT) density in humans (McCann et al., 1998; Semple et al., 1999) adding weight to the argument that it is neurotoxic.

Fenfluramine is also an analogue of dexamphetamine and is of particular interest because it differs pharmacologically from the large majority of amphetamines. It does not cause psychomotor stimulation (Tessel et al., 1975), or have the abuse potential of other amphetamines. Fenfluramine selectively induces a loss of 5-HT axonal markers in rats that is evident for weeks (Johnson and Nichols, 1990) or even months (Kleven et al., 1988) after drug discontinuation. These effects are unlikely to be related to the acute pharmacological effects of fenfluramine as the half-life of this compound and its metabolites is relatively short (Brownsill et al., 1991), suggesting that fenfluramine is neurotoxic, like MDMA, regardless of the different behavioral and stimulant effects it may cause. This neurotoxicity is determined by a sustained decrease in the content of 5-HT, 5-HIAA (5-hydroxyindoleacetic acid), and 5-HTT density in neuronal tissues (Johnson and Nichols, 1990; Kleven et al., 1988).

Fenfluramine can induce either hypo- or hyperthermia in humans, rats, mice, rabbits, and chickens (Clark and Lipton, 1986), depending on the ambient temperature at which the

* Corresponding author. Tel.: +64-3-479-7459.
E-mail address: dick.laverty@stonebow.otago.ac.nz (R. Laverty).

PII: S0091-3057(01)00454-3

animals were dosed. For example, in an early study, fenfluramine was given to rats at 4°C, 20°C, and 37°C; at the lower temperatures hypothermia resulted and at 37°C hyperthermia was produced (Yehuda and Wurtman, 1972). A recent study using biotelemetry has shown that fenfluramine causes hypothermia in the rat at a normal ambient temperature of 24°C and, in contrast to MDMA, neurotoxicity still occurs (Malberg and Seiden, 1997). This is markedly different from other amphetamines, such as methamphetamine (METH) and MDMA, because hypothermia protects against their monoamine depleting effects (Bowyer et al., 1994; Miller and O'Callaghan, 1995).

Earlier work established that fenfluramine does not produce acute locomotor stimulation, unlike other *meta* substituted *N*-ethylamphetamines, such as *m*-chloro-*N*-ethylamphetamine (Tessel et al., 1975). In fact, dexfenfluramine (Even and Nicolaidis, 1986) and the racemate have been found to suppress locomotor activity in animals (Callaway et al., 1993) and produces sedation or lethargy in humans (Aulakh et al., 1988).

Experiments have shown that core body temperature effects of MDMA are also dependent on ambient temperature. Treatment of rats with MDMA and raised ambient temperature causes hyperthermia, while giving MDMA at a lower ambient temperature results in hypothermia (Broening et al., 1995; Gordon et al., 1991; Malberg and Seiden, 1998). MDMA administration also increases locomotor activity (Gordon et al., 1991) along with signs of sympathetic stimulation, such as mydriasis, salivation, piloerection, and hyperthermia (Frith et al., 1987; Hardman et al., 1973).

N-methyl-D-aspartate (NMDA) antagonists have been used in experiments to prevent fenfluramine- or MDMA-induced changes in the CNS; it has been suggested that NMDA receptor activity may play an important role in the consequent neurotoxicity (Dragunow et al., 1991; Sabol et al., 1992). The noncompetitive channel blocking NMDA antagonist, MK-801, prevents fenfluramine from increasing striatal neurotensin levels and Fos expression, (Guerra et al., 1998; Hanson et al., 1991), although fenfluramine-induced decreases in 5-HT content are not prevented by MK-801 (Farfel and Seiden, 1995b).

MK-801 also prevents MDMA-induced neurotoxicity. However, several investigators have suggested that the neuroprotection provided is solely dependent on the ability of MK-801 to produce hypothermia (Farfel and Seiden, 1995b; Miller and O'Callaghan, 1994). Moreover, this type of NMDA antagonist has a number of other effects, such as increasing locomotor activity, that may not be directly related to NMDA antagonism (Morrow et al., 1995).

Alternative neuroprotective agents may be those acting at the strychnine-insensitive glycine site of the NMDA receptor, such as *R*(+)-3-aminohydroxypyrrolidin-2-one [(*R*)-HA-966] and 5-nitro-6,7-dichloro-2,3-quinoxalinedione (ACEA 1021). Glycine site-specific NMDA antagonists such as (*R*)-HA 966 have shown protective effects against the toxicity induced by MPTP and cocaine (Kanthasamy et al., 1997; Matsumoto et al., 1997). They have a significantly different spectrum of activity than noncompetitive NMDA antagonists, for example, they do not possess the rewarding properties (Papp et al., 1996) or the stimulant effects (Morrow et al., 1995) of MK-801. The lack of stimulant effects may play an important role in these studies because amphetamine-induced hyperthermia may result from the build up of heat following increased locomotor and consequent muscular activity (Cox, 1977).

These experiments were designed to test if the glycine site-specific NMDA antagonists protect against the toxicity induced by the stereoisomers dexfenfluramine or (*S*)-MDMA without altering locomotor activity or causing a profound decrease in core body temperature.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (35–42 days old) were obtained from the animal breeding station, University of Otago. Animals were housed in pairs with plain shredded newsprint bedding that was changed every second day. Drug administration was always carried out on the day following bedding changes. The animal house was kept at a constant 23 ± 1°C with a 12-h dark/light cycle. Standard laboratory food and water was constantly available to all animals. A period of 2 days was allowed for the animals to become accustomed to their new surroundings before surgery and all surgical procedures allowed a 4-day recovery period before drug administration. The University of Otago Animal Ethics Committee approved all experiments.

2.2. Drugs

(*S*)-MDMA HCl was synthesized, and its chemical identity and purity confirmed by ^{1}H-NMR, melting point analysis, and measurement of optical rotation following standard methods (Nichols et al., 1986). Servier donated dexfenfluramine HCl. Cosensys of California donated ACEA 1021 and TOCRIS UK supplied (*R*)-HA 966.

2.3. Doses and regimen

To compare the neurotoxicity produced by both (*S*)-MDMA and dexfenfluramine a four-dose regimen was chosen for most experiments. Many investigators have previously used a four dose, 2 hourly regimen (Bowyer et al., 1994; Shankaran and Gudelsky, 1998). Farfel and Seiden (1995a) used a 40 mg/kg total dose of MDMA or 50 mg/kg of fenfluramine, split over four doses. Splitting the dose of (*S*)-MDMA into four also decreases the risk of causing potentially lethal hyperthermia (Broening et al., 1995).

(*R*)-HA 966 at 30 mg/kg was chosen on the basis of earlier experiments (Kanthasamy et al., 1997) that found that a single dose provided significant protection against MPTP-induced dopaminergic damage. For this particular experiment, it was decided to test a regimen with only two doses of dexfenfluramine and (*R*)-HA 966.

