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The Spin Trap Reagent PBN Attenuates Degeneration of 5-HT Neurones in Rat Brain Induced by *p*-Chloroamphetamine but not Fenfluramine

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Summary—Dark Agouti rats injected with either *p*-chloroamphetamine (PCA; 2.5 mg/kg i.p.) or fenfluramine (15 mg/kg i.p.) had substantial decreases (approximately 50%) in the concentration of 5-HT and 5-HIAA and binding of [^{3}H]paroxetine in the cerebral cortex 7 days later. This indicates that both compounds had produced neurodegeneration of 5-HT axon terminals. Two doses of α-phenyl-*N*-tert-butyl nitrone (PBN; 150 mg/kg i.p.) 130 min apart had no effect on cortical 5-HT content or [^{3}H]paroxetine binding. However, when PBN (150 mg/kg) was given 10 min before and 120 min after PCA (2.5 mg/kg) it attenuated the PCA-induced neurodegeneration. In contrast, PBN was without significant effect on the fenfluramine-induced damage. Changes in rectal temperature following either the neurotoxins or neurotoxins+PBN were no more than ± 1°C of saline-injected control rats. These data indicate that PCA, like MDMA, probably induces neurotoxic degeneration because of the formation of catechol or quinone metabolites and subsequent reactive free radical formation. Such a mechanism does not appear to explain fenfluramine-induced damage to 5-HT neurones. Copyright

Keywords—*p*-Chloroamphetamine, fenfluramine, [^{3}H]paroxetine binding, free radicals, α-phenyl-*N*-tert butyl nitrone (PBN), 5-hydroxytryptamine, neurodegeneration.

There are several compounds that produce long-term neurotoxic loss of 5-hydroxytryptamine (5-HT) in regions of rodent brain, even after a single dose. Such compounds include 3,4-methylenedioxymethamphetamine (MDMA), also known as ecstasy (e.g. Schmidt, 1987; Colado *et al*., 1995; and see Green *et al*., 1995 for review), *p*-chloroamphetamine (PCA) (Sanders-Bush *et al*., 1972; Fuller *et al*., 1975) and fenfluramine (Harvey and McMaster, 1975; Neckers *et al*., 1976). The mechanism involved in producing this change is not clear; however, there is substantial morphological and biochemical evidence that the 5-HT loss reflects neurodegenerative changes that occur (Molliver *et al*., 1990; Hewitt and Green, 1994).

We have shown previously that the MDMA-induced damage could be prevented by administration of the GABA-enhancing compound, chlormethiazole (Colado *et al*., 1993). The damage was also attenuated by administration of the NMDA antagonist dizocilpine (Colado *et al*., 1993). In contrast, chlormethiazole and dizocilpine were unable to prevent degeneration induced by injection of either PCA (Henderson *et al*., 1992; Colado *et al*., 1993) or fenfluramine (Sabol *et al*., 1992; Colado *et al*., 1993).

Recently we observed that administration to rats of the spin trap reagent α-phenyl-*N*-tert-butylnitrone (PBN) also prevented MDMA-induced neurodegeneration of 5-HT neurones in cortex and hippocampus (Colado and Green, 1995) and have therefore now investigated whether PBN will also protect against the neurodegenerative changes which follow injection of PCA and fenfluramine. Some of these results have been presented in preliminary form to a meeting of the British Pharmacological Society (Murray *et al*., 1996).

MATERIALS AND METHODS

Animals and compounds

Male Dark Agouti (DA) strain rats (Harlan U.K. Ltd., Bicester) weighing 160–200 g were used. They were housed in conditions of constant temperature (20 $\pm$ 1°C)

