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Chlormethiazole, Dizocilpine and Haloperidol Prevent the Degeneration of Serotonergic Nerve Terminals Induced by Administration of MDMA ('Ecstasy') to Rats

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Summary—An investigation has been made into the effect of 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') administration on the concentration of 5-hydroxytryptamine (5-HT), uptake of [^{3}H]5-HT and [^{3}H]paroxetine binding in rat cerebral cortex tissue. Four days after 2 injections of MDMA (20 mg/kg i.p., 6 hr apart) the concentrations of 5-HT and its metabolite 5-HIAA were reduced by 60%. The binding of [^{3}H]paroxetine to the presynaptic 5-HT transporter was decreased and high affinity uptake of [^{3}H]5-HT was reduced by a similar amount, indicating neurodegeneration of 5-HT terminals. Pretreatment with chlormethiazole (100 mg/kg i.p.), 10 min before each MDMA injection prevented the decrease in both [^{3}H]parotextine binding and uptake of [^{3}H]5-HT. The loss in 5-HT and 5-HIAA content was also attenuated. Pretreatment with dizocilpine (1 mg/kg i.p.) or haloperidol (2 mg/kg i.p.) also prevented the MDMA-induced loss of [^{3}H]paroxetine binding and attenuated the loss of 5-HT and 5-HIAA content. All three compounds also decreased the degree of hyperthermia that follows MDMA administration, although previous studies suggest that the long term neurodegeneration is not associated with the acute hyperthermic response. These data support the findings of others that MDMA injection produces degeneration of 5-HT nerve terminals in the cortex, confirm that chlormethiazole, dizocilpine and haloperidol attenuate MDMA-induced neurotoxic loss of 5-HT and demonstrate for the first time that these compounds prevent the neurodegeneration of 5-HT nerve terminals that follows MDMA administration.

Keywords—Ecstasy, neurodegeneration, neuroprotection, chlormethiazole, dizocilpine, haloperidol, [^{3}H]paroxetine binding, 5-hydroxytryptamine, 3,4-methylenedioxymethamphetamine.

Several amphetamine-related compounds are neurotoxic and their administration to rodents results in a profound and long lasting decrease in the concentration of monoamines in several regions of the brain. For example, administration of several high doses of methamphetamine results in a loss of dopamine in the striatum (Koda and Gibb, 1973) and 5-hydroxytryptamine (5-HT) in the striatum, cortex and hippocampus (Hotchkiss and Gibb, 1980). In contrast, 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') is relatively selective in sparing dopamine while producing neurotoxic damage to 5-HT neurones (Stone *et al.*, 1986; Schmidt, 1987; Battaglia *et al.*, 1987).

MDMA is a widely used recreational drug of abuse (Peroutka, 1987) and administration of the compound to rats or primates results in a marked long term decrease in the concentration of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in several brain regions (Schmidt *et al.*, 1986; Stone *et al.*, 1986; Battaglia *et al.*, 1987; Colado *et al.*, 1993), a loss of 5-HT uptake sites (Battaglia *et al.*, 1987; Battaglia *et al.*, 1988; Sharkey *et al.*, 1991) and a reduction in 5-HT immunoreactive axon terminals (Battaglia *et al.*, 1987; Commins *et al.*, 1987; Molliver *et al.*, 1990).

The long term loss of 5-HT content induced by MDMA administration has been reported to be attenuated by administration of the dopamine antagonist haloperidol (Schmidt *et al.*, 1990), the GABA potentiating drug chlormethiazole (Colado *et al.*, 1993) and the *N*-methyl-D-aspartate (NMDA) antagonist dizocilpine (Colado *et al.*, 1993). Neither dizocilpine nor chlormethiazole had any effect on the acute (4 hr) loss of 5-HT induced by MDMA (Colado and Green, 1994).