ACEA 1021 (20–30 mg/kg) effectively reduced infarct volumes and hemiparesis without inducing catalepsy in a model of cerebral ischaemia (Warner et al., 1995). In experiments using ACEA 1021, a minimal amount of Tween 20 was used to solubilise the drug in sterile saline. The same amount of Tween 20 was added to the sterile saline before being given to animals used as controls. All other drugs were dissolved in sterile saline and given as a dose of 1 ml/kg. Doses were calculated and given as the salt.

2.4. Dissection

Rats were killed by decapitation without anaesthesia 7 days after drug treatment. The brain was quickly removed from the skull and chilled in ice-cold isotonic saline for 1 min before the cerebellum was carefully removed and discarded. The brain was frozen over dry ice, carefully wrapped in aluminium foil, and stored at −80°C until dissection.

Serial transverse sections of the brain were cut at −12°C and identified using the atlas of Paxinos and Watson (1997). Beginning at the section corresponding with Plates 7, 11, 19, 30, or 40, 12 consecutive 20-μm thick slices and a 250-μm thick slice were taken. The 20-μm thick slices were thaw-mounted on gelatin-subbed slides and fan dried at room temperature for 1 h before being frozen at −20°C until autoradiographic analysis. The 250-μm thick slice was dissected while still frozen using the micropunch method of Palkovits and Brownstein (1988) for HPLC analysis.

2.5. HPLC analysis

Micropunch samples were stored in a vial containing 50 μl of ice-cold tissue buffer, capped, and immediately frozen on dry ice. Tissue buffer was composed of 0.05 M disodium hydrogen phosphate, 0.03 M citric acid monohydrate, and 15% methanol in water. Samples were stored at −80°C until required.

The HPLC system used consisted of a Hewlett-Packard 1100 system composed of an online degasser, high-pressure pump flow rate 0.6 ml/min, cooled autosampler 4°C, column oven 27°C, Phenomonex prodigy 150 × 4.6 mm 3 μm C_{18} column with Hewlett-Packard Series 1100 Chemstation software. The analytical detection system (ECD) used an ESA 5200 Coulochem II with a Guard cell 5020 at 375 mV, Conditioning cell 5021 at 350 mV, High sensitivity analytical cell 5011 set with $E_1 = -150$ mV and $E_2 = +350$ mV.

The mobile phase in this system consisted of 0.05 M disodium hydrogen phosphate, 0.03 M citric acid monohydrate, 0.1 mM Na_2EDTA, 1.29 mM octanesulphonic acid in 12.5% methanol made to volume with water. HPLC-grade chemicals were used wherever possible.

Samples were homogenized using a cooled ultrasonic homogenizer. Samples were then centrifuged at 4°C for 10 min (18,200 × *g*). The supernatant was injected onto the column by autosampler and protein content determined on the remaining pellet.

Standards for dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-HIAA, and 5-HT were used, *N*-ω-5-HT was used as an internal standard. Sample monoamine and metabolite concentrations were calculated from the percentage recovery of the internal standard and related to the amount of protein determined in each sample.

Protein was determined using a commercially supplied Bio-Rad DC Protein Assay kit. Monoamine and metabolite concentrations are expressed as picogram/microgram of protein and compared well with published data (Colado and Green, 1994; Palkovits et al., 1979) suggesting an accurate, comparable, and reliable method for measurement.

2.6. Autoradiographic analysis

An autoradiographic method was used to determine 5-HTT density (Hrdina et al., 1990). ^{3}H-paroxetine was chosen to label the 5-HTT because it is a more selective ligand than alternatives, such as imipramine (Hrdina et al., 1990), in addition to being extensively used by others in this field, for example Appel et al. (1990) and Scheffel and Ricaurte (1990).

Briefly, the method is as follows. For total binding, the slides were incubated for 2 h in Tris buffer with 120 mM NaCl, 5 mM KCl at pH 7.4 with 0.2 nM ^{3}H-paroxetine. For nonspecific binding alternate slides were incubated for 2 h with 0.2 nM ^{3}H-paroxetine and 30 μM fluoxetine in Tris buffer with 120 mM NaCl, 5 mM KCl at pH 7.4. Following this, the slides were air-dried, then exposed to Amersham Hyperfilm in cassettes at 4°C for 7 weeks. Standards were incubated in each cassette along with all samples. Sample and standard images were digitized for further analysis using a Sony DXC151-AP digital video camera controlled by a 486 PC with Scion Image software and LG-3 frame grabber card.

Areas measured correspond with those dissected by micropunch from which tissue was removed for HPLC analysis. Results obtained compare well with (Appel et al., 1990; De Souza and Kuyatt, 1987).

2.7. Radiotelemetry

A Dataquest 3 Automated Data Acquisition System (Mini-mitter, OR, USA) was used. Mini-mitter transmitters model VM-FH were coated with an inert paraffin/elvax film supplied by Mini-mitter that is biocompatible and does not cause inflammation. Transmitters were surgically implanted using aseptic techniques. Transmitter calibration was checked

with a temperature controlled water bath placed over a receiver pad.

This information was recorded and collected by an IBM compatible 486 PC for later analysis. Minimitters have a resolution of ±0.01°C (Clement et al., 1989) and have been shown to be valid and reliable (Dilsaver et al., 1992) for measuring core temperature. Locomotor activity was also measured using the same system.

2.8. Statistics

All statistics for data obtained by HPLC and autoradiography were carried out by GraphPad Prism version 2.01. The statistical significance of differences between groups was carried by a one-way analysis of variance (ANOVA) followed by a Neuman–Keuls post-hoc comparison.

Radiotelemetry data was analysed by SPSS for Windows Ver. 8.0. Between group comparisons were determined by calculating the mean temperature and mean activity count (MAC) for individual animals in each group over 120 min, then using a 2-way ANOVA with repeated measures followed by Student–Neuman–Keuls post-hoc comparison.

3. Results

3.1. Effect of dexfenfluramine, (R)-HA 966, and ACEA 1021 on the regional content of 5-HT, 5-HIAA content, and 5-HTT density and core body temperature

Four doses of dexfenfluramine (15 mg/kg) induced (after 7 days) decreases in 5-HT, 5-HIAA content, and 5-HTT density in the sensory cortex, CA1, caudate putamen, and hypothalamus (Table 1).

Basal levels in the sensory cortex of 5-HT were 3.2 ± 0.3 pg/μg, 5-HIAA 2.7 ± 0.2 pg/μg, and 5-HTT 59.1 ± 4.7 fmol/mg of protein. Basal levels in the CA1 region of 5-HT were 8.9 ± 0.9 pg/μg, 5-HIAA 6.5 ± 0.6 pg/μg, and 5-HTT 87.1 ± 11.4 fmol/mg of protein. Basal levels in the hypothalamus of 5-HT were 10.0 ± 0.6 pg/μg, 5-HIAA 6.9 ± 0.5 pg/μg, and 5-HTT 80.7 ± 5.1 fmol/mg of protein. Basal levels in the caudate putamen of DA were 86.7 ± 8.0 pg/μg, DOPAC 10.4 ± 1.0 pg/μg, 5-HT 7.0 ± 0.6 pg/μg, 5-HIAA 4.7 ± 0.2 pg/μg, and 5-HTT 65.6 ± 9.1 fmol/mg of protein.

Dexfenfluramine significantly decreased 5-HT basal levels in the sensory cortex [$F(3,26) = 14.4$, $P < .05$], CA1 [$F = 13.6$, $P < .05$], caudate putamen [$F(3,28) = 6.0$, $P < .05$], and hypothalamus ($F = 5.9$, $P < .05$), 5-HIAA basal levels were significantly decreased in the sensory cortex ($F = 6.0$, $P < .05$), CA1 ($F = 15.9$, $P < .05$), caudate putamen [$F(3,28) = 4.4$, $P < .05$], and hypothalamus ($F = 10.0$, $P < .05$). The 5-HTT density was significantly decreased in the sensory cortex [$F(3,24) = 4.38$, $P < .05$], CA1 [$F(3, 27) = 4.55$, $P < .05$], and hypothalamus [$F(3,28) = 21.23$, $P < .05$].

ACEA 1021 was unable to prevent this depletion when given 30 min before dexfenfluramine. A further experiment using the same dosing regimen was carried out using a lower dose of dexfenfluramine (10 mg/kg) in combination with ACEA 1021 (4 × 30 mg/kg). However, ACEA 1021 was still unable to prevent dexfenfluramine-induced decreases in 5-HT, 5-HIAA, and 5-HTT density (data not shown).

3.2. Effect of dexfenfluramine and (R)-HA 966 on the regional content of 5-HT, 5-HIAA content, and 5-HTT density and core body temperature

Basal levels in the cingulate cortex of 5-HT were 0.9 ± 0.1 pg/μg, 5-HIAA 0.5 ± 0.1 pg/μg, and 5-HTT 224.6 ± 27.7 fmol/mg of protein. Basal levels in the CA1 region of 5-HT were 18.9 ± 3.0 pg/μg, 5-HIAA 18.0 ± 2.3 pg/μg, and 5-HTT 194.1 ± 33.0 fmol/mg of protein. Basal levels in the caudate putamen of DA were 156.9 ± 13.5 pg/μg, DOPAC 23.4 ± 2.8 pg/μg, 5-HT 1.7 ± 0.4 pg/μg, 5-HIAA, 2.9 ± 0.3 pg/μg, and 5-HTT 118.1 ± 24.1 fmol/mg of protein.

Dexfenfluramine significantly decreased 5-HT levels in the cingulate cortex [$F(3,18) = 5.5$, $P < .05$], caudate putamen [$F(3,19) = 5.8$, $P < .05$], and 5-HIAA levels in the

Table 1
Regional change in the concentration of 5-HT, 5-HIAA, and the density of ^{3}H-paroxetine labeled 5-HTT 7 days after giving intraperitoneal injections of ACEA 1021 (30 mg/kg) 30 min before dexfenfluramine (15 mg/kg)

	Sensory cortex			CA1			Caudate putamen					Hypothalamus		
Region	5-HT	5-HIAA	5-HTT	5-HT	5-HIAA	5-HTT	DA	DOPAC	5-HT	5-HIAA	5-HTT	5-HT	5-HIAA	5-HTT
Dexfenfluramine, 15 mg/kg	46.2* (6.5)	55.6* (4.5)	76.4 (12.4)	48.6* (6.9)	52.2* (4.8)	59.1* (4.1)	113.0 (7.4)	113.5 (5.7)	66.0* (5.8)	77.6 (7.9)	73.0 (6.8)	68.1* (10.4)	62.2* (6.4)	75.8* (6.5)
ACEA 1021, 30 mg/kg	99.8 (7.5)	93.7 (19.2)	114.8 (12.4)	109.0 (9.5)	100.1 (8.0)	104.3 (15.8)	119.9 (7.4)	112.5 (9.36)	80.1 (9.7)	85.1 (8.8)	98.6 (18.8)	92.5 (9.4)	80.6* (5.8)	131.3 (8.8)
Dexfenfluramine, 15 mg/kg and ACEA 1021, 30 mg/kg	44.4* (8.2)	45.2* (7.7)	69.4 (6.7)	56.9* (5.1)	56.8* (4.2)	58.55 (12.6)	116.9 (10.2)	118.0 (8.4)	57.3* (4.2)	68.0* (3.5)	57.4 (11.3)	57.6* (6.2)	60.0* (4.4)	63.7* (6.1)

Injections of both ACEA 1021 and dexfenfluramine were repeated after 120, 240, and 360 min ($n = 8$). Data represent the mean ± S.E.M. expressed as a percentage of the corresponding control group of saline-treated rats.

* $P < .05$ significance of difference from saline-treated rats determined by ANOVA on concentrations.

Table 2
Regional change in 5-HT, 5-HIAA concentrations, and ^{3}H-paroxetine-labeled 5-HTT density 7 days after giving intraperitoneal injections of (*R*)-HA 966 (30 mg/kg) 30 min before dexfenfluramine (10 mg/kg)

	Cingulate cortex			CA1			Caudate putamen		
Region	5-HT	5-HIAA	5-HTT	5-HT	5-HIAA	5-HTT	5-HT	5-HIAA	5-HTT
Dexfenfluramine, 10 mg/kg	60.3* (6.4)	78.1 (14.9)	59.8* (9.4)	71.6 (11.3)	63.5 (10.1)	64.4* (9.4)	31.5* (6.1)	47.1* (3.8)	57.1* (4.8)
(*R*)-HA 966, 30 mg/kg	108.5 (14.0)	111.3 (7.8)	122.2 (7.1)	88.7 (17.0)	115.0 (28.6)	124.4 (23.0)	77.7 (8.3)	80.7 (6.8)	87.4 (14.3)
Dexfenfluramine, 10 mg/kg and (*R*)-HA 966, 30 mg/kg	56.5* (9.3)	96.1 (15.9)	45.6* (9.1)	74.7 (6.0)	78.0 (6.5)	41.5* (6.7)	47.9* (10.5)	53.4* (6.0)	36.1* (16.2)

Injections of both (*R*)-HA 966 and dexfenfluramine were repeated after 120 min ($n=6$). Data represent the mean ± S.E.M. expressed as a percentage of the corresponding control group of saline-treated rats.

* $P<.05$ significance of difference from saline-treated rats determined by ANOVA.

caudate putamen [$F(3,19)=12.2$, $P<.05$]. The 5-HTT density was also significantly different decreased in the cingulate cortex [$F(3,20)=4.9$, $P<.05$], CA1 [$F(3,20)=5.9$, $P<.05$], and caudate putamen ($F=3.8$, $P<.05$).

Two doses of dexfenfluramine (10 mg/kg) caused depletion of 5-HT and 5-HIAA and 5-HTT density in the cingulate cortex and caudate putamen (Table 2). Dexfenfluramine also appears to have decreased 5-HT content in the CA1; although this was not statistically significant, the decrease in 5-HTT density suggested that damage to serotonergic neurons did occur. (*R*)-HA 966 failed to prevent depletion of 5-HT, 5-HIAA, or 5-HTT if given before dexfenfluramine.

There was a significant drug effect on temperature relative to controls over the entire test period in experiments using dexfenfluramine combined with either ACEA 1021 (Fig. 1) [$F(3,5)=5.1$, $P<.029$] or (*R*)-HA 966 (Fig. 2) [$F(3,5)=44.8$, $P<.0005$]. Dexfenfluramine alone caused a significant decrease in body temperature compared to control animals beyond 180 min in both experiments ($\bar{x}=37.0$°C, $P<.05$, Fig. 1; $\bar{x}=36.9$°C $P<.05$, Fig. 2).

In the group given (*R*)-HA 966 alone, no significant change in body temperature occurred at any time point. In contrast, the group given ACEA 1021 showed a significant decrease in temperature ($\bar{x}=36.3$°C, $P<.05$) between 180 and 300 min compared to animals given saline only ($\bar{x}=37.8$°C).

However, dexfenfluramine combined with (*R*)-HA 966 significantly decreased body temperature between 0 and 120 min ($\bar{x}=37.1$, $P<.05$) that further decreased between 180 and 300 min ($\bar{x}=36.2$, $P<.05$).

In the group given ACEA 1021 before dexfenfluramine, a significant decrease in body temperature occurred that further decreased until the period between 380 and 500 min ($\bar{x}=35.6$°C, $P<.05$).

Body temperature slowly returned to approximately 37.8°C by 700 min in groups given either ACEA 1021 or (*R*)-HA 966 before dexfenfluramine.

Locomotor activity was monitored in both experiments, and although small significant changes were measured in some groups, the results were not considered biologically significant.

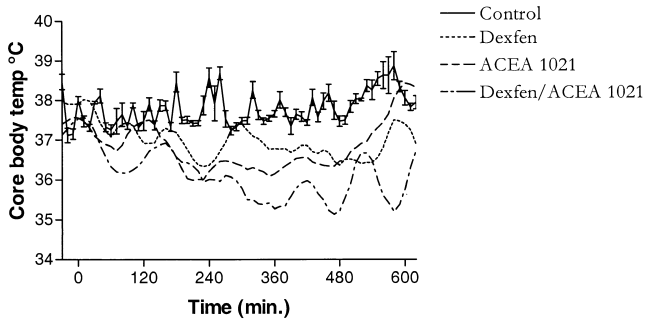

Fig. 1. Effects of dexfenfluramine and ACEA 1021 on core body temperature. Animals were injected intraperitoneally with ACEA 1021 (30 mg/kg) 30 min before dexfenfluramine (15 mg/kg), at 0 min, and again at 120, 240, and 360 min. Data points represent mean core body temp for 10-min periods ± S.E.M. ($n=3$). The saline control group is shown with error bars (S.E.M.), drug groups are shown without error bars for simplicity. Variability of data is comparable in all groups.

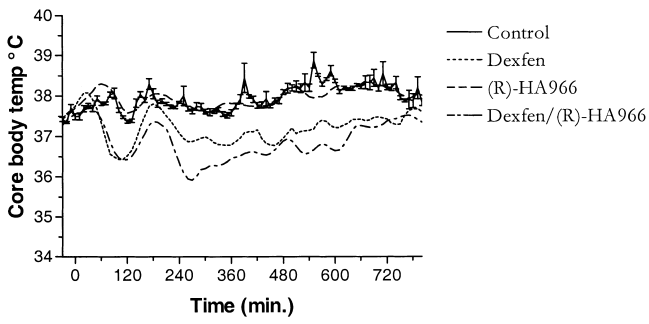

Fig. 2. Effects of dexfenfluramine and (R)-HA 966 on core body temperature. Animals were injected intraperitoneally, with (R)-HA 966 (30 mg/kg) 30 min before dexfenfluramine (10 mg/kg), at 0 min, and again at 120 min. Data points represent mean core body temp for 10-min periods ± S.E.M. ($n=3$). The saline control group is shown with error bars (S.E.M.), drug groups are shown without error bars for simplicity. Variability of data is comparable in all groups.

Table 3
Regional change in DA, DOPAC, 5-HT, 5-HIAA concentrations and ^{3}H-paroxetine-labeled 5-HTT density 7 days after giving intraperitoneal injections of ACEA 1021 (30 mg/kg) 30 min before (*S*)-MDMA (10 mg/kg)

	Caudate putamen					Cingulate cortex			CA1		
Region	DA	DOPAC	5-HT	5-HIAA	5-HTT	5-HT	5-HIAA	5-HTT	5-HT	5-HIAA	5-HTT
(*S*)-MDMA, 10 mg/kg	60.4* (7.8)	73.5 (15.5)	44.8* (5.2)	48.1* (5.5)	34.2 * (6.2)	57.2* (10.3)	56.9* (9.1)	98.0 (7.4)	39.2* (9.9)	55.7* (11.0)	46.5 * (9.4)
ACEA 1021, 30 mg/kg	95.2 (3.5)	86.6 (2.4)	79.0* (4.3)	84.6 (9.0)	83.2 (10.9)	98.5 (15.9)	87.5 (10.8)	94.6 (14.7)	92.5 (7.6)	100.2 (6.7)	81.3 (7.7)
(*S*)-MDMA, 10 mg/kg and ACEA 1021, 30 mg/kg	95.8† (9.0)	95.3 (9.7)	73.5* † (7.7)	77.8 (8.1)	56.4* (9.6)	118.4† (13.7)	93.9 (14.4)	84.2 (8.9)	109.5† (6.3)	103.2† (16.8)	66.1* (6.8)

Injections of both ACEA 1021 and (*S*)-MDMA were repeated after 120, 240, and 360 min ($n=6$). Data represent the mean ± S.E.M. expressed as a percentage of the corresponding control group of saline-treated rats.

* $P<.05$ significance of from saline-treated rats determined by ANOVA on concentrations.

† $P<.05$ significance of difference from (*S*)-MDMA treated rats determined by ANOVA on concentrations.

