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A study of the mechanism of MDMA ('Ecstasy')-induced neurotoxicity of 5-HT neurones using chlormethiazole, dizocilpine and other protective compounds

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1 An investigation has been made in rats into the neurotoxic effect of the relatively selective 5-hydroxytryptamine (5-HT) neurotoxin, 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') using chlormethiazole and dizocilpine, both known neuroprotective compounds and also γ-butyrolactone, ondansetron and pentobarbitone.

2 Administration of MDMA (20 mg kg^{-1}, i.p.) resulted in a 50% loss of cortical and hippocampal 5-HT and 5-hydroxyindole acetic acid (5-HIAA) 4 days later. This reflects the long term neurotoxic loss of 5-HT that occurs. Injection of γ-butyrolactone (GBL; 400 mg kg^{-1}, i.p.) 5 min before and 55 min after the MDMA provided substantial protection. Pentobarbitone (25 mg kg^{-1}, i.p.) using the same dose regime was also protective, but ondansetron (0.5 mg kg^{-1} or 0.1 mg kg^{-1}, i.p.) was without effect.

3 MDMA (20 mg kg^{-1}) had no significant effect on striatal dopamine concentration 4 days later but did produce a small decrease in 3,4-dihydroxyphenylacetic acid (DOPAC) content. There were few significant changes in rats given MDMA plus GBL, ondansetron or pentobarbitone.

4 A single injection of MDMA (20 mg kg^{-1}, i.p.) resulted in a greater than 80% depletion of 5-HT in hippocampus and cortex 4 h later, reflecting the initial rapid release that had occurred. None of the neuroprotective compounds (chlormethiazole, 50 mg kg^{-1}; dizocilpine, 1 mg kg^{-1}; GBL, 400 mg kg^{-1}; pentobarbitone, 25 mg kg^{-1}) given 5 min before and 55 min after the MDMA injection, altered the degree of 5-HT loss.

5 Acute MDMA injection increased striatal dopamine content (28%) and decreased the DOPAC content. In general, administration of the drugs under investigation did not significantly alter these MDMA-induced changes. Both chlormethiazole and GBL produced a greater increase in dopamine than MDMA alone, but this was apparently an additive effect to the action of either drug alone.

6 The 5-HT loss 4 h following administration of the neurotoxin p-chloroamphetamine (2.5 mg kg^{-1}, i.p.) was not affected by chlormethiazole or dizocilpine. *p*-Chloroamphetamine did not appear to alter striatal dopamine metabolism.

7 None of the protective drugs inhibited the initial 5-HT loss following MDMA, rendering unlikely any proposal that they are protective because they inhibit 5-HT release and the subsequent formation of a toxic indole derivative. All the protective compounds (unlike ondansetron) probably inhibit dopamine release in the striatum. Since the neurotoxic action of some substituted amphetamines is dependent on the integrity of nigro-striatal neurones, this fact may go some way to explain the protective action of this diverse group of compounds.

Keywords: 3,4-Methylenedioxymethamphetamine; chlormethiazole; dizocilpine; γ-butyrolactone; pentobarbitone; neurotoxicity; brain monoamines; neuroprotection; 'Ecstasy'

Introduction

We recently reported that the neurotoxic effects of 3,4 methylenedioxymethamphetamine (MDMA or 'Ecstasy') on 5-hydroxytryptamine (5-HT) terminals in the rat cortex and hippocampus could be prevented by administration of chlormethiazole or dizocilpine (Colado *et al.*, 1993). Both drugs prevented the long term loss of 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) which occurs in both brain regions following a single dose of MDMA. In contrast, neither chlormethiazole nor dizocilpine protected against the effect of two other 5-HT neurotoxins, namely *p*-chloroamphetamine (PCA) and fenfluramine (Colado *et al.*, 1993). Others have also recently reported on the failure of dizocilpine to protect against the toxic effect of fenfluramine (Sabol *et al.*, 1992) and PCA (Henderson *et al.*, 1992).

The neurochemical actions of chlormethiazole and dizocilpine in the brain are recognised to be very different. Chlormethiazole, a sedative, hypnotic and anticonvulsant agent (see Evans *et al.*, 1986), potentiates γ-aminobutyric acid (GABA) function (Harrison & Simmonds, 1983; Ogren, 1986; Cross *et al.*, 1989). Dizocilpine, in contrast, is a non-competitive N-methyl-D-aspartate (NMDA) antagonist (Wong *et al.*, 1986). While both biochemical (Cross *et al.*, 1993a) and electrophysiological (Addae & Stone, 1988) studies have indicated that chlormethiazole does not interact with the NMDA receptor complex, it nevertheless antagonizes various NMDA receptor-mediated events such as NMDLA-induced seizures (Cross *et al.*, 1993a), the harmaline-induced increase in cerebellar guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Cross *et al.*, 1993b) and NMDA-induced derangement of sensory evoked potentials (Thorén & Sjölander, 1993).

MDMA administration produces a marked and rapid (3–4 h) depletion of 5-HT in several brain regions (Schmidt, 1987; Gibb *et al.*, 1990). There is a recovery of brain 5-HT concentration by 24 h but this is followed by a long term decrease which is unequivocal by 3–4 days and which lasts for several months (Schmidt, 1987; see also McKenna & Peroutka, 1990). This second phase of monoamine loss reflects neurodegenerative changes that have occurred and

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which have been demonstrated by immunocytochemical and visualisation studies (see Molliver *et al.*, 1990). Similar acute and long term changes in 5-HT occur after administration of both PCA and fenfluramine (Sanders-Bush *et al.*, 1972; Fuller *et al.*, 1975; Harvey & McMaster, 1975; Neckers *et al.*, 1976).

The neurotoxic action of methamphetamine on 5-HT neurones in hippocampus and cortex and dopamine neurones in striatum is dependent on the integrity of nigrostriatal neurones (Schmidt *et al.*, 1985; and see McKenna & Peroutka, 1990). This also seems to be true of MDMA toxicity (Stone *et al.*, 1988; Johnson *et al.*, 1991) even though MDMA is a relatively specific 5-HT neurotoxin (Schmidt *et al.*, 1986; Stone *et al.*, 1986; Schmidt, 1987). Furthermore the short term changes in the concentration of dopamine and its metabolites which occur following MDMA administration have been shown to be blocked by certain neuroprotective agents (Nash, 1990; Schmidt *et al.*, 1990; Callaway *et al.*, 1991).

Since dopamine neurones in the substantia nigra are modulated by both glutamate and GABA (see Dray, 1979) it seemed possible that the neuroprotective action of chlormethiazole and dizocilpine against MDMA-induced toxicity that we observed (Colado *et al.*, 1993) might be due to an inhibition of dopamine release as we have previously suggested to be the case in the investigation of methamphetamine-induced neurotoxicity (Green *et al.*, 1992).

We have now extended our earlier findings (Colado *et al.*, 1993) to examine whether another GABA potentiating drug (pentobarbitone) will also confer protection and also investigated the effect of two compounds known to interfere with dopamine release in the brain, namely γ-butyrolactone (Gessa *et al.*, 1966; Anden *et al.*, 1973) and ondansetron (Butler *et al.*, 1988). The former inhibits dopamine release in the striatum (see for example Chrapusta *et al.*, 1992), while the latter is a 5-HT_3 antagonist (Butler *et al.*, 1988) and inhibits dopamine release in the nucleus accumbens (Kilpatrick *et al.*, 1987; Costall *et al.*, 1987).

A study has also been made of the effects of chlormethiazole, dizocilpine, pentobarbitone, γ-butyrolactone (GBL) and ondansetron on the acute release of 5-HT by MDMA (as measured by the 5-HT and 5-HIAA content in hippocampus and cortex 4 h after MDMA) to determine whether any of the drugs prevented neurotoxicity by inhibiting the release of 5-HT, since it has been proposed that indolic neurotoxins might be formed as a result of the massive 5-HT release which follows administration of substituted amphetamines (Commins *et al.*, 1987a,b; Wrona & Dryhurst, 1991; Wrona *et al.*, 1992) and Azmitia *et al.* (1990) have proposed that toxicity is related to 5-HT release.

Methods

Animals and drug administration

Adult male Lister Hooded rats (Harlan Olac, Bicester) weighing 220–300 g were used. They were housed in groups, in conditions of constant temperature (21°C) and a 12 h light/dark cycle (lights on 07 h 00 min) and given free access to food and water.

The effects of MDMA (20 mg kg^{-1}, i.p.) on cerebral indoleamine content were examined either 4 h or 4 days later. The putative protective agents were always given 5 min before and 55 min after the MDMA injection. The following agents were examined: di-chlormethiazole ethanedisulphonate (50 mg kg^{-1}, i.p.); dizocilpine HCl (1 mg kg^{-1}, i.p.); pentobarbitone Na (25 mg kg^{-1}, i.p.); γ-butyrolactone (400 mg kg^{-1}, i.p.); ondansetron (0.5 and 0.1 mg kg^{-1}, i.p.).

Measurement of monoamines and their metabolites

Rats were killed by cervical dislocation and decapitation, the brains removed and cortex, hippocampus and striatum dissected out. Tissue was homogenized and 5-HT, 5-HIAA, dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) measured by high performance liquid chromatography (h.p.l.c.) with electrochemical detection by the method previously reported in detail elsewhere (Green *et al.*, 1992; Colado *et al.*, 1993).

Drugs

Drugs were obtained from the following sources (in parenthesis): *p*-Chloroamphetamine, γ-butyrolactone, 3,4-methylenedioxymethamphetamine, pentobarbitone Na (Sigma Chemical Co, Poole); dizocilpine HCl (Semat Technical (U.K.) Ltd, St Albans) di-chlormethiazole ethanedisulphonate (Astra Arcus, Södertälje, Sweden), ondansetron (gift from Glaxo Group Research, Ware). All drugs were dissolved in saline (0.9% w/v NaCl) and all doses refer to the concentration of the base.

Statistics

All biochemical data were analysed by analysis of variance (1 way), followed by *post-hoc* 2-tailed *t* tests. Because of slight variation in monamine levels from experiment to experiment, some values have been presented as a percentage change from the control group in the experiment. All statistics were performed on original data, not following percentage transformation.

Results

Effect of pentobarbitone on MDMA-induced neurotoxicity

Administration of MDMA (20 mg kg^{-1}) resulted in a substantial depletion of 5-HT and 5-HIAA in the hippocampus and cortex 4 days later (Figure 1a).

Two injections of pentobarbitone (25 mg kg^{-1}) 60 min apart did not alter cerebral 5-HT or 5-HIAA 4 days later (data not shown) but did attenuate the neurotoxic effect of MDMA on the indole concentrations (Figure 1a), other than the 5-HIAA content in cortex.

Effect of γ-butyrolactone or ondansetron on MDMA-induced neurotoxicity

Neither GBL (400 mg kg^{-1}) nor ondansetron (0.5 mg kg^{-1}) administration altered cerebral indole concentrations in hippocampus or cortex 4 days later (data not shown). However, GBL (400 mg kg^{-1}) given before and after MDMA (20 mg kg^{-1}) provided substantial protection against the neurotoxic depletion of 5-HT and 5-HIAA (Figure 1b).

In contrast, ondansetron either at a dose of 0.5 mg kg^{-1} (Figure 1c) or 0.1 mg kg^{-1} (data not shown) failed to provide any protection.

Effect of MDMA on striatal dopamine and DOPAC content 4 days later and influence of pentobarbitone, GBL and ondansetron

MDMA (20 mg kg^{-1}) injection had no significant effect on striatal dopamine content 4 days later. Pentobarbitone ondansetron and GBL did not alter dopamine content while pentobarbitone produced a modest increase (10%; $P<0.01$). There were no significant changes in animals given these compounds in combination with MDMA (data not shown).

MDMA did produce a decrease in DOPAC content (saline: 2979 ± 220 ng g^{-1} tissue ($n=6$); MDMA: $1882 \pm 99(6)$; $P<0.01$). This change was less pronounced in rats also given pentobarbitone, GBL and ondansetron (data not shown).

Acute effect of MDMA on cerebral 5-HT content

A single injection of MDMA (20 mg kg^{-1}) resulted in a greater than 80% depletion of 5-HT in the hippocampus and cortex 4 h later (Figure 2). The percentage decrease in 5-HIAA content was somewhat smaller in both regions (Figure 2).

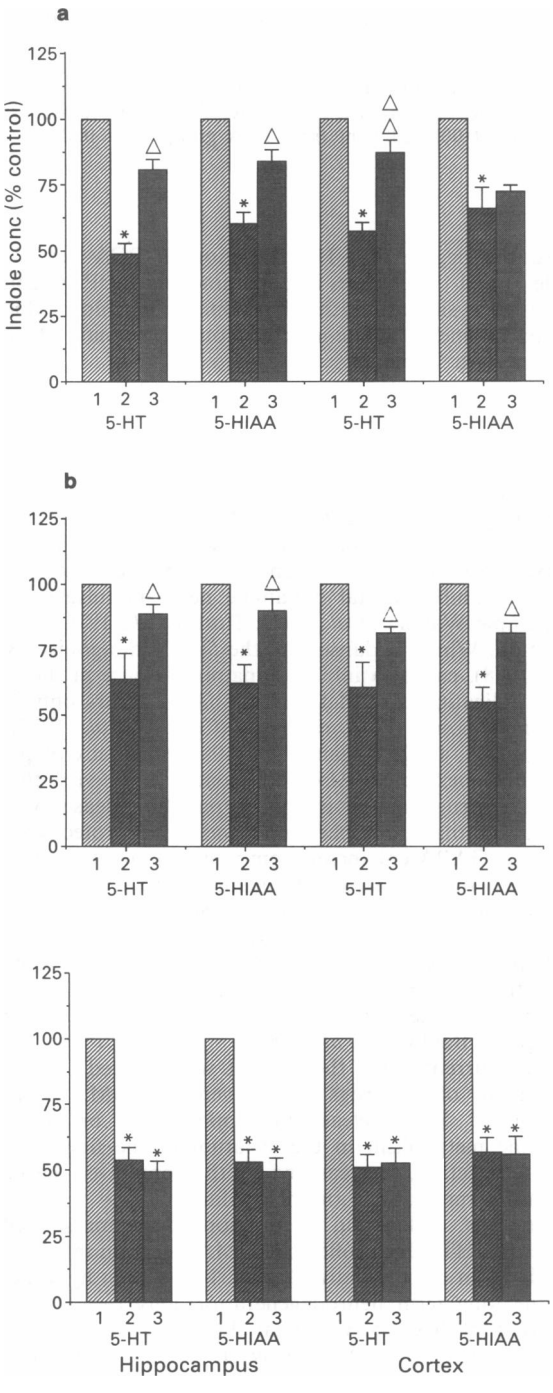

Figure 1 The effect of pentobarbitone, γ-butyrolactone and ondansetron on the 3,4-methylenediomethyoxyamphetamine (MDMA)-induced decrease in hippocampal and cortical indole concentration. Results show % change in brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentration compared to saline-injected control animals; shown as mean ± s.e.mean ($n = 5-6$). Group (1) saline injected; group (2) MDMA injected; group (3) MDMA + experimental drug injected. (a) Effect of pentobarbitone (25 mg kg^{-1}); (b) effect of γ-butyrolactone (400 mg kg^{-1}) and (c) effect of ondansetron (0.5 mg kg^{-1}) given 5 min before and 55 min after MDMA (20 mg kg^{-1}). Different from saline: *$P<0.001$; different from MDMA: $^{\Delta}P<0.05$; $^{\Delta\Delta}P<0.01$.

Effect of the chlormethiazole, dizocilpine, pentobarbitone, GBL and ondansetron on the MDMA-induced decrease of 5-HT in the brain

None of the compounds under investigation altered the 5-HT or 5-HIAA content of the hippocampus or cortex 4 h later with the exception of chlormethiazole which produced an increase in 5-HT content of hippocampus and cortex of approximately 20% (data not shown) and γ-butyrolactone which produced a similar increase in 5-HIAA content in both these regions. Furthermore none of the compounds influenced the acute decrease of 5-HT and 5-HIAA content induced by MDMA injection in either brain region (Figure 2) with the exception of GBL which produced a modest attenuation of the fall in 5-HIAA content (Figure 2).

Effect of MDMA on striatal dopamine and DOPAC concentrations and the action of chlormethiazole, dizocilpine, pentobarbitone, GBL and ondansetron

Administration of chlormethiazole, dizocilpine and GBL increased striatal dopamine concentrations 4 h later (Table 1).

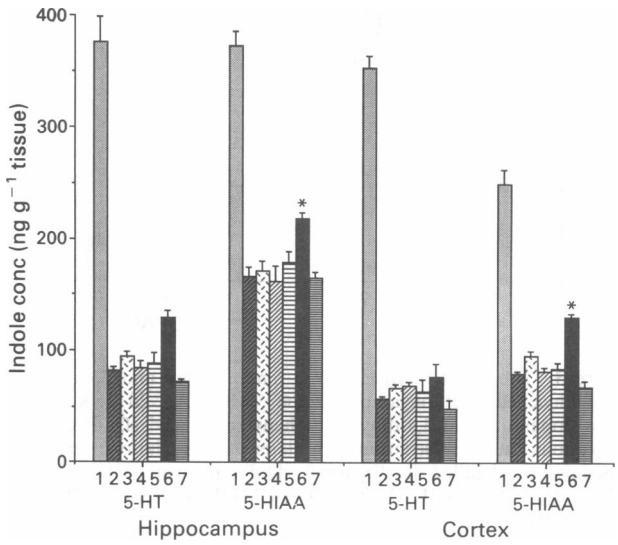

Figure 2 Effect of drugs given 5 min before and 55 min after 3,4-methylenediomethyoxyamphetamine (MDMA, 20 mg kg^{-1}) on brain indole concentration 4 h later. Column (1) saline injected; (2) MDMA; (3) MDMA + chlormethiazole (50 mg kg^{-1}); (4) MDMA + dizocilpine (1 mg kg^{-1}); (5) MDMA + pentobarbitone (25 mg kg^{-1}); (6) MDMA + γ-butyrolactone (400 mg kg^{-1}); (7) MDMA + ondansetron (0.5 mg kg^{-1}). Decrease in brain indole concentration different from control ($P<0.001$) after all treatments. *Different from MDMA alone: $P<0.05$. Results shown as mean ± s.e.mean ($n = 5-6$).

Table 1 Effect of various compounds on striatal dopamine and 3,4-dihydroxyphenylacetic acid DOPAC concentrations 4 h later

	Striatum	
	Dopamine	*DOPAC*
Saline	7161 ± 240 (5)	3212 ± 143 (5)
Chlormethiazole	9539 ± 436 (5)**	1247 ± 94 (5)**
Dizocilpine	8987 ± 80 (5)*	2100 ± 158 (5)**
Pentobarbitone	7794 ± 348 (6)	2569 ± 114 (6)*
γ-Butyrolactone	10495 ± 732 (4)*	3294 ± 391 (5)
Ondansetron	6749 ± 318 (6)	2947 ± 103 (6)

Rats were injected with the compounds under investigation 5 min before and 55 min after a saline injection and striatal dopamine and DOPAC concentration measured 4 h after the saline injection. Different from saline: *$P<0.05$; **$P<0.01$.

Table 2 Monoamine and metabolite levels in rat brain 4 h after *p*-chloroamphetamine (PCA) and effect of chlormethiazole and dizocilpine

		Hippocampus		*Cortex*		*Striatum*	
Treatment	n	*5-HT*	*5-HIAA*	*5-HT*	*5-HIAA*	*Dopamine*	*DOPAC*
Saline	4	363 ± 15	378 ± 8	369 ± 22	220 ± 9	7882 ± 330	2073 ± 43
PCA	5	100 ± 5**	172 ± 10**	63 ± 3**	82 ± 3**	8613 ± 227	2269 ± 155
PCA + chlormethiazole	5	125 ± 4**	220 ± **†	75 ± 3**	116 ± 4**†	11223 ± 403**†	1011 ± 25†
PCA + dizocilpine	5	105 ± 7**	172 ± 9**	62 ± 5**	89 ± 3**	10160 ± 285*	1920 ± 108

Rats were injected with saline or PCA (2.5 mg kg^{-1}) and monoamine levels measured 4 h later. Rats treated with chlormethiazole (50 mg kg^{-1}) or dizocilpine (1 mg kg^{-1}) were given the drug 5 min before and 55 min after the PCA. Results expressed as mean ± s.e.mean in ng g^{-1} tissue. Different from saline group: *$P<0.01$; **$P<0.001$; different from PCA group: †$P<0.05$.

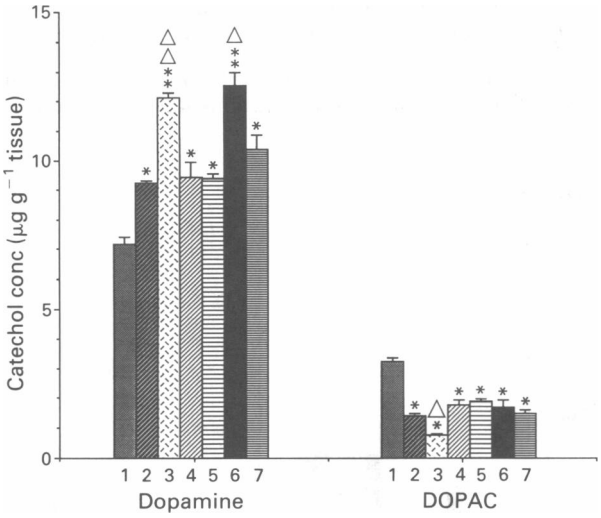

Figure 3 The effect of drugs given 5 min before and 55 min after 3,4-methylenediomethoxyamphetamine (MDMA, 20 mg kg^{-1}) on brain dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in the striatum 4 h later. Abbreviation and dose schedules as detailed in Figure 2. Different from saline-injected: *$P<0.01$; **$P<0.001$. Different from MDMA-injected, $^{\Delta}P<0.01$, $^{\Delta\Delta}P<0.001$.

Dizocilpine and pentobarbitone both decreased the DOPAC concentration, while chlormethiazole administration resulted in a substantial decrease in the concentration of that metabolite (Table 1).

The administration of MDMA (20 mg kg^{-1}) produced a rise in striatal dopamine content and a decrease in DOPAC concentration (Figure 3). In general, administration of the drugs under investigation in the current study did not significantly alter these changes. Both chlormethiazole and GBL administration to the MDMA-treated animals produced a greater increase than MDMA alone (Figure 3), while the DOPAC concentration in the MDMA plus chlormethiazole-treated rats was lowered by a greater amount than that in rats given MDMA alone (Figure 3). However these changes appeared to be additive (see Table 1 and Figure 3).

The effect of chlormethiazole and dizocilpine on the acute changes in brain monamine content following p-*chloroamphetamine administration*

Four hours after injection of PCA (2.5 mg kg^{-1}, i.p.) the 5-HT concentration in cortex and hippocampus had decreased substantially while dopamine metabolism appeared unaltered (Table 2). Neither chlormethiazole nor dizocilpine altered the degree of 5-HT depletion in PCA-treated rats (Table 2) and the changes in dopamine and DOPAC concentration were similar to those produced by chlormethiazole and dizocilpine in saline-injected control animals (Table 2, compare with Table 1).

Discussion

Consistent with the proposal that chlormethiazole and dizocilpine might protect against MDMA-induced neurotoxicity by inhibiting striatal dopamine release has been the current observation that γ-butyrolactone, a compound known for its ability to inhibit striatal dopamine nerve impulse flow (see for example Walters *et al.*, 1973; Roth *et al.*, 1976) afforded protection. While, ondansetron, the 5-HT$_3$ antagonist (Butler *et al.*, 1988), can also inhibit dopamine release in the brain, all the indications are that this inhibition occurs only in the n. accumbens not the n. caudatus (Costall *et al.*, 1987; Kilpatrick *et al.*, 1987; Hagan *et al.*, 1990) so a lack of protection is not unexpected. Also consistent with the view that the protective drugs might inhibit dopamine release was the observation that pentobarbitone was neuroprotective. This drug is a GABA-enhancing compound thought to act at a site near, but perhaps not identical to that at which chlormethiazole is active (Ogren, 1986; Cross *et al.*, 1989).

However a major problem with the proposals above is the fact that using *in vivo* microdialysis we found only a modest attenuation of dopamine release in the striatum in rats given methamphetamine and chlormethiazole and no effect at all of dizocilpine (Baldwin *et al.*, 1993).

In the current study therefore it was decided to examine whether any of the drugs that prevented MDMA neurotoxicity altered 5-HT release in hippocampus or cortex or dopamine metabolism in the striatum in the first 4 h after MDMA injection, as measured by the content of the neurotransmitter monoamine or metabolite.

There was, in fact, no attenuation of the massive (80%) release of 5-HT which follows MDMA injection in any of the animals given the protective agents. It has been hypothesized that the long term neurodegeneration may result from the formation of toxic 5-HT metabolites such as 5,6- or 5,7-dihydroxytryptamine (Wrona & Dryhurst, 1991) or 5,5-dihydroxy 4,4′ bitryptamine (Wrona *et al.*, 1992) following the substantial release of 5-HT by substituted amphetamines and Commins *et al.* (1987a,b) detected 5,6-dihydroxytryptamine in rat brain after substituted amphetamine administration. However, none of the drugs in the current study appear to be preventing neurotoxicity by blocking 5-HT release.

It does appear that some of the compounds do have acute effects on striatal dopamine metabolism. Chlormethiazole, in agreement with an earlier report (Ogren, 1986), produced both a rise in dopamine and a decrease in DOPAC, probably indicative of inhibition of dopamine release. γ-Butyrolactone

also produced a rise in striatal dopamine, and while it did not appear to decrease striatal DOPAC content, it does have very time-dependent effects, Chrapusta *et al.* (1992) reporting a decrease at 30 min and return by 4 h. Thus chlormethiazole and GBL share distinct similarities. Pentobarbitone and ondansetron however appeared to have little effect. The effect of dizocilpine was small but this is not surprising given that blockade of NMDA receptors can initially enhance dopamine tone (Yoshida *et al.*, 1991; Moore *et al.*, 1993) which would then presumably result in subsequent decrease in dopamine synthesis. The problem with ascribing importance to this change in dopamine biochemistry, at least as produced by chlormethiazole and GBL administration, is that MDMA also increased striatal dopamine content and decreased DOPAC concentration, in agreement with the findings of others (Schmidt *et al.*, 1986; Johnson *et al.*, 1991). Therefore far from opposing the neurochemical actions of MDMA in the striatum, both chlormethiazole and GBL appear to have enhanced them.

While measures of dopamine metabolites do not allow accurate interpretation in terms of dopamine release, it seems likely that all the neuroprotective compounds examined are probably inhibiting dopamine function which may go some way towards explaining the protective action of the diverse group of compounds in the current study. However, they are clearly not reversing the acute effect of MDMA on dopamine and DOPAC content as has been shown to occur following administration of other protective drugs such as 5-HT_2 antagonists (Schmidt *et al.*, 1990) and fluoxetine (Callaway *et al.*, 1991).

We previously reported that neither dizocilpine nor chlormethiazole were able to prevent PCA-mediated neurotoxicity (Colado *et al.*, 1993). The current study has further demonstrated that neither drug altered the marked release of 5-HT induced by PCA injection. Interestingly PCA did not appear to alter dopamine metabolism in the striatum. One wonders therefore if this may go some way to providing an explanation for the failure of chlormethiazole and dizocilpine to provide protection from the neurotoxicity since there may not be a perturbation to this system following PCA.

After many years of research there have been few clues as to the mechanisms by which substituted amphetamines produce their neurotoxic effects. There is however a substantial body of evidence as to what drugs afford protection. In the case of MDMA neurotoxicity the current study strengthens the view that the same group of compounds protect against both methamphetamine and MDMA neurotoxicity while PCA- and fenfluramine-induced neurotoxicity are different.

What is also clear is that in the case of MDMA-induced toxicity, the protective effect of chlormethiazole, barbiturates, GBL and dizocilpine is unlikely to have been due to an inhibition of the initial marked release of 5-HT.

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