It has been generally assumed that the 5-HT and 5-HIAA loss seen several days after MDMA injection reflects neurodegenerative changes, and therefore that prevention of loss indicates neuroprotection. However it is possible to envisage a situation whereby damage has occurred but this is not detected by measurement of 5-HT content because the 'protective' drug has increased 5-HT

concentration and turnover in the remaining nerve terminals. It is for this reason that most investigators refer to drugs producing protection from neurotoxic loss of amine, rather than neuroprotection (e.g. Schmidt *et al.*, 1990; Colado *et al.*, 1993).

We have therefore now examined the effect of chlormethiazole, dizocilpine and haloperidol not only on 5-HT and 5-HIAA content in rat brain 4 days after MDMA administration, but also on a marker of intact 5-HT terminal structure, namely binding of [^{3}H]paroxetine to the presynaptic 5-HT transporter (see Habert *et al.*, 1985). [^{3}H]5-HT uptake into rat cortical tissue was also measured in the study on chlormethiazole.

We also measured the ability of the three putative neuroprotective agents to prevent the marked hyperthermia that follows the administration of MDMA (see e.g. Nash *et al.*, 1988; Gordon *et al.*, 1991; Colado *et al.*, 1993; Dafters, 1994) since hyperpyrexia can be a major clinical problem in cases of acute MDMA toxicity (Henry *et al.*, 1992).

METHODS

Animals and drug treatment

Adult male Lister Hooded rats (Harlan Olac, Bicester) weighing 200–300 g were used. They were housed in groups in conditions of constant temperature (21°C) and controlled lighting (lights on: 07.00–19.00 hr) and given free access to food and water.

All drugs were given intraperitoneally. Because our intitial experiment showed that a single dose of MDMA (20 mg/kg) was producing a smaller loss of 5-HT than that seen in our earlier studies, two injections of MDMA (20 mg/kg) were given 6 hr apart. Putative protective agents were given 10 min before each MDMA injection. Drugs given were chlormethiazole (100 mg/kg), dizocilpine (1 mg/kg), and haloperidol (2 mg/kg).

Measurement of rectal temperature

Temperature was measured by use of a rectal probe inserted 2.5 cm and a thermocouple with digital readout.

Biochemical analyses

To be consistent with our previous study (Colado *et al.*, 1993) animals were killed by cervical dislocation and decapitation 4 days following drug treatment and the brains removed for biochemical analyses. The cortex was dissected out and used for all measures.

The concentration of 5-HT and 5-HIAA was measured by HPLC with electrochemical detection exactly as described previously (Colado *et al.*, 1993).

The binding of [^{3}H]paroxetine was essentially by the method described by Nash *et al.* (1991). Briefly, cortical tissue was homogenized in ice-cold Tris HCl (50 mM; pH 7.4) containing NaCl (120 mM) and KCl (5 mM) using an Ultra–Turrax. The homogenate was centrifuged at 30,000 *g* for 10 min at 4°C. The supernatant was discarded and this wash process was repeated twice. The pellet was finally resuspended in the Tris buffer at a concentration of 10 mg tissue/ml. The assay solution (1 ml) contained [^{3}H]paroxetine (1 nM) and 800 μl tissue preparation with addition of 5-HT (100 μM) for determination of non-specific binding. The incubation was for 60 min at room temperature. Assays were terminated by rapid filtration through presoaked (0.05% polyethyleneimine) glass fibre filters using a Skatron cell harvester. After drying the filters radioactivity was counted by scintillation spectrometry.

Rat cortical tissue was also used for measurement of [^{3}H]5-HT uptake. The tissue was homogenized in ice cold sucrose (0.32 M) at a concentration of 100 mg tissue/ml sucrose, using a Potter homogenizer. The homogenate was centrifuged at 4000 *g* for 5 min at 4°C and 1 ml of supernatant diluted 10- fold with Krebs Ringer phosphate buffer (pH 7.4). The samples were incubated at 37°C for 15 min before assay. The assay solution (200 μl) contained [^{3}H]5-HT (48 nM) and 160 μl of the tissue preparation with imipramine (10 μM) added to determine non-specific uptake. This assay was incubated at 37°C for 5 min and terminated by rapid filtration through glass fibre filters using a Skatron cell harvester. Filters were dried and radioactivity measured by scintillation spectrometry.

Drugs

Drugs used (sources in brackets) were: di-chlormethiazole ethane disulphonate (Astra Arcus, Södertälje, Sweden) dizocilpine hydrogen maleate (Semat Technical (U.K.) Ltd, St Albans), haloperidol ('Haldol', Janssen Pharmaceutical, Grove, Oxford), and MDMA (Sigma Chemical Co., Poole). All drugs were dissolved in 0.9% NaCl w/v (saline) and doses are reported in terms of the free base.

Statistics

All data were analysed by Analysis of Variance (ANOVA) followed by Tukey test.

RESULTS

Effect of MDMA on cortical 5-HT and 5-HIAA content, [^{3}H]paroxetine binding and [^{3}H]5-HT uptake

Four days following administration of MDMA (20 mg/kg × 2, 6 hr apart), the concentrations of 5-HT and 5-HIAA in the cortex were reduced by approx 60%, compared to saline injected control animals [Fig. 1(a) and (b)]. Similar reductions were observed in both [^{3}H]paroxetine binding [Fig. 1(c)] and uptake of [^{3}H]5-HT [Fig. 1(d)].

Effect of chlormethiazole administration on the reduction in indole amine biochemical parameters which follow MDMA injection

When chlormethiazole (100 mg/kg) was injected 10 min before each MDMA administration there was a

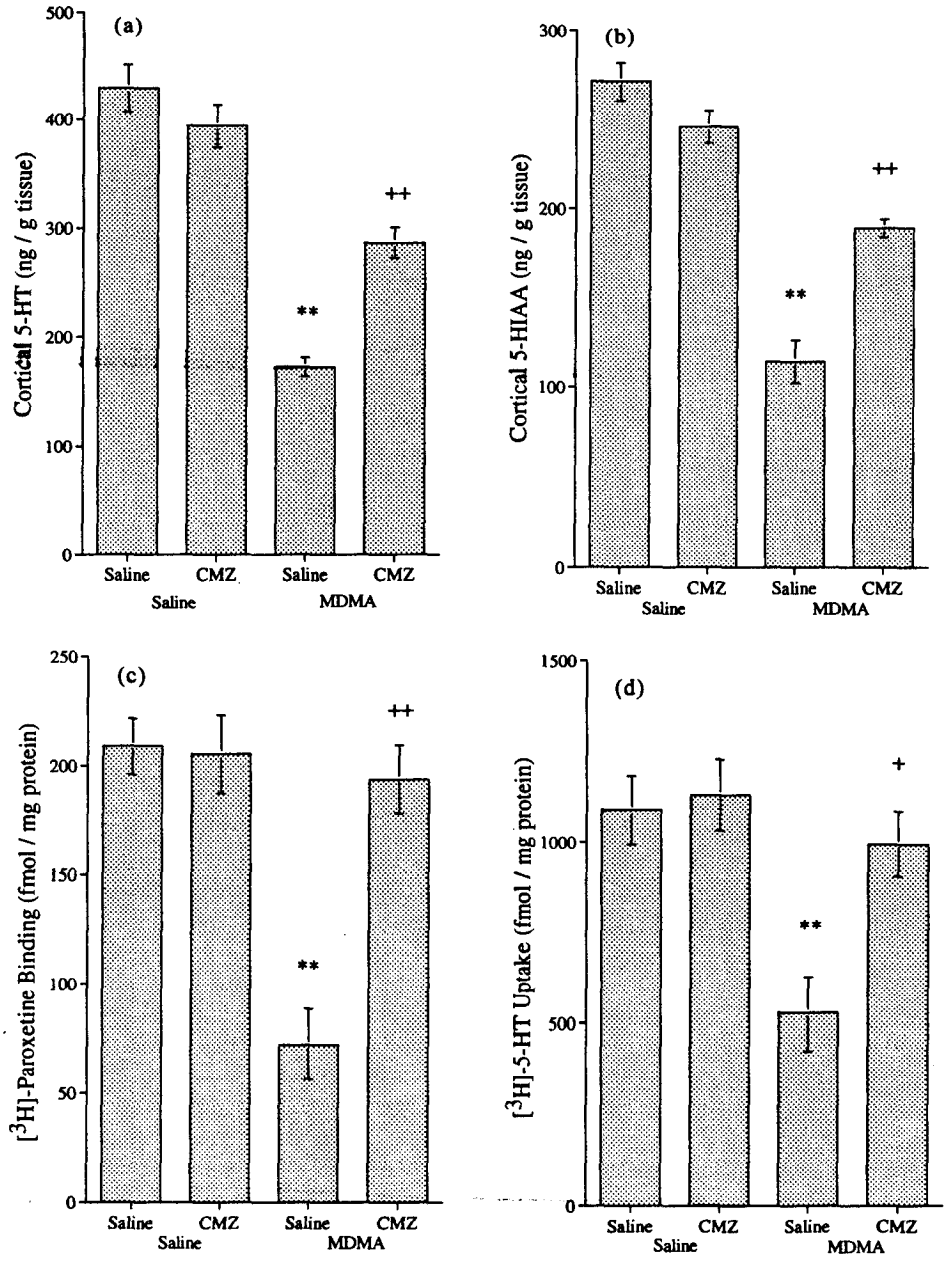

Fig. 1. Effects of chlormethiazole (CMZ) treatment on MDMA-induced depletion of cortical (a) 5-HT content, (b) 5-HIAA content, (c) [^{3}H]paroxetine binding, and (d) [^{3}H]5-HT uptake. Results expressed as mean ± SEM ($n = 7$–10/group). Different from control groups: $^{**}P < 0.01$. Different from saline/MDMA group: $^{++}P < 0.01$, $^{+}P < 0.05$.

significant attenuation of the MDMA-induced loss of both 5-HT and 5-HIAA [Fig. 1(a) and (b)]. Chlormethiazole pretreatment provided almost total protection from the MDMA-induced loss of [^{3}H]paroxetine binding sites [Fig. 1(c)] and the reduction in high affinity uptake of [^{3}H]5-HT [Fig. 1(d)].

Effect of dizocilpine and haloperidol on 5-HT nerve terminal loss following MDMA

Two injections 6 hr apart of dizocilpine (1 mg/kg) or haloperidol (2 mg/kg) resulted in a modest increase in cortical 5-HT content 4 days later [Fig. 2(a)]. The concentration of 5-HIAA was however unaffected [Fig. 2(b)], as was [^{3}H]paroxetine binding [Fig. 2(c)]. Both compounds when given 10 min before MDMA (20 mg/kg) provided significant protection against the loss of 5-HT and 5-HIAA content [Fig. 2(a) and (b)] and prevented the loss in [^{3}H]paroxetine binding [Fig. 2(c)].

Effect of chlormethiazole, dizocilpine and haloperidol on the hyperthermia induced by MDMA administration

It was previously shown that chlormethiazole (100 mg/kg) given 20 min after MDMA (20 mg/kg) reversed the hyperthermic effect of the MDMA.

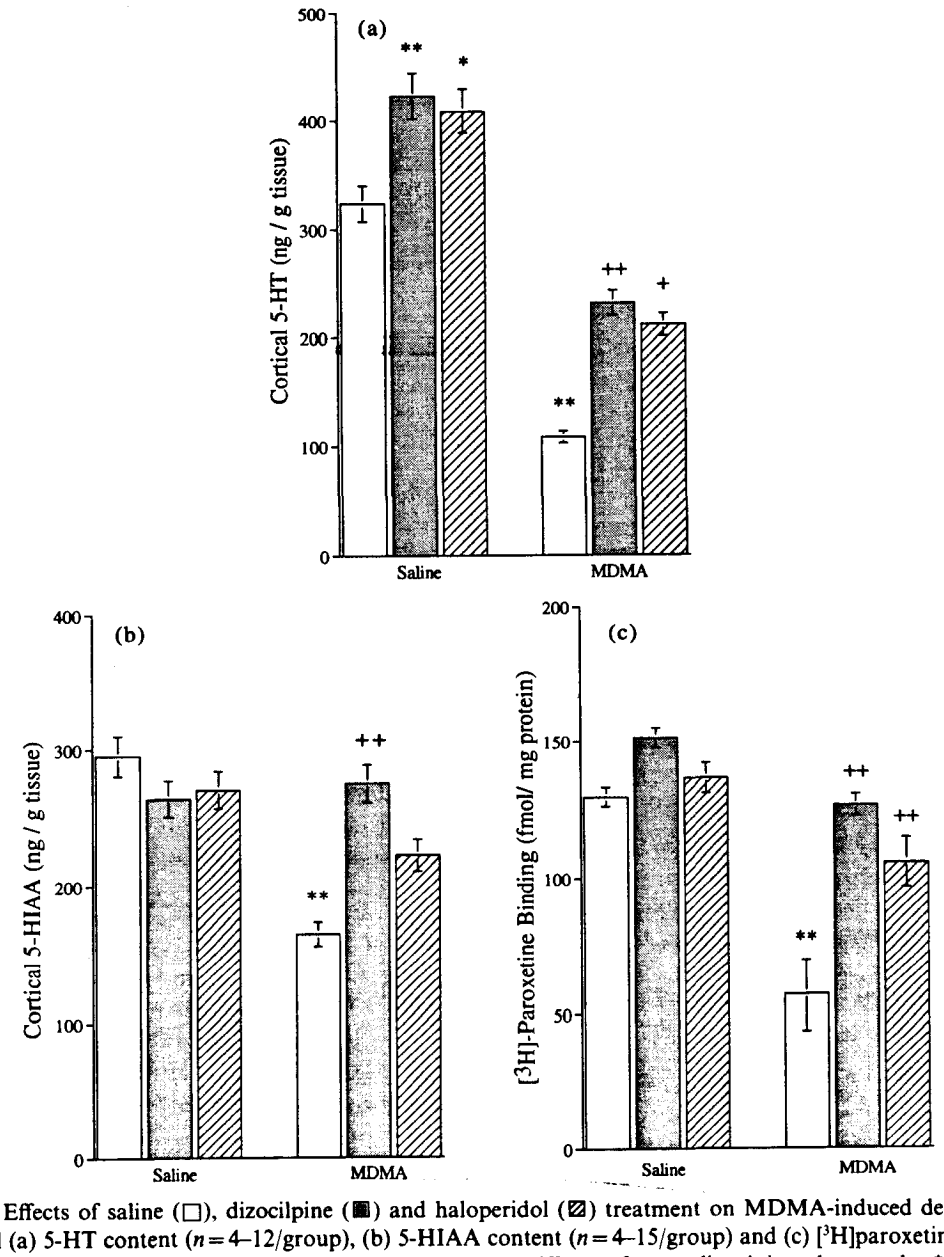

Fig. 2. Effects of saline (□), dizocilpine (■) and haloperidol (▨) treatment on MDMA-induced depletion of cortical (a) 5-HT content ($n=4$–12/group), (b) 5-HIAA content ($n=4$–15/group) and (c) [^{3}H]paroxetine binding ($n=4$–10/group). Results are expressed as mean ± SEM. Different from saline injected controls: $^{**}P<0.01$, $^{*}P<0.05$. Different from saline/MDMA treated animals: $^{++}P<0.01$, $^{+}P>0.05$.

Chlormethiazole (100 mg/kg) alone had little effect on rectal temperature (Colado *et al.*, 1993).

In the current study we examined the effect of pretreatment with chlormethiazole, dizocilpine or haloperidol on the MDMA induced rise in rectal temperature.

Dizocilpine (1 mg/kg) and haloperidol (2 mg/kg) had no significant effects on temperature compared to saline injection (data not shown). Pretreatment with both compounds however reduced by approx 60% the average 1.5°C rise in rectal temperature seen 40 min after MDMA (20 mg/kg) (Fig. 3). Chlormethiazole totally abolished the hyperthermic response of the animals to MDMA injection (Fig. 3).

DISCUSSION

Since 5-HT uptake sites are located predominantly on 5-HT nerve terminals (Kuhar and Aghaganian, 1973), 5-HT nerve terminal loss can be examined by measuring either high affinity uptake of [^{3}H]5HT or binding of [^{3}H]paroxetine to the 5-HT transporter (Habert *et al.*, 1985). MDMA administration to rats resulted in a marked loss in both these neurochemical markers of 5-HT nerve terminals. The loss of [^{3}H]paroxetine binding is in full agreement with the earlier reports (Battaglia *et al.*, 1987; Nash *et al.*, 1991; Sharkey *et al.*, 1991) and consistent with the histological evidence (Battaglia *et al.*,

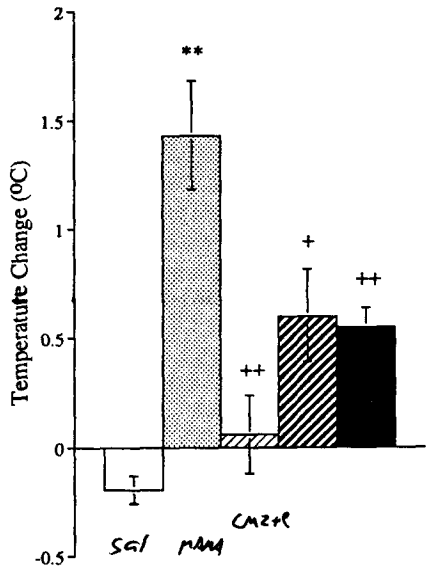

Fig. 3. Changes in rectal temperature 40 minutes after initial administration of (a) saline/saline (□), (b) saline/MDMA (■), (c) chlormethiazole (CMZ)/MDMA (▨), (d) dizocilpine/MDMA (□), and (e) haloperidol/MDMA (■). Results expressed as mean ± s.e. mean ($n = 4$–10/group). Different from saline controls: $^{**}P < 0.01$. Different from saline/MDMA treated animals: $^{++}P < 0.01$. $^{+}P < 0.05$.

1987; Commins *et al.*, 1987; Molliver *et al.*, 1990) that MDMA produces degeneration of 5-HT neurones in the cortex.

When chlormethiazole was injected prior to the MDMA it prevented the MDMA-induced decrease in both uptake of [^{3}H]5-HT and [^{3}H]paroxetine binding. These data provide the strongest support for our earlier proposal (Colado *et al.*, 1993), based entirely on measurement of 5-HT and 5-HIAA concentrations, that chlormethiazole protects against MDMA induced neurodegeneration. Indeed in the current investigation [^{3}H]paroxetine binding and uptake of [^{3}H]5-HT in MDMA/chlormethiazole treated rats were unchanged while 5-HT and 5-HIAA concentrations were still somewhat decreased. This decrease in indole concentrations occurring with little evidence for nerve terminal loss probably reflects the fact that MDMA produces long term inhibition of tryptophan hydroxylase (Schmidt and Taylor, 1987). It would therefore take several days for 5-HT to be replenished following the depletion that occurs immediately after an MDMA injection (Stone *et al.*, 1986; Schmidt, 1987; Colado and Green, 1994).

A previous study (Habert *et al.*, 1985) using 5,7-dihydroxytryptamine to produce a lesion of 5-HT pathways demonstrated a concordance between neurone loss and [^{3}H]paroxetine binding. Our results support the contention (e.g. Nash *et al.*, 1991) that [^{3}H]paroxetine binding is a better determinant of 5-HT neurone integrity than 5-HT concentrations when investigating MDMA neurotoxicity. The problem of interpreting results when the putative neuroprotective drug has itself altered 5-HT concentrations [Fig. 2(a)] is also circumvented.

[^{3}H]Paroxetine binding studies demonstrated that two other compounds which diminish the MDMA-induced neurotoxic loss of 5-HT, namely dizocilpine (Colado *et al.*, 1993) and haloperidol (Schmidt *et al.*, 1990) are also neuroprotective. These data are, we believe, the first direct biochemical evidence that these three compounds are preventing neurogeneration.

There is increasing evidence to support the contention that MDMA administration might produce neurodegeneration of 5-HT in not only rats and primates but also in humans. For example, people who have regularly used MDMA recreationally show a significant (25%) decrease of 5-HIAA in the cerebrospinal fluid (Ricaurte *et al.*, 1990), have altered sleep patterns (Allen *et al.*, 1993) and also a possible blunting of their prolactin response to L-tryptophan administration (Price *et al.*, 1989). Of more immediate concern however is the acute, often fatal, toxic response of some users to MDMA. Predominant among the toxic problems is hyperpyrexia (Henry *et al.*, 1992; Randall, 1992). We therefore briefly examined whether the drugs under investigation would abolish the hyperthermic response of the animals to the first MDMA injection. The ability of chlormethiazole to block effectively the hyperthermic response of rats to MDMA (Colado *et al.*, 1993) was confirmed and both dizocilpine and haloperidol were found to attenuate the response. It should however be noted that previous studies have indicated that the hyperthermia is probably not associated with the long term neurotoxicity produced by administration of MDMA or other substituted amphetamines (Green *et al.*, 1992; Colado *et al.*, 1993).

The mechanisms by which these three diverse compounds produce their neuroprotective effects remains unclear. Decreasing dopamine function in the striatum by inhibiting synthesis or release appears to provide protection against 5-HT loss in the cortex (Schmidt *et al.*, 1991; Schmidt and Kehne, 1990; Johnson *et al.*, 1991). Haloperidol is presumably acting by blocking the function of released dopamine. This suggests that it is increased dopamine transmission that is important to the neurotoxic action of MDMA, not merely increased concentration of released dopamine as was perhaps intimated by the enhancement of MDMA-neurotoxicity by concomitant L-dopa administration (Schmidt *et al.*, 1991). It has been proposed that chlormethiazole, by increasing GABA function, would also inhibit dopamine function in the striatum (Colado *et al.*, 1993; Colado and Green, 1994). Dizocilpine may be acting to inhibit the neurotoxic action of glutamate, a transmitter implicated in the neurodegenerative changes resulting from the action of certain substituted amphetamines (Johnson *et al.*, 1989; Sonsalla *et al.*, 1989; Sonsalla *et al.*, 1991; Finnegan *et al.*, 1990; Green *et al.*, 1992; Colado *et al.*, 1993). However it should also be remembered that

chlormethiazole, although not an NMDA antagonist (Cross *et al.*, 1993a) can inhibit certain NMDA-receptor mediated events (Cross *et al.*, 1993a; Cross *et al.*, 1993b; Thorén and Sjölander, 1993).

In conclusion therefore it has been confirmed that chlormethiazole, dizocilpine and haloperidol decrease the neurotoxic loss of 5-HT in the cortex which follows MDMA administration and it has been shown that this is because these compounds are preventing the neurodegeneration that results from injection of this commonly used recreational drug.

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can we measure 5-HTT genes in our ss?

Lesch '96 Science 274:1527-31

Are there l/s 5HTTLPR in rats or other animals?

Kahn & Wetzler Biol Psychiatry 30: 1139-1166

increas. ↓ SHT transmission → ↑ anxiety (panic attacks) in panic disorder patients

l/l 5HTTLPR ~~[illegible]~~ schizophrenics have higher intensity of hallucinations

s/s 5HTTLPR is linked to alcoholism

chronic alcohol abuse ↑ 5HTT binding but no Δ to 5HTT mRNA

mice w/ no 5HTT have lower SHT

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in vitro PKC activation → ↓ V_{max} of 5-HT uptake