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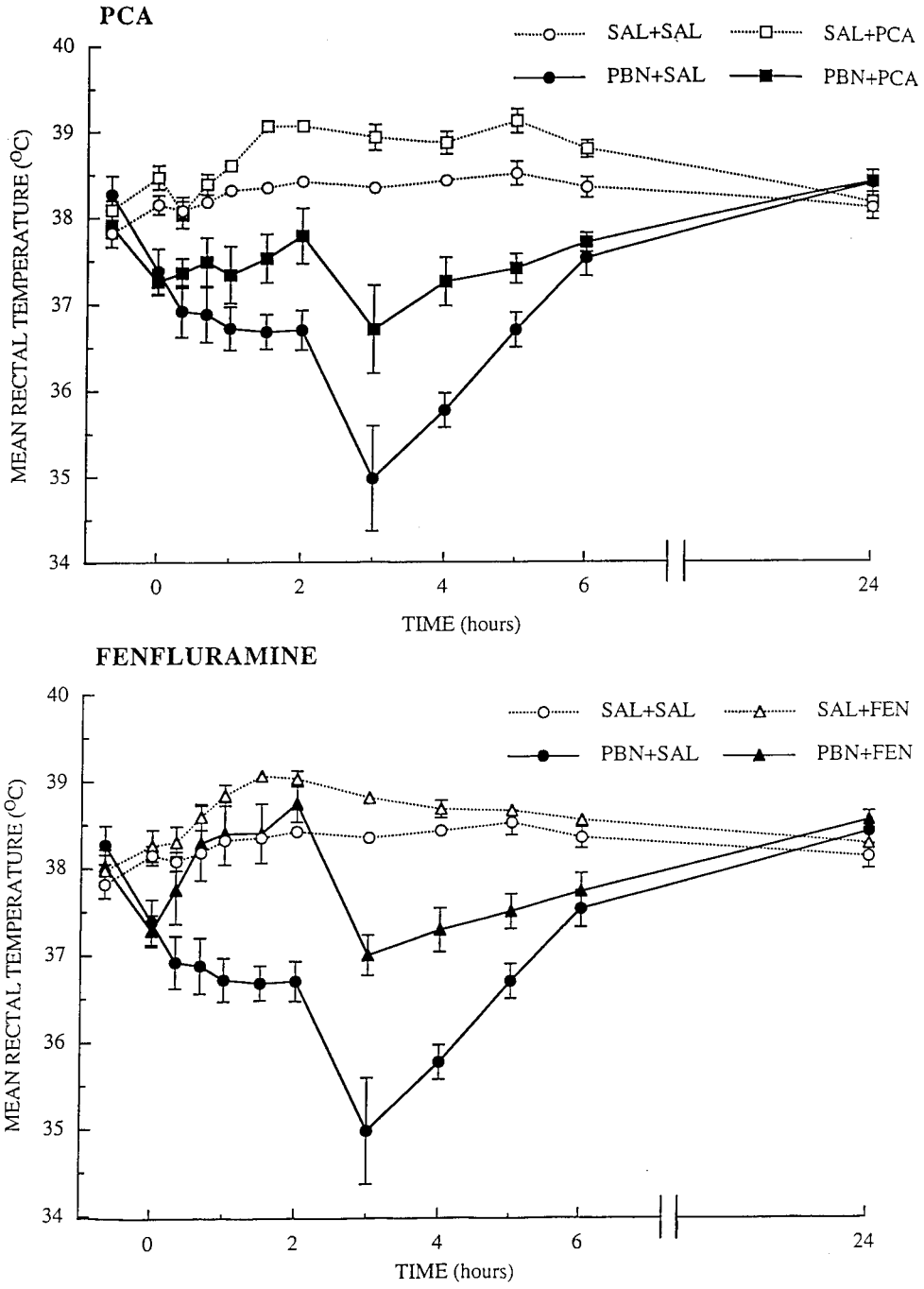

Fig. 1. The rectal temperature of rats injected with saline (○), PCA 2.5 mg/kg i.p. (□) or fenfluramine 15 mg/kg (△) at time 0. The effect of PBN (150 mg/kg i.p.) administration at −10 min and +120 min is also shown (filled symbols). Basal rectal temperature did not differ between the groups [$F(5,36) = 0.75$]. For PCA (upper graph), there was a significant effect of treatment on temperature [$F(3,22) = 10.4$, $p < 0.002$). For fenfluramine (lower graph), there was a significant effect of treatment on temperature [$F(3,24) = 10.4$, $p < 0.002$).

and lighting (lights on: 07.00–19.00 hr) and given free access to food and water. Six groups of animals were investigated. The experimental groups received either PCA (2.5 mg/kg i.p.) or fenfluramine (15 mg/kg i.p.), with saline or PBN (150 mg/kg i.p.) being given 10 min before the neurotoxin and 120 min after the neurotoxin. The control groups received saline in place of the neurotoxin, with either saline or PBN (150 mg/kg i.p.) 10 min before and 120 min afterwards, as in the experimental groups.

Rectal temperature was measured over a period of 24 hr following the neurotoxin administration. Temperature was measured by insertion of a thermocouple probe, connected to a digital readout, 2.5 cm into the rectum, the

Table 1. Effect of *p*-chloroamphetamine (PCA) and fenfluramine on content of 5-HT and 5-HIAA in rat cortex and [^{3}H]paroxetine binding and time effect of PBN treatment on these parameters

	Indole concentration (ng/g tissue)		
Injected	5-HT	5-HIAA	[^{3}H]Paroxetine (fmol/mg protein)
Saline	367 ± 11 (12)	271 ± 10 (12)	53.1 ± 4.6 (11)
PBN	365 ± 13 (11)	289 ± 8 (11)	57.4 ± 4.5 (11)
PCA	167 ± 9 (15)**	130 ± 9 (15)*	24.5 ± 1.8 (12)**
PCA/PBN	248 ± 18 (13)**,[a]	197 ± 7 (13)**,[a]	42.7 ± 3.5 (13)[a]
Fenfluramine	194 ± 14 (8)**	117 ± 7 (8)**	28.7 ± 3.6 (8)**
Fenfluramine/PBN	216 ± 9 (8) **	170 ± 16 (8)**,[b]	36.9 ± 3.4 (8)*

Results show cortical amine content and [^{3}H]paroxetine binding 7 days following injection of PBN (150 mg/kg × 2), PCA (2.5 mg/kg) and fenfluramine (15 mg/kg). For full experimental details see Methods section.

Results expressed as mean ± SEM (*n*). There was a significant effect of treatment group as follows: 5-HT: $F(5,61) = 46.4$, $p < 0.0001$; 5-HIAA: $F(5,61) = 55$, $p < 0.0001$; [^{3}H]paroxetine binding: $F(5,57) = 12.6$, $p < 0.0001$. *Post-hoc* comparisons, values different from saline-injected group; $*p < 0.05$, $**p < 0.01$. Values different from PCA-treated group; [a]$p < 0.01$ and from fenfluramine-treated group; [b]$p < 0.05$.

rat being lightly restrained by holding in the hand. A steady temperature readout was obtained within 10 sec of probe insertion; this was recorded and the probe removed. This was repeated at every time point. Basal temperature was measured before the intial injection, between 09.30 hr and 10.00 hr.

Animals were kept for 7 days following treatment at which time they were killed, the brain and cerebral cortex dissected out for analysis of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT and [^{3}H]paroxetine binding.

Fenfluramine, PCA and PBN were obtained from Sigma Chemical Co., Poole, U.K. and [^{3}H]paroxetine (20.2 Ci/mmol) from NEN, Stevenage, U.K.

Analytical methods

5-HT and 5-HIAA were measured by high performance liquid chromatography (HPLC) with electrochemical detection. Tissue was homogenised in perchloric acid (0.2 M), containing sodium metabisulphite (0.1%), cysteine (0.1%) and ethylenediamine tetraacetic acid (EDTA, 0.01%). Homogenates were centrifuged at 11 000 *g* for 10 min in an Eppendorf bench centrifuge. A 50 μl sample of the supernatant was injected into the HPLC system. The chromatographic system comprised an ACS isocratic pump and Rheodyne 7125 injector with 50 μl loop. The column was a reversed phase 150 × 4.6 mm, ODS1, 5 μm (Spherisorb) and detection was by a Coulochem electrochemical cell. The mobile phase (flow rate 1 ml/min) comprised sodium-1-octane sulphonic acid (0.75 mM), EDTA (0.1 mM), methanol (15%) and KH_2PO_4 (0.1 M), which had been adjusted to pH 3.0 with phosphoric acid, filtered and degassed. Standards contained known concentrations of 5-HT and 5-HIAA in the perchloric acid mixture.

[^{3}H]Paroxetine binding was measured by the method described by Hewitt and Green (1994). Briefly, cortical tissue was homogenised 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. Protein concentration was determined by the method of Lowry *et al*. (1951).

Statistics

Statistical analysis was performed using the statistical computer package BMDP/386 Dynamic (Statistical Solutions, Cork, Eire). Temperature data were analysed by repeated measures ANOVA (program 2V), with drug treatment as the between subjects factor and time as the repeated measure. Neurochemical data were analysed by one-way analysis of variance (program 7D), with treatment as the between subjects factor and either amine level or paroxetine binding as the within subjects factor. Where a significant effect of group occurred, *post hoc* pairwise comparisons (two-tailed *t*-tests) were carried out.

RESULTS

Effect of neurotoxins on temperature

The first injection of PBN produced a decrease of rectal temperature of approximately 0.5°C and the second dose resulted in a further decrease (Fig. 1). Administration of both PCA and fenfluramine resulted in an increase in rectal temperature that was no more than 0.5°C above saline-injected controls (Fig. 1). The temperature of the fenfluramine-injected rats given PBN was lower than that of the animals given fenfluramine alone only after the second PBN injection. Animals given PCA and PBN had rectal temperatures approximately 1.5°C lower than those given PCA alone for several hours after the PCA

injection (Fig. 1). At no time did the temperature of the neurotoxin-treated rats injected with PBN fall significantly below 37°C (Fig. 1).

Effect of PCA, fenfluramine and PBN on cortical 5-*HT content and [*^{3}*H]paroxetine binding*

Administration of either PCA or fenfluramine resulted in an approximate 50% loss of 5-HT and its metabolite 5-HIAA in cerebral cortex 7 days later (Table 1). [^{3}H]Paroxetine binding decreased by a similar amount (Table 1).

PBN alone had no effect on brain monoamine content or [^{3}H]paroxetine binding. However, it markedly attenuated the PCA-induced loss of 5-HT and 5-HIAA and produced a proportionally greater protection against loss of [^{3}H]paroxetine binding (Table 1).

In contrast, the fenfluramine-induced loss of 5-HT content was not significantly altered by PBN, nor was the loss in [^{3}H]paroxetine binding (Table 1).

DISCUSSION

In previous studies we found that DA rats were more sensitive than Lister Hooded to the neurotoxic effects of MDMA (Colado *et al*., 1995; Colado and Green, 1995). This appears to be true also for other 5-HT neurotoxins, since there was a greater 5-HT loss in the DA rats following both PCA and fenfluramine than seen in our earlier study using Lister Hooded rats, despite using lower doses of the compounds (cf. this paper with Colado *et al*., 1993 and Hewitt and Green, 1994).

Both neurotoxins produced an approximate 50% loss in [^{3}H]paroxetine binding in the cortex, indicating strongly that they had caused degeneration of 5-HT neurones. In PCA-treated rats the spin trap reagent PBN attenuated the loss of [^{3}H]paroxetine binding, the protection as measured with this marker being somewhat greater than that indicated by measures of 5-HT content. A similar situation was seen in MDMA-treated rats when neuroprotection had been produced by either chlormethiazole (Hewitt and Green, 1994) or PBN (Colado and Green, 1995). This is probably because MDMA (Schmidt and Taylor, 1987) and PCA (Sanders-Bush *et al*., 1972) produce not only neurodegeneration, but also a long-term inhibition of tryptophan hydroxylase, the rate limiting enzyme in the synthesis of 5-HT. This inhibitory action would not be expected to be affected by PBN, and thus amine levels would still be somewhat depleted even if PBN had achieved a significant neuroprotective effect.

In contrast to PCA, the neurodegeneration induced by fenfluramine was not significantly prevented by PBN administration. Of course it is difficult to claim with certainty that PBN does not protect against fenfluramine-induced damage in the absence of dose-response studies. Such studies would, however, be difficult to undertake since our earlier investigation suggested that lower doses of PBN were not protective against MDMA-induced degeneration (Colado and Green, 1995), while higher doses were toxic. Given therefore that the same dose of PBN protected against both MDMA- and PCA-induced damage, it seems reasonable to propose that PBN does not protect against the neurotoxic effects of fenfluramine. Examination of the metabolic fates of fenfluramine and PCA gives a possible explanation for this differential effect of PBN. Fenfluramine is metabolised predominantly by a pathway that leaves the stable ring unaltered with the formation of norfenfluramine and trifluoromethylbenzoic acid (Campbell, 1971; Caccia *et al*., 1982). PCA, in contrast, has been shown to be metabolised to 3-chloro-4-hydroxyamphetamine (Parli and Schmidt, 1975), and produces reactive intermediates (Miller *et al*., 1986) and can be predicted to be metabolised to catechol metabolites (Jerina and Daly, 1974) structurally similar to those produced during MDMA metabolism. Such catechol and quinone metabolites can, on further metabolism, give rise to free radicals (Chiueh *et al*., 1993; Hiramatsu *et al*., 1990). Formation of neurotoxic free radicals has been proposed as the basis of the neurodegenerative action of MDMA (Colado and Green, 1995). PBN protects against oxidative damage to the central nervous system by a free radical scavenger action (Carney and Floyd, 1991) and it seems reasonable, therefore, to propose that both PCA and MDMA are metabolised to products which generate free radicals and which in turn cause damage to 5-HT nerve terminals. Concomitant administration of L-Dopa with PCA or MDMA would be expected to increase the damage produced by the amphetamine since, by forming catecholamines in serotonergic neurones, it would further deplete the cerebral tissue mechanisms which trap free radicals. In contrast, L-Dopa administration would not be expected to exacerbate fenfluramine-induced damage. This is indeed what happens (Schmidt *et al*., 1991).

There are other data which support this proposal that the neurotoxic damage to 5-HT neurones which occurs after MDMA and PCA results from free radical formation. Firstly, administration of MDMA increases the formation of thiobarbituric acid-reactive substances in rat brain, which are indicators of lipid peroxidation (Sprague and Nichols, 1995). Elevated levels of free radicals within the 5-HT nerve terminal would result in membrane lipid peroxidation, leading to 5-HT terminal degeneration. Secondly, the reduction in cerebral 5-HT levels in rats given PCