Br. J. Pharmacol. (1993), **108**, 590–596

Striatal dopamine release *in vivo* following neurotoxic doses of methamphetamine and effect of the neuroprotective drugs, chlormethiazole and dizocilpine

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1 Administration to rats of methamphetamine (15 mg kg^{-1}, i.p.) every 2 h to a total of 4 doses resulted in a neurotoxic loss of striatal dopamine of 36% and of 5-hydroxytryptamine (5-HT) in the cortex (43%) and hippocampus (47%) 3 days later.

2 Administration of chlormethiazole (50 mg kg^{-1}, i.p.) 15 min before each dose of methamphetamine provided complete protection against the neurotoxic loss of monoamines while administration of dizocilpine (1 mg kg^{-1}, i.p.) using the same dose schedule provided substantial protection.

3 Measurement of dopamine release in the striatum by *in vivo* microdialysis revealed that methamphetamine produced an approximate 7000% increase in dopamine release after the first injection. The enhanced release response was somewhat diminished after the third injection but still around 4000% above baseline. Dizocilpine (1 mg kg^{-1}, i.p.) did not alter this response but chlormethiazole (50 mg kg^{-1}, i.p.) attenuated the methamphetamine-induced release by approximately 40%.

4 Dizocilpine pretreatment did not influence the decrease in the dialysate concentration of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) produced by administration of methamphetamine while chlormethiazole pretreatment decreased the dialysate concentration of these metabolites still further.

5 The concentration of dopamine in the dialysate during basal conditions increased modestly during the course of the experiment. This increase did not occur in chlormethiazole-treated rats. HVA concentrations were unaltered by chlormethiazole administration.

6 Chlormethiazole (100–1000 μM) did not alter methamphetamine (100 μM) or K^+ (35 mM)-evoked release of endogenous dopamine from striatal prisms *in vitro*.

7 Several NMDA antagonists prevent methamphetamine-induced neurotoxicity; however chlormethiazole is not an NMDA antagonist. Inhibition of striatal dopamine function prevents methamphetamine-induced toxicity of both dopamine and 5-HT pathways. Therefore the attenuation of the enhanced dopamine release which occurs in animals given chlormethiazole may be associated with the protective action of this drug against methamphetamine-induced neurotoxicity.

Keywords: Dopamine release *in vivo*; chlormethiazole; dizocilpine; methamphetamine; neurotoxicity; 5-hydroxytryptamine; dopamine; *in vivo* microdialysis; striatum

Introduction

It has been known for some time that large doses of methamphetamine are neurotoxic and result in a long term loss of dopamine content in the striatum (Koda & Gibb, 1973). Subsequent research established that 5-hydroxytryptamine (5-HT) is also lost, not only in the striatum (Hotchkiss & Gibb, 1980), but also in cortex and hippocampus (see Gibb *et al.*, 1990). Immunocytochemical and visualisation studies have indicated that the loss of biochemical markers such as enzyme activity and monoamine content reflect neurodegenerative changes that have occurred (Ricuarto *et al.*, 1982; Molliver *et al.*, 1990).

Recently Sonsalla *et al.* (1989) demonstrated that the non-competitive N-methyl-D-aspartate antagonist, dizocilpine, protected dopaminergic neurones in the striatum from methamphetamine-induced neurotoxicity and this work was recently extended to show that this protective property was shared by other competitive and non-competitive NMDA antagonists (Sonsalla *et al.*, 1991). Dizocilpine has also been found to prevent the toxic effects of methamphetamine on striatal 5-HT systems (Johnson *et al.*, 1989).

Green *et al.* (1992) confirmed the protective effect of dizocilpine against methamphetamine-induced loss of striatal dopamine and extended the work of Johnson *et al.* (1989) to demonstrate that dizocilpine would also protect against 5-HT loss in the hippocampus and cortex. However this study also demonstrated that chlormethiazole was a very effect protective agent against methamphetamine-induced toxicity to both dopamine in the striatum and 5-HT in cortex and hippocampus (Green *et al.*, 1992).

While there is now considerable evidence to show that chlormethiazole potentiates the action of γ-aminobutyric acid (GABA) by interacting with the $GABA_A$ receptor complex (Harrison & Simmonds, 1983; Ogren, 1986; Cross *et al.*, 1989; Moody & Skolnick, 1989; Vincens *et al.*, 1989) there is no evidence that the drug interacts directly with the NMDA receptor complex (Cross *et al.*, 1992b). The protective action of chlormethiazole could not therefore be ascribed to a direct action involving decreased NMDA-receptor-mediated events.

It is well established that inhibiting dopamine function in the striatum either with dopamine antagonists (Buening & Gibb, 1974) or dopamine synthesis inhibitors (Gibb & Kogan, 1979) prevents methamphetamine-induced neurotoxicity of striatal dopamine and 5-HT (Schmidt *et al.*, 1985). Therefore, using a methamphetamine dose paradigm which we show produces neurotoxicity, we have examined whether protective doses of chlormethiazole and dizocilpine alter

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dopamine release by using *in vivo* microdialysis in the striatum. This has not been previously examined in the case of chlormethiazole, while such data as exist with dizocilpine have been conflicting, with Weihmuller *et al.* (1991; 1992) reporting that the drug attenuated dopamine release and Kashihara *et al.* (1991) finding no alteration of release. In addition we have examined the concentration of the major dopamine metabolites in the dialysate and the effect of chlormethiazole on methamphetamine- and potassium-evoked release of dopamine *in vitro* using striatal slices.

The doses of protective drugs given in the current study were 50 mg kg^{-1} i.p. for chlormethiazole and 1 mg kg^{-1} i.p. for dizocilpine. These doses have previously been shown to be effective against neurotoxic damage induced by methamphetamine in this laboratory (Green *et al.*, 1992). They are also effective doses in protecting against neurotoxicity produced by injection of 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') (Colado *et al.*, 1993) and ischaemia-induced neurodegeneration (Gill *et al.*, 1987; Cross *et al.*, 1991).

A preliminary account of some of this work has been reported to the British Pharmacological Society (Baldwin *et al.*, 1992).

Methods

Animals and drug treatment

Adult male Lister Hooded rats (Harlan Olac, Bicester) weighing 250–350 g were used. They were housed in groups of 5 in conditions of constant temperature (21°C) and controlled lighting (light period: 07 h 00 min–19 h 00 min) and given free access to food and water.

Neurotoxicity was induced by the injection of methamphetamine (15 mg kg^{-1}, i.p.) at 2 h intervals to a total of four injections. In the studies on the effects of neuroprotective drugs, chlormethiazole (50 mg kg^{-1}, i.p.) or dizocilpine (1 mg kg^{-1}, i.p.) were given 15 min before each dose of methamphetamine, control rats being given saline (NaCl 0.9% w/v) in place of the neuroprotective drug.

Measurement of monoamines and metabolites

Three days after methamphetamine administration, rats were killed by cervical dislocation, brains removed and cortex, hippocampus and striatum dissected out. Subsequent measurement of the monoamines (dopamine and 5-hydroxytryptamine) and their metabolites: 5-hydroxyindoleacetic acid (5-HIAA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was by high performance liquid chromatography (h.p.l.c.) with electrochemical detection as described previously (Green *et al.*, 1992).

Implantation of dialysis probes

Rats were implanted with microdialysis probes (3.5 mm × 200 μm; Hospal) in the striatum as described by Baldwin *et al.* (1991). On the following day probes were perfused with artificial cerebrospinal fluid (aCSF) at 1 μl min^{-1} and samples were collected from the freely moving animals at 20 min intervals (Baldwin *et al.*, 1991). The 'dead space' volume of the system was approximately 10 μl. This means that there was an approximate 10 min delay between any response and its detection. Samples were stored at −70°C until analysis for dopamine, DOPAC and HVA by h.p.l.c. with electrochemical detection (Green *et al.*, 1992).

In the study on basal concentrations of dopamine and its metabolites, dopamine concentrations were some 200 times lower than DOPAC. There was therefore marked interference with the dopamine peak unless the DOPAC was eliminated with the solvent front. It was felt that dopamine was the important measure in the study and DOPAC could not therefore be determined.

Measurement of dopamine release from tissue slices

The effect of chlormethiazole on endogenous dopamine release from rat brain tissue prisms was performed by a modification of the method of Auerbach & Lipton (1985) as described in detail by Robinson *et al.* (1989). Briefly, tissue prisms (300 × 300 μm, 45°C) were prepared by use of a McIlwain tissue chopper. Slices were suspended in Krebs-Ringer bicarbonate buffer containing nomifensine (10 μM) and pargyline (10 μM). Tissue suspension was distributed between plastic test tubes with nylon mesh bases. These baskets were incubated in vials containing buffer (basal release) followed by vials containing KCl (35 mM) for K^+-evoked release or methamphetamine (100 μM). Supernatants from the incubation were analysed for dopamine content. In the current study basal release (absence of K^+ or methamphetamine) was measured during a 2 min rather than 5 min period as in the study by Robinson *et al.* (1989).

Drugs

Drugs were obtained from the following (sources in parentheses): (+)-methamphetamine (Sigma Chemical Co, Poole), dichlormethiazole ethanedisulphonate (Astra Arcus, Södertälje, Sweden), dizocilpine hydrogen maleate (Semat Technical (UK) Ltd, St Albans). All other chemicals were obtained from Sigma Chemical Co. or Merck Ltd, Dagenham.

Drugs were dissolved in saline (0.9% w/v NaCl) and all doses refer to the concentration of the base.

Statistics

In vivo dialysis data were analysed by Analysis of Variance (ANOVA). The effects of methamphetamine on dialysate concentrations of dopamine, DOPAC and HVA were determined by separate one-way ANOVAs with 'time' as the repeated measure. The effects of chlormethiazole and dizocilpine on the basal and methamphetamine-induced concentrations of dopamine, DOPAC and HVA were determined by separate two-way ANOVAs with 'drug' as the between groups factor and 'time' as the repeated measure. Comparison of cerebral concentrations of monoamines and their metabolites following drug treatments was by Student's *t* test (unpaired).

Results

Effect of repeated doses of methamphetamine on monoamine content of striatum, hippocampus and cortex and effect of chlormethiazole or dizocilpine pretreatment

Rats were injected with methamphetamine (15 mg kg^{-1}, i.p.) every 2 h to a total of four doses. Three days later the striatal dopamine content was decreased by 36% and the 5-HT content decreased in both hippocampus and cortex by 47% and 43% respectively (Table 1). The concentration of the major 5-HT metabolite, 5-HIAA, was similarly decreased in both regions (Table 1).

Administration of chlormethiazole (50 mg kg^{-1}, i.p.) or dizocilpine (1 mg kg^{-1}, i.p.) every 2 h to a total of four doses did not alter either striatal dopamine content or the indole content in hippocampus or cortex 3 days later except for a modest rise in hippocampal 5-HT following dizocilpine (Table 1). However, administration of chlormethiazole (50 mg kg^{-1}) 15 min before each dose of methamphetamine totally prevented the catecholamine and indoleamine loss (Table 1). Dizocilpine (1 mg kg^{-1}) given 15 min before each dose of methamphetamine, was a reasonably effective protec-

Table 1 The effect of methamphetamine (15 mg kg^{-1}) given 4 times at 2 hourly intervals on brain monoamine concentrations 3 days later and the effects of chlormethiazole (50 mg kg^{-1}) or dizocilpine (1 mg kg^{-1}) given 15 min before each methamphetamine dose

Injected	*Striatum* dopamine	*Hippocampus* 5-HT	*Hippocampus* 5-HIAA	*Cortex* 5-HT	*Cortex* 5-HIAA
Saline	5776 ± 289 (8)	294 ± 12 (8)	355 ± 15 (8)	261 ± 12 (8)	181 ± 8 (8)
Chlormethiazole	6300 ± 463 (8)	321 ± 22 (7)	349 ± 13 (8)	276 ± 13 (8)	186 ± 7 (8)
Dizocilpine	6751 ± 2161 (8)	379 ± 13 (8)‡	335 ± 7 (8)	283 ± 9 (8)	178 ± 4 (8)
Methamphetamine	3721 ± 317 (4)†	155 ± 16 (4)†	201 ± 17 (4)†	150 ± 9 (4)†	104 ± 6 (4)†
Methamphetamine + chlormethiazole	5983 ± 346 (7)	312 ± 20 (7)	354 ± 21 (7)	243 ± 13 (7)	184 ± 12 (7)
Methamphetamine + dizocilpine	6518 ± 231 (6)	277 ± 21 (7)*ø	252 ± 14 (7)*	208 ± 15 (6)*ø	134 ± 8 (7)*ø

Results shown as mean ± s.e.mean (n) ng g^{-1} tissue (wet weight).
Different from saline injected; †$P < 0.001$; ‡$P < 0.01$. Different from dizocilpine injected; *$P < 0.001$. Different from methamphetamine injected; ø$P < 0.03$ or better.

tive agent but, unlike chlormethiazole did not always produce monoamine concentrations in the brain of methamphetamine-treated rats that were not different from control values (Table 1).

Effect of chlormethiazole and dizocilpine on methamphetamine-induced release of dopamine in the striatum

Three baseline samples of dialysate were collected from all rats. One group was then injected with saline followed 15 min later by methamphetamine (15 mg kg^{-1}). This procedure was repeated two further times at 2 h intervals. Further groups were injected with chlormethiazole (50 mg kg^{-1}) or dizocilpine (1 mg kg^{-1}) 15 min before each dose of methamphetamine and the procedure repeated twice more at 2 h intervals. Sample collections were made over an 8 h period.

The first doses of methamphetamine produced a massive release of dopamine of somewhat greater than 7000% above baseline (Figure 1). At the end of 2 h the dialysate dopamine was still more than 4000% above baseline. The second methamphetamine injection produced another peak which was similar to the first. The third injection however produced a somewhat smaller peak, albeit still around 4500% above basal (Figure 1).

Pretreatment with dizocilpine before each dose of methamphetamine did not influence the pattern of dopamine release (Figure 1). Chlormethiazole pretreatment did not alter the first peak concentration but did produce a more rapid return towards baseline and a lower dopamine dialysate concentration than the methamphetamine alone group (Figure 1). The second peak was attenuated and the third methamphetamine-induced peak nearly abolished (Figure 1). Statistical analysis indicated that chlormethiazole had markedly inhibited the dopamine release ($P < 0.0001$, see legend to Figure 1). Whilst not quantified, observation of the behaviour of the animals during the dialysis experiment was undertaken. Injection of methamphetamine led to prolonged and severe locomotor activity which was diminished by the time the next dose was given. Both chlormethiazole and dizocilpine abolished the behavioural excitation, the animals being apparently sedated most of the time. Some behavioural excitation was beginning to appear in the 20 min before the next injection of chlormethiazole or dizocilpine.

Effect of methamphetamine on dialysate HVA and DOPAC concentration and the effect of chlormethiazole and dizocilpine

The first methamphetamine injection resulted in a marked decrease in dialysate DOPAC concentration (Figure 2a) and a modest decrease in HVA concentration (Figure 2b). The diminished concentration of these metabolites continued throughout the collection period with the subsequent methamphetamine injections not inducing any further major effects (Figure 2). Pretreatment with dizocilpine before each methamphetamine injection did not influence the HVA or DOPAC concentration at all (Figure 2). Chlormethiazole pretreatment however produced a further decrease in the dialysate concentration of both HVA and DOPAC compared with methamphetamine (Figure 2).

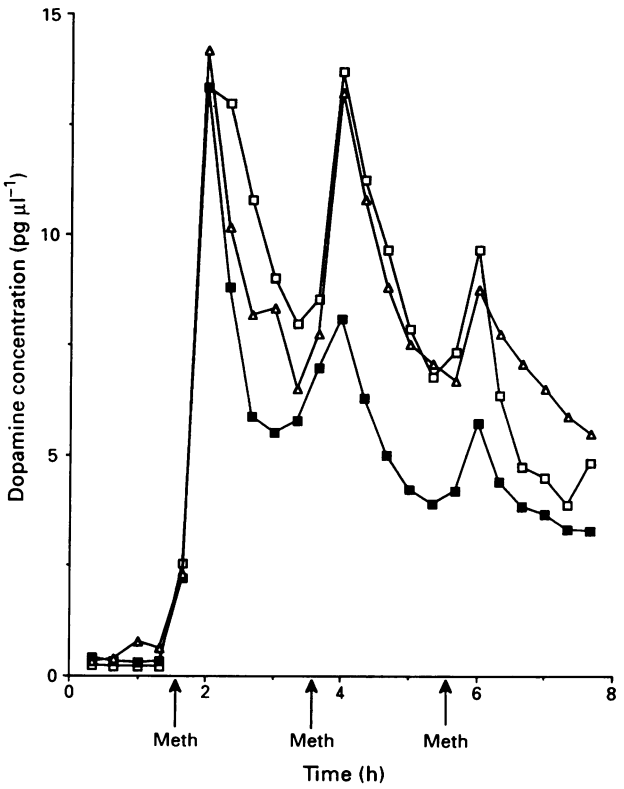

Figure 1 Effect of chlormethiazole (CMZ; 50 mg kg^{-1}, i.p.) and dizocilpine (Diz; 1 mg kg^{-1}, i.p.) given 15 min before each dose of methamphetamine (Meth; 15 mg kg^{-1}, i.p.) on dopamine release in the striatum: (□) Meth; (■) Meth/CMZ; (△) Meth/Diz. Values are mean dopamine concentrations in the dialysate (pg µl^{-1}). There was a highly significant effect of Meth on dopamine release (one way ANOVA F (18, 36) = 24.2, $P < 0.0001$). A separate 2-way ANOVA on the dopamine concentrations following the first methamphetamine injection only showed that there was no effect of CMZ on this peak. However, ANOVA on the remaining data showed that CMZ significantly prevented the dopamine release following the 2nd and 3rd Meth injections (2 way ANOVA 'time' × 'CMZ' interaction F (9, 54) = 2.8, $P < 0.01$). Dizocilpine had no effect on dialysate dopamine concentrations. Mean ± s.e.mean basal concentrations were: Meth 0.22 ± 0.05 (n = 4); Meth/CMZ 0.36 ± 0.05 (n = 5); Meth/Diz 0.35 ± 0.09 (n = 5).

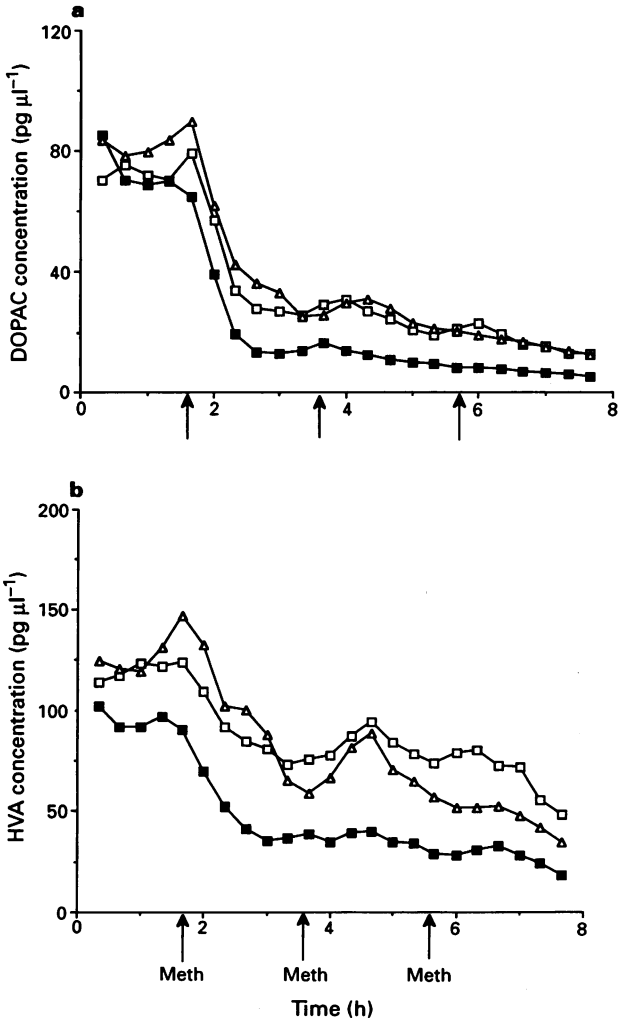

Figure 2 (a) Effect of chlormethiazole (CMZ; 50 mg kg^{-1}, i.p.) and dizocilpine (Diz; 1 mg kg^{-1}, i.p.) on dihydroxyphenylacetic acid (DOPAC) concentrations in the striatum following methamphetamine (Meth; 15 mg kg^{-1}, i.p.): (□) Meth; (■) Meth/CMZ; (△) Meth/Diz. Values are mean DOPAC concentrations in the dialysate (pg μl^{-1}). There was a significant reduction in DOPAC concentrations following methamphetamine administration (one-way ANOVA F (19, 59) = 58.6, $P < 0.0001$). Chlormethiazole further reduced DOPAC concentrations (two-way ANOVA 'CMZ' F (1, 6) = 13.3, $P < 0.05$) whilst dizocilpine had no effect on DOPAC levels in the dialysate. Mean ± s.e.mean basal concentrations were: Meth 72.45 ± 2.22 (n = 4); Meth/CMZ 69.45 ± 3.76 (n = 5); Meth/Diz 80.56 ± 5.82 (n = 5). (b) Effect of chlormethiazole (CMZ; 50 mg kg^{-1}, i.p.) and dizocilpine (Diz; 1 mg kg^{-1}, i.p.) on homovanillic acid (HVA) concentrations in the striatum following methamphetamine (Meth; 15 mg kg^{-1}, i.p.): (□) Meth; (■) Meth/CMZ; (△) Meth/Diz. Values are mean HVA concentrations in the dialysate (pg μl^{-1}). There was a significant reduction in HVA concentrations following methamphetamine administration (one-way ANOVA F (19, 38) = 2.0, $P < 0.05$). Chlormethiazole further reduced HVA concentrations (two-way ANOVA 'CMZ' F (1, 6) = 11.3, $P < 0.05$) whilst dizocilpine had no effect on HVA levels in the dialysate. Mean ± s.e.mean basal concentrations were: Meth 118.18 ± 7.90 (n = 4); Meth/CMZ 91.01 ± 12.08 (n = 5); Meth/Diz 115.92 ± 21.38 (n = 5).

Effect of chlormethiazole and dizocilpine on basal concentrations of dopamine and HVA in the dialysate

Rats were injected with saline in place of the methamphetamine but injected with either chlormethiazole (50 mg kg^{-1}), dizocilpine (1 mg kg^{-1}) or saline as before. Saline-injected animals showed a small rise in basal dopamine over time which was prevented by chlormethiazole (Figure 3a) but not dizocilpine (data not shown). There was no significant effect of chlormethiazole (Figure 3b) or dizocilpine (data not shown) injections on basal HVA concentrations. DOPAC could not be measured in this part of the study for reasons detailed in the Methods.

Effect of chlormethiazole on methamphetamine- or K^+-evoked release of endogenous dopamine from brain tissue prisms

The release of endogenous dopamine from tissue prisms prepared from the striatum induced by the addition of either K^+ (35 mM) or methamphetamine (100 μM) to the medium was unaffected by the presence of 100 μM or 1000 μM chlormethiazole (Table 2).

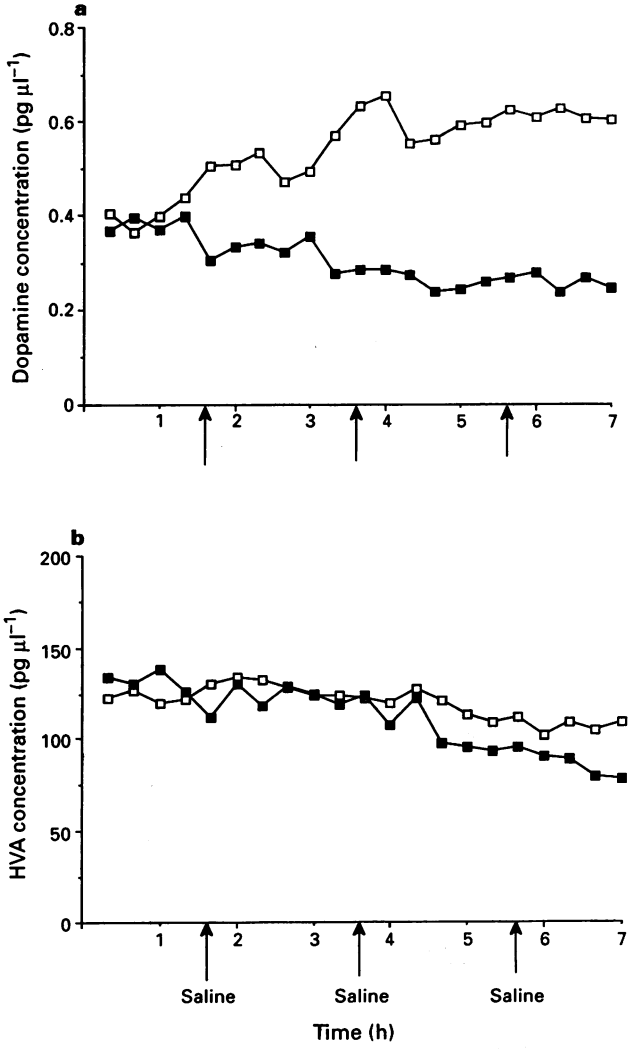

Figure 3 (a) Effect of chlormethiazole (CMZ; 50 mg kg^{-1}, i.p.) given 15 min before each saline injection on basal dopamine release in the striatum: (□) saline; (■) CMZ. Values are mean dopamine concentrations in the dialysate (pg μl^{-1}). Chlormethiazole significantly reduced dialysate dopamine concentrations compared with saline injections alone (2 way ANOVA 'time' × 'CMZ' interaction F (19, 133) = 3.2, $P < 0.0001$). Mean ± s.e.mean basal concentrations were: saline 0.39 ± 0.05 (n = 5); CMZ 0.38 ± 0.07 (n = 4). (b) Effect of chlormethiazole (CMZ; 50 mg kg^{-1}, i.p.) given 15 min before each saline injection on basal homovanillic acid (HVA) levels in the striatum: (□) saline; (■) CMZ. Values are mean HVA concentrations in the dialysate (pg μl^{-1}). There was no significant effect of chlormethiazole on HVA concentrations compared with saline injections alone. Mean ± s.e.mean basal concentrations were: saline 119.96 ± 13.50 (n = 5); CMZ 138.68 ± 4.52 (n = 4).

Table 2 Endogenous dopamine release from striatal slices. Effect of chlormethiazole (CMZ) on methamphetamine (100 μM) and potassium (35 mM) stimulated release

Condition	CMZ concentration (μM)	Dopamine concentration (ng mg^{-1} protein)
Basal	–	3.0 ± 0.6
Methamphetamine	–	16.2 ± 0.9
Methamphetamine	100	14.9 ± 2.8
Methamphetamine	1000	14.1 ± 3.9
K^+	–	18.5 ± 0.6
K^+	100	15.2 ± 3.3
K^+	1000	16.2 ± 0.6
Basal	1000	3.2; 3.1

Results shown as mean ± s.e.mean of 3 observations except basal in presence of chlormethiazole which shows the two observation values. The presence of chlormethiazole did not alter either methamphetamine- or potassium-evoked release.

Discussion

There is substantial evidence to support the contention that the loss of monoamines which occurs in several brain regions following high dose methamphetamine is a reflection of neurodegeneration. This has been demonstrated using biochemical (Stone *et al.*, 1986; 1988) and importantly morphological (Jonsson & Nzanze, 1982; Ricuarto *et al.*, 1982; Molliver *et al.*, 1990) techniques.

In our previous study (Green *et al.*, 1992) four doses of methamphetamine (15 mg kg^{-1}) were given at 3 h intervals. However, the logistics of this protocol when applied to collection of microdialysis samples was difficult and it was therefore decided to administer the methamphetamine at 2 h intervals in the current investigation. The neurotoxic effects of substituted amphetamines has been shown to be dependent on both the dose and treatment regime (see McKenna & Peroutka, 1990) and so the first part of the study examined the severity of monoamine loss following the protocol to be used in the rest of the investigation.

Three days after the repeated methamphetamine injection there was around a 45% loss of striatal dopamine and cortical and hippocampal 5-HT (Table 1). This decrease was somewhat less than seen when the methamphetamine was given at 3 h intervals (65–75%; Green 1992) but was nevertheless a substantial neurotoxic effect. Consistent with the earlier study was the fact that the loss of both 5-HT and dopamine was similar. This part of the study confirmed both the protective effect of dizocilpine against methamphetamine-induced neurotoxicity as previously reported (Sonsalla *et al.*, 1989; Johnson *et al.*, 1989; Green *et al.*, 1992) and also that, at the doses used, chlormethiazole was a more effective protective agent than dizocilpine (Green *et al.*, 1992).

Administration of methamphetamine produced a massive release of dopamine in the striatum. This response was attenuated somewhat by the third injection as one might expect if neuronal stores were being depleted. Dizocilpine pretreatment failed to have any effect on this release. Weihmuller *et al.* (1991, 1992) in contrast, have reported that dizocilpine administration inhibited dopamine release after every injection of methamphetamine. Two points should perhaps be made here. The methamphetamine dose in their investigation was lower (4 mg kg^{-1}) than the current study and produced a rise in dialysate dopamine concentrations around 10 times less than our study. This might explain why the final injection in their investigation was able to produce the largest rise (Weihmuller *et al.*, 1992). In contrast, in our study, methamphetamine produced a much smaller increase by the third injection, presumably as stores had become depleted. This dosing difference makes direct comparison difficult. However, the second point is that the Weilmuller *et al.* (1991, 1992) studies do not agree with Kashihara *et al.* (1991) who used a similar dose of methamphetamine (6.25 mg kg^{-1}) and saw no evidence for an inhibition of dopamine release after a single injection of methamphetamine, consistent with our failure to detect any inhibition at all. The fact remains that in our investigation dizocilpine was an effective protective agent against the consequences of high dose methamphetamine and no inhibition of dopamine release was seen at any time.

Chlormethiazole, in contrast, produced a substantial decrease in dopamine release throughout the whole experiment (around 35%) and, with the exception of the first peak around a 40% decrease in the subsequent peaks (above basal).

The effect of chlormethiazole, but not dizocilpine, on dopamine release was further illustrated by the measures of dopamine metabolites in the dialysate. The concentrations of HVA and DOPAC were unaffected by dizocilpine while they declined further after chlormethiazole. These data confirm the observation of Kashihara *et al.* (1991) that the DOPAC concentration in the dialysate after dizocilpine plus methamphetamine is the same as that following methamphetamine alone.

The question arose as to what effect chlormethiazole would have on extracellular striatal dopamine concentrations in untreated rats. Basically it appeared to induce a small inhibition of the modest rise that occurred during the study, consistent with the drug being a GABA-mimetic agent since other GABA-mimetic agents are known to inhibit dopamine release (Fuxe *et al.*, 1979). However this effect on release cannot explain the inhibition of methamphetamine-induced dopamine release since the inhibition of release by GABA-mimetic drugs is presumably due to a presynaptic effect involving nerve-impulse flow. In contrast, amphetamine-evoked release involves uptake of the amphetamine, probable reversal of the uptake pump and release of newly synthesized transmitter (see McMillen, 1983). This is a Ca^{2+}-independent mechanism not requiring nerve impulse flow (see McMillen, 1983; Robinson *et al.*, 1989). Dizocilpine was not observed to alter basal dopamine release consistent with the report of Weihmuller *et al.* (1992).

Interestingly, we were unable to show any effect of chlormethiazole on either K^+- or methamphetamine-evoked release of endogenous dopamine from tissue prisms *in vitro*. These *in vitro* data therefore indicate that chlormethiazole does not have a direct effect on dopamine release at the nerve ending and is acting through neuronal loops requiring intact anatomical architecture. Dizocilpine has previously been shown not to alter methamphetamine-induced release of dopamine from brain slices (Bowyer *et al.*, 1991). These studies further indicate that chlormethiazole has no direct effect on uptake of methamphetamine into nerve terminals *in vitro*. It is reasonable therefore to conclude that the inhibition of dopamine release after methamphetamine and chlormethiazole *in vivo* cannot be because the drug is interfering with the uptake of methamphetamine into the nerve ending.

Both chlormethiazole and dizocilpine inhibited the methamphetamine-induced behavioural changes and dizocilpine at least is not doing so by directly decreasing dopamine release. It therefore seems likely that dizocilpine and possibly also chlormethiazole (given that there is still considerable

dopamine release) are acting 'downstream' to attenuate the behavioural responses. These data again conflict with Weihmuller *et al.* (1991) who reported that dizocilpine did not inhibit the methamphetamine-induced behaviour but not the report of Farfel *et al.* (1991) who noted an attenuation by dizocilpine of methamphetamine-induced stereotyped behaviour. We, have also recently observed that dizocilpine and chlormethiazole inhibit the behavioural abnormalities induced by administration of three other neurotoxic substituted amphetamines namely, *p*-chloramphetamine, fenfluramine and 3,4-methylenedioxy-methamphetamine (MDMA or Ecstasy) albeit only the MDMA neurotoxicity of 5-HT pathways was prevented by chlormethiazole and dizocilpine (Colado *et al.*, 1993).

Whilst the concentration of chlormethiazole used in the medium might appear high, the IC_{50} value for inhibition of [^{3}H]-TPBS binding to the chloride channel is over 10^{-4} M (Cross *et al.*, 1989) and peak plasma and brain concentrations are also greater than 10^{-4} M following an injection of chlormethiazole of 50–100 mg kg^{-1} (Kalant & Khanna, 1986; Cross *et al.*, unpublished observations).

What the current experiments do provide is partial explanation for why chlormethiazole prevents methamphetamine-induced neurotoxicity. There is substantial evidence that inhibiting striatal dopamine function by use of either dopamine synthesis inhibitors or receptor antagonists prevents not only the subsequent damage to striatal dopamine (Buening & Gibb, 1974; Gibb & Kogan, 1979; Gibb *et al.*, 1990) but also brain 5-hydroxytryptaminergic systems (Schmidt *et al.*, 1985; Gibb *et al.*, 1990). The ability of chlormethiazole to attenuate the dopamine release caused by methamphetamine may well therefore be involved in the protective effect. Dizocilpine and other NMDA antagonists, in contrast, presumably act by preventing NMDA receptor 'overactivity' downstream to the dopamine release. That having been said, the degree of inhibition of dopamine release by chlormethiazole was only partial. There is now increasing evidence from both experiments on N-methyl-DL-aspartate-induced seizures (Cross *et al.*, 1992b) and harmaline-induced guanosine 3':5'-cyclic monophosphate (cyclic GMP) formation in the cerebellum (Cross *et al.*, 1992a) that chlormethiazole will inhibit NMDA-mediated functional responses even though it does not interact with the NMDA receptor directly (Cross *et al.*, 1992b). It is possible therefore that the protective effect of chlormethiazole involves *both* the inhibition of dopamine release and antagonism of NMDA receptor-mediated function. Consistent with this proposal is the fact that chlormethiazole is also an effective protective agent against MDMA-induced neurotoxicity of 5-HT terminals in the cortex and hippocampus (Colado *et al.*, 1993). MDMA is a weak releaser of striatal dopamine (see Schmidt & Kehne, 1990) so inhibition of dopamine release is probably an unsatisfactory proposal in order to explain entirely the neuroprotection by chlormethiazole in this model.

While these data allow one to propose that chlormethiazole is protective in methamphetamine-induced neurotoxicity because of it inhibiting dopamine release, in addition to its reported effect of inhibiting at least some NMDA-mediated effects, no explanation can be given at present as to the mechanism by which it inhibits methamphetamine-induced dopamine release other than to say that this does not appear to be due to a direct effect at dopamine nerve terminals.

Both chlormethiazole (Cross *et al.*, 1991) and dizocilpine (Gill *et al.*, 1987; 1988; Cross *et al.*, 1991) are protective agents in the gerbil model of global ischaemia and both are protective against methamphetamine- (Green *et al.*, 1992; this paper) and MDMA-induced (Colado *et al.*, 1993) neurotoxicity. The involvement of dopamine as well as glutamate in ischaemia-induced neurodegeneration has been proposed (Globus *et al.*, 1988) so the current investigation may assist in the search for the mechanisms by which chlormethiazole is neuroprotective against ischaemia-induced damage.

M.I.C. thanks the European Science Foundation and DGICYT (Spain) for financial support.

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(Received August 3, 1992
Revised September 30, 1992
Accepted October 14, 1992)