3.3. Effect of (S)-MDMA and ACEA 1021 on the regional content of monoamines, their metabolites, 5-HTT density core body temperature, and locomotor activity

Basal levels in the cingulate cortex of 5-HT were 2.0 ± 0.3 pg/μg, 5-HIAA 1.7 ± 0.3 pg/μg, and 5-HTT 176.9 ± 12.7 fmol/mg of protein. Basal levels in the CA1 region of 5-HT were 18.9 ± 3.1 pg/μg, 5-HIAA pg/μg 18.0 ± 2.3 pg/μg, and 5-HTT 140.6 ± 5.0 fmol/mg of protein. Basal levels in the caudate putamen of 5-HT were 3.9 ± 0.3 pg/μg, 5-HIAA 5.1 ± 0.7 pg/μg, and 5-HTT 101.9 ± 9.7 fmol/mg of protein.

(*S*)-MDMA significantly decreased DA levels in the caudate putamen [$F=(3,20)=7.0$, $P<.05$], 5-HT levels in the cingulate cortex [$F(3,20)=3.8$, $P<.05$], caudate putamen ($F=13.8$, $P<.05$), CA1 ($F=9.4$, $P<.05$), and 5-HIAA levels in the caudate putamen [$F(3,20)=5.2$, $P<.05$] and the CA1 ($F=3.6$, $P<.05$). The 5-HTT density was significantly decreased in caudate putamen [$F(3,20)$, $P<.05$], CA1 ($F=10.0$, $P<.05$), and cingulate cortex ($F=10.1$, $P<.05$).

ACEA 1021 provided complete protection against (*S*)-MDMA-induced 5-HT and 5-HIAA depletion in the cingulate cortex and CA1 region, while only providing partial protection in the caudate putamen.

ACEA 1021 also appears to attenuate an (*S*)-MDMA-induced decrease in 5-HTT density in the caudate putamen and CA1 region, although this was not statistically significant. In this experiment, (*S*)-MDMA did not deplete the density of 5-HTT in the cingulate cortex (Table 3); however, this has occurred in other experiments (results not published).

There was a significant drug effect on temperature over the entire test period [$F(3,5)=14.0$, $P<.001$] (Fig. 3). A significant increase in core body temperature occurred between 30 and 90 min after the injection series began in the groups given (*S*)-MDMA ($\bar{x}=38.7$°C, $P<.05$) relative to the group given saline ($\bar{x}=37.8$°C). This group exhibited a further increase ($\bar{x}=39.9$°C, $P<.05$) that continued until 450 min before the temperature returned to baseline 600 min from the beginning of the test period.

During the time period between 90 and 210 min, a significant decrease in body temperature occurred in the group given ACEA 1021 ($\bar{x}=36.4$°C, $P<.05$) compared to those given saline that returned to baseline approximately 360 min after the beginning of the test period.

The group given (*S*)-MDMA and ACEA 1021 combined did not exhibit a significant decrease in core body temperature.

Over the test period, drug treatment caused a significant change in the MAC [$F(3,5)=16.0$, $P<.001$] (Fig. 4). There was a significant increase in MAC in animals given (*S*)-MDMA ($\bar{x}=60$, $P<.05$) by 90 min.

The group given combined ACEA 1021 and (*S*)-MDMA also showed a significant increase after 90 min ($\bar{x}=34$, $P<.05$) when compared with the group given saline ($\bar{x}=15$). The increase in MAC continued between 90 and 210 min ($\bar{x}=74$, $\bar{x}=58$, $P<.05$, respectively)

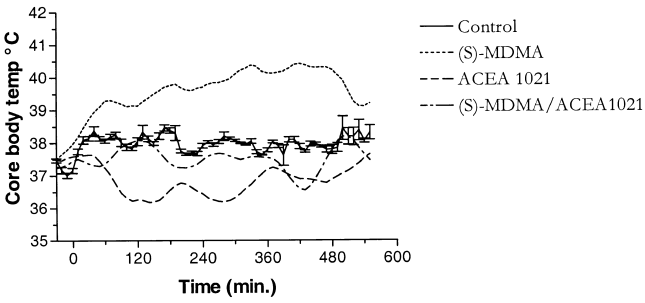

Fig. 3. Effects of (*S*)-MDMA and ACEA 1021 on core body temperature. Animals were injected intraperitoneally with ACEA 1021 (30 mg/kg) 30 min before (*S*)-MDMA (10 mg/kg), at 0 min, and again at 120, 240, and 360 min. Data points represent mean core body temp for 10-min periods ± S.E.M. ($n=3$). The saline control group is shown with error bars (S.E.M.), drug groups are shown without error bars for simplicity. Variability of data is comparable in all groups.

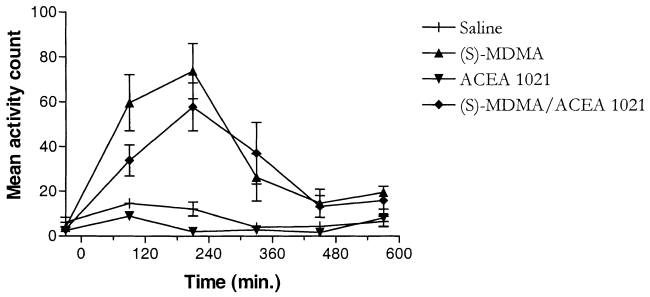

Fig. 4. Effects of (*S*)-MDMA and ACEA 1021 on locomotor activity (MAC). Animals were injected intraperitoneally with ACEA 1021 (30 mg/kg) 30 min before (*S*)-MDMA (10 mg/kg), at 0 min, and again at 120, 240, and 360 min. Data points represent the MAC for 120-min sample periods ± S.E.M. ($n = 3$).

compared to the group given saline or ACEA 1021 alone. After 330 min, the MAC was no longer significantly different from controls.

4. Discussion

These experiments have clearly shown that glycine site-specific NMDA antagonists, ACEA 1021 and (*R*)-HA 966, are unable to prevent dexfenfluramine-induced serotonergic depletion. It has also been demonstrated that ACEA 1021 can prevent MDMA-induced dopaminergic and serotonergic toxicity.

Dexfenfluramine depleted 5-HT, 5-HIAA content, and 5-HTT density 7 days after drug treatment, as found in other laboratories (McCann et al., 1994; Stewart et al., 1997). Dexfenfluramine (10 mg/kg) did not produce a consistent depletion of serotonergic markers in all regions, consequently, the dose was increased to 15 mg/kg, which produced more consistent results (see Table 1).

The failure of ACEA 1021 or (*R*)-HA 966 to prevent dexfenfluramine-induced serotonergic depletion indicates that glycine site-specific NMDA antagonists are unable to modulate dexfenfluramine-induced serotonergic toxicity. Investigation has shown that dexfenfluramine causes widespread 5-HT and extracellular glutamate release in vivo (Rocher et al., 1999), although MDMA does not (Nash and Yamamoto, 1992). In comparison with dexfenfluramine, METH also increases glutamate release and produces damage (Nash and Yamamoto, 1992), and glycine site-specific NMDA antagonists also do not prevent METH-induced neurotoxicity (Layer et al., 1993). This suggests that dexfenfluramine or METH-induced neurotoxicity is unrelated to NMDA receptor activation and does not involve excitatory amino acids, in particular glutamate (GLU).

Dexfenfluramine produced minor decreases in core body temperature of approximately 1°C that do not appear to be dose-dependent (see Figs. 1 and 2). Following administration of the fenfluramine racemate, small changes in ambient temperature can induce hypo or hyperthermia (Malberg and Seiden, 1997). In the experiments by Malberg and Seiden (1997), the first dose of fenfluramine decreased body temperature by more than 1.5°C, considerably more than the minor decreases observed in these experiments. This suggests that the fenfluramine racemate, through the L-isomer, has a greater effect on body temperature than dexfenfluramine. The difference in temperature effects is unlikely to be a dose effect because Malberg and Seiden (1997) used a dose of 12.5 mg/kg ip, which is comparable to the present study. However, Malberg and Seiden (1997) did give one dose each hour, four times, as opposed to one dose every 2 h as in the present experiments, consequently the greater total decrease of −2.8°C could be the result of cumulative dosing.

These experiments have demonstrated that when given alone the glycine site-specific NMDA antagonist ACEA 1021 causes significant hypothermia (Fig. 3). Although (*R*)-HA 966 did not produce a significant change in core body temperature when given alone, if given before dexfenfluramine, significant hypothermia occurred (Fig. 2). In addition, dexfenfluramine combined with either ACEA 1021 or (*R*)-HA 966 induced a significantly greater decrease in core body temperature than dexfenfluramine alone (Figs. 1 and 2), without significantly altering locomotor activity (data not shown).

When using this method of radiotelemetry as a measure of locomotor activity, the monitor counts changes in position of the radiotransmitter over a series of receivers beneath the animal cage. Hence, when recording movement counts by this method, the animal may be moving over small distances, for example, swaying, or exhibiting stereotyped behavior, so the count may not be the result of increased locomotor activity but a reflection of these other behaviors. Consequently, the recorded MAC may not be a true reflection of locomotor activity unless the MAC is very high and/or the animal is hyperactive and moving about the cage. Dexfenfluramine, ACEA 1021, and (*R*)-HA 966 produced only minor differences in locomotor activity when compared with saline (data not shown). So, although dexfenfluramine induced significant differences in MAC that were modified by ACEA 1021 and (*R*)-HA 966, the changes were not considered to be biologically significant.

(*S*)-MDMA-induced regional decreases in 5-HT and 5-HIAA content are comparable to those found by other laboratories (Colado and Green, 1994; Schmidt, 1987). A significant decrease in the regional density of 5-HTT also compares well with figures shown by other laboratories (Battaglia et al., 1987; De souza and Battaglia, 1989). (*S*)-MDMA also caused an unexpected significant decrease in DA and DOPAC content in the striatum. In the past, this laboratory has observed an occasional inconsistent decrease in DA content in rats after giving the MDMA racemate (R. Laverty, unpublished observations). Early work examining the neurotoxicity caused by (*S*)-MDMA also observed regional DA depletion (Commins et al., 1987; Schmidt,

1987; Slikker et al., 1988). In general, however, MDMA has been usually regarded as a selective serotonergic neurotoxin in rats.

In the majority of studies carried out, (±)-MDMA is used. (*S*)-MDMA is more toxic than (*R*)-MDMA and causes DA depletion (Commins et al., 1987; Schmidt et al., 1987). (*S*)-MDMA is also a more potent releaser of DA than (*R*)-MDMA, although both are similar in potency with respect to their ability to induce 5-HT release (Johnson et al., 1986). Consequently, (±)-MDMA administration would produce differing effects on DA and 5-HT release from those caused by an individual isomer.

The neurotoxicity of amphetamine analogues increases with their ability to increase DA release (Schmidt and Kehne, 1990), so, given the relative potencies of the two MDMA stereoisomers in this respect, it is not surprising that (*S*)-MDMA is more toxic than (*R*)-MDMA. Behavioral studies have also demonstrated that the (*S*)-MDMA isomer is more potent than (*R*)-MDMA (Glennon et al., 1987). In addition, the two isomers of MDMA and the racemate produce different responses in humans. For example, subjects stated that the effects of (*S*)-MDMA were slower in onset than those of the racemate (Nichols et al., 1986). The fact that different behavioral responses in both animals and humans alone are induced by individual isomers suggests there are significant differences in their neurochemical effects.

Toxicity may also vary within major structures, such as the striatum, with the result that subtle changes within regions could be missed. For example, the results here show a (*S*)-MDMA-induced decrease in DA content in one specific portion of the caudate, but it is uncertain if this depletion is evident within all areas of the caudate. Incorrect assumptions could also have been made about the mechanism behind MDMA-induced neurotoxicity. Higher doses of MDMA may also be required to induce dopaminergic damage in rats. Regardless, the assumption that MDMA is selective only for serotonergic markers requires revision.

(*S*)-MDMA induced a significant hyperthermia, as has been previously demonstrated with (±)-MDMA (Broening et al., 1995; Farfel and Seiden, 1995b; Gordon et al., 1991). The hyperthermia produced by (±)-MDMA is dependent on ambient temperature and changes of less than 4°C can make the difference between inducing hyper- or hypothermia (Malberg et al., 1996). This appears to be the first study examining core body temperature that has used the (*S*)-MDMA isomer and not the racemate.

The glycine site-specific NMDA antagonist ACEA 1021 alone produced hypothermia. However, when combined with (*S*)-MDMA, it prevented (*S*)-MDMA-induced hyperthermia, which is possibly how it protects against (*S*)-MDMA-induced DA and 5-HT depletion.

Administration of the noncompetitive channel-blocking NMDA antagonist MK-801 produces hypothermia. Indeed, many investigators attribute the neuroprotective properties of this class of drug to its ability to produce hypothermia because, by maintaining the hyperthermia produced by MDMA, the neuroprotective properties of MK-801 were abolished (Farfel and Seiden, 1995b; Miller and O'Callaghan, 1995). Although experiments conducted by these groups used either competitive or noncompetitive channel-blocking NMDA antagonists rather than a glycine site-specific NMDA antagonist.

ACEA 1021 provides a neuroprotective effect when combined with (*S*)-MDMA without producing marked hypothermia. It may be that the ability of this class of drug to provide protection in this model is reliant on its ability to maintain a normal body temperature. However, it has been shown that MDMA can cause neurotoxicity without inducing hyperthermia (Broening et al., 1995).

(*S*)-MDMA caused a significant increase in locomotor activity, an effect also observed by many others. Behavioral studies have been carried out examining schedule-controlled responding that indicate that (*S*)-MDMA is more potent than its stereoisomer (*R*)-MDMA (Callaway and Geyer, 1992).

The finding that ACEA 1021 blocks serotonergic and dopaminergic neurotoxicity induced by (*S*)-MDMA was of interest, because another group (Layer et al., 1993) using glycine site-specific antagonists, i.e., (*R*)-HA 966 (50 mg/kg) and ACPC, were unable to protect against METH-induced neurotoxicity in mice. This could be accounted for by differences between the neurotoxic mechanism behind METH or MDMA-induced neurotoxicity or the sensitivity of neurons in either mice or rats to MDMA-induced DA depletion, which commonly occurs in mice but is rarely observed in rats (Logan et al., 1988).

Glycine site-specific NMDA antagonists, while blocking the same receptor function as channel-blocking NMDA antagonists, have different physiological effects, and so their neuroprotective mechanisms are probably different. This suggests that NMDA receptors are not a key factor in neuroprotection against amphetamine-induced neurotoxicity. These experiments illustrate the interplay between physiological and pharmacological factors, for instance body temperature, activity, and receptor type, all of which need careful control to perform useful experiments. They also demonstrate that while many amphetamine derivatives induce neurotoxicity, they do so by different mechanisms susceptible to different antagonists.

References

Appel NM, Mitchell WM, Contrera JF, De Souza EB. Effects of high-dose fenfluramine treatment on monoamine uptake sites in rat brain: assessment using quantitative autoradiography. Synapse 1990;6:33–44.

Aulakh CS, Hill JL, Wozniak KM, Murphy DL. Fenfluramine-induced suppression of food intake and locomotor activity is differentially altered by the selective type A monoamine oxidase inhibitor clorgyline. Psychopharmacology (Berlin) 1988;95:313–7.

Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ, De Souza EB. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neu-

rodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. J Pharmacol Exp Ther 1987;242:911–6.

Bowyer JF, Davies DL, Schmued L, Broening HW, Newport GD, Slikker W, Holson RR. Further studies of the role of hyperthermia in methamphetamine neurotoxicity. J Pharmacol Exp Ther 1994;268:1571–80.

Broening HW, Bowyer JF, Slikker W. Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (±)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. J Pharmacol Exp Ther 1995; 275:325–33.

Brownsill R, Wallace D, Taylor A, Campbell B. Study of human urinary metabolism of fenfluramine using gas chromatography–mass spectrometry. J Chromatogr 1991;562:267–77.

Callaway CW, Geyer MA. Stimulant effects of 3,4-methylenedioxymethamphetamine in the nucleus accumbens of rat. Eur J Pharmacol 1992;214:45–51.

Callaway CW, Wing LL, Nichols DE, Geyer MA. Suppression of behavioral activity by norfenfluramine and related drugs in rats is not mediated by serotonin release. Psychopharmacology (Berlin) 1993;111:169–78.

Clark WG, Lipton JM. Changes in body temperature after administration of adrenergic and serotonergic agents and related drugs including antidepressants: II. Neurosci Biobehav Rev 1986;10:153–220.

Clement JG, Mills P, Brockway B. Use of telemetry to record body temperature and activity in mice. J Pharmacol Methods 1989;21:129–40.

Colado MI, Green AR. A study of the mechanism of MDMA ('ecstasy')-induced neurotoxicity of 5-HT neurones using chlormethiazole, dizocilpine and other protective compounds. Br J Pharmacol 1994;111:131–6.

Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS. Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 1987;241:338–45.

Cox B. Pharmacologic control of temperature regulation. Annu Rev Pharmacol Toxicol 1977;17:341–53.

De Souza EB, Battaglia G. Effects of MDMA and MDA on brain serotonin neurons: evidence from neurochemical and autoradiographic studies. NIDA Res Monogr 1989;94:196–222.

De Souza EB, Kuyatt BL. Autoradiographic localization of ^{3}H-paroxetine-labeled serotonin uptake sites in rat brain. Synapse 1987;1:488–96.

Dilsaver SC, Overstreet DH, Peck JA. Measurement of temperature in the rat by rectal probe and telemetry yields compatible results. Pharmacol, Biochem Behav 1992;42:549–52.

Dragunow M, Logan B, Laverty R. 3,4-Methylenedioxymethamphetamine induces Fos-like proteins in rat basal ganglia: reversal with MK 801. Eur J Pharmacol 1991;206:255–8.

Even P, Nicolaidis S. Dextrofenfluramine increases energy cost of muscular effort. Pharmacol, Biochem Behav 1986;24:647–55.

Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA, Hutt AJ. Stereospecific analysis and enantiomeric disposition of 3,4-methylenedioxymethamphetamine (Ecstasy) in humans. Clin Chem 1999;45: 1058–69.

Farfel GM, Seiden LS. Role of hypothermia in the mechanism of protection against serotonergic toxicity: I. Experiments using 3,4-methylenedioxymethamphetamine, dizocilpine, CGS 19755 and NBQX. J Pharmacol Exp Ther 1995a;272:860–7.

Farfel GM, Seiden LS. Role of hypothermia in the mechanism of protection against serotonergic toxicity: II. Experiments with methamphetamine, *p*-chloroamphetamine, fenfluramine, dizocilpine and dextromethorphan. J Pharmacol Exp Ther 1995b;272:868–75.

Frith CH, Chang LW, Lattin DL, Walls RC, Hamm J, Doblin R. Toxicity of methylenedioxymethamphetamine (MDMA) in the dog and the rat. Fundam Appl Toxicol 1987;9:110–9.

Glennon RA, Little PJ, Rosecrans JA, Yousif M. The effect of MDMA ("Ecstasy") and its optical isomers on schedule-controlled responding in mice. Pharmacol, Biochem Behav 1987;26:425–6.

Gordon CJ, Watkinson WP, O'Callaghan JP, Miller DB. Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. Pharmacol, Biochem Behav 1991;38:339–44.

Guerra MJ, Liste I, Labandeira-Garcia JL. Interaction between the serotonergic, dopaminergic, and glutamatergic systems in fenfluramine-induced Fos expression in striatal neurons. Synapse 1998;28:71–82.

Hanson GR, Singh N, Bush L, Gibb JW. Response of extrapyramidal and limbic neuropeptides to fenfluramine administration: comparison with methamphetamine. J Pharmacol Exp Ther 1991;259:1197–202.

Hardman HF, Haavik CO, Seevers MH. Relationship of the structure of mescaline and seven analogs to toxicity and behavior in five species of laboratory animals. Toxicol Appl Pharmacol 1973;25:299–309.

Hatzidimitriou G, McCann UD, Ricaurte GA. Altered serotonin innervation patterns in the forebrain of monkeys treated with (±)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. J Neurosci 1999;19:5096–107.

Henry JA, Fallon JK, Kicman AT, Hutt AJ, Cowan DA, Forsling M. Low-dose MDMA ("Ecstasy") induces vasopressin secretion. Lancet 1998;351:1784.

Hrdina PD, Foy B, Hepner A, Summers RJ. Antidepressant binding sites in brain: autoradiographic comparison of [^{3}H]paroxetine and [^{3}H]imipramine localization and relationship to serotonin transporter. J Pharmacol Exp Ther 1990;252:410–8.

Johnson MP, Nichols DE. Comparative serotonin neurotoxicity of the stereoisomers of fenfluramine and norfenfluramine. Pharmacol, Biochem Behav 1990;36:105–9.

Johnson MP, Hoffman AJ, Nichols DE. Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices. Eur J Pharmacol 1986;132:269–76.

Kanthasamy AG, Kanthasamy A, Matsumoto RR, Vu TQ, Truong DD. Neuroprotective effects of the strychnine-insensitive glycine site NMDA antagonist (*R*)-HA-966 in an experimental model of Parkinson's disease. Brain Res 1997;759:1–8.

Kleven MS, Schuster CR, Seiden LS. Effect of depletion of brain serotonin by repeated fenfluramine on neurochemical and anorectic effects of acute fenfluramine. J Pharmacol Exp Ther 1988;246:822–8.

Layer RT, Bland LR, Skolnick P. MK-801, but not drugs acting at strychnine-insensitive glycine receptors, attenuate methamphetamine nigrostriatal toxicity. Brain Res 1993;625:38–44.

Lenton S, Boys A, Norcross K. Raves, drugs and experience: drug use by a sample of people who attend raves in Western Australia. Addiction 1997;92:1327–37.

Logan BJ, Laverty R, Sanderson WD, Yee YB. Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity. Eur J Pharmacol 1988;152:227–34.

Malberg JE, Seiden LS. Administration of fenfluramine at different ambient temperatures produces different core temperature and 5-HT neurotoxicity profiles. Brain Res 1997;765:101–7.

Malberg JE, Seiden LS. Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. J Neurosci 1998;18:5086–94.

Malberg JE, Sabol KE, Seiden LS. Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. J Pharmacol Exp Ther 1996;278:258–67.

Matsumoto RR, Brackett RL, Kanthasamy AG. Novel NMDA/glycine site antagonists attenuate cocaine-induced behavioral toxicity. Eur J Pharmacol 1997;338:233–42.

McCann U, Hatzidimitriou G, Ridenour A, Fischer C, Yuan J, Katz J, Ricaurte G. Dexfenfluramine and serotonin neurotoxicity: further preclinical evidence that clinical caution is indicated. J Pharmacol Exp Ther 1994;269:792–8.

McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet 1998;352:1433–7.

Miller DB, O'Callaghan JP. Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. J Pharmacol Exp Ther 1994;270: 752–60.

Miller DB, O'Callaghan JP. The role of temperature, stress, and other factors in the neurotoxicity of the substituted amphetamines 3,4-methylenedioxymethamphetamine and fenfluramine. Mol Neurobiol 1995;11:177–92.

Morrow BA, Taylor JR, Roth RH. *R*-(+)-HA-966, an antagonist for the glycine/NMDA receptor, prevents locomotor sensitization to repeated cocaine exposures. Brain Res 1995;673:165–9.

Nash JF, Yamamoto BK. Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. Brain Res 1992;581:237–43.

Nichols DE, Hoffman AJ, Oberlender RA, Jacob PD, Shulgin AT. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: representatives of a novel therapeutic class. J Med Chem 1986;29:2009–15.

Palkovits M, Brownstein MJ. Maps and guide to microdissection of the rat brain Elsevier, New York 1988.

Palkovits M, Zaborszky L, Brownstein MJ, Fekete MI, Herman JP, Kanyicska B. Distribution of norepinephrine and dopamine in cerebral cortical areas of the rat. Brain Res Bull 1979;4:593–601.

Papp M, Moryl E, Maccecchini ML. Differential effects of agents acting at various sites of the NMDA receptor complex in a place preference conditioning model. Eur J Pharmacol 1996;317:191–6.

Paxinos G, Watson C. The rat brain in stereotoxic coordinates Academic London. 1997.

Ricaurte GA, DeLanney LE, Irwin I, Langston JW. Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration. Brain Res 1988;446:165–8.

Rocher C, Jacquot C, Gardier AM. Simultaneous effects of local dexfenfluramine application on extracellular glutamate and serotonin levels in rat frontal cortex: a reverse microdialysis study. Neuropharmacology 1999;38:513–23.

Sabol KE, Richards JB, Seiden LS. The NMDA receptor antagonist MK-801 does not protect against serotonin depletions caused by high doses of DL-fenfluramine. Brain Res 1992;582:129–33.

Scheffel U, Ricaurte GA. Paroxetine as an in vivo indicator of 3,4-methylenedioxymethamphetamine neurotoxicity: a presynaptic serotonergic positron emission tomography ligand? Brain Res 1990;527:89–95.

Schmidt CJ. Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its *N*-desmethyl and *N*-ethyl analogs. Eur J Pharmacol 1987;136:81–8.

Schmidt CJ, Kehne JH. Neurotoxicity of MDMA: neurochemical effects. Ann NY Acad Sci 1990;600:665–80 (Discussion 680-1).

Schmidt CJ, Levin JA, Lovenberg W. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem Pharmacol 1987;36:747–55.

Semple DM, Ebmeier KP, Glabus MF, O'Carroll RE, Johnstone EC. Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('Ecstasy') users. Br J Psychiatry 1999;175:63–9.

Shankaran M, Gudelsky GA. Effect of 3,4-methylenedioxymethamphetamine (MDMA) on hippocampal dopamine and serotonin. Pharmacol, Biochem Behav 1998;61:361–6.

Slikker W, Ali SF, Scallet AC, Frith CH, Newport GD, Bailey JR. Neurochemical and neurohistological alterations in the rat and monkey produced by orally administered methylenedioxymethamphetamine (MDMA). Toxicol Appl Pharmacol 1988;94:448–57.

Stewart CW, Bowyer JF, Slikker W. Elevated environmental temperatures can induce hyperthermia during D-fenfluramine exposure and enhance 5-hydroxytryptamine (5-HT) depletion in the brain. J Pharmacol Exp Ther 1997;283:1144–50.

Tessel RE, Woods JH, Counsell RE, Lu M. Structure–activity relationships between meta-substituted *N*-ethylamphetamines and locomotor activity in mice. J Pharmacol Exp Ther 1975;192:310–8.

Warner DS, Martin H, Ludwig P, McAllister A, Keana JF, Weber E. In vivo models of cerebral ischemia: effects of parenterally administered NMDA receptor glycine site antagonists. J Cereb Blood Flow Metab 1995;15:188–96.

Webb E, Ashton CH, Kelly P, Kamali F. Alcohol and drug use in UK university students. Lancet 1996;348:922–5.

Yehuda S, Wurtman RJ. The effects of D-amphetamine and related drugs on colonic temperatures of rats kept at various ambient temperatures. Life Sci 1972;11:851–9 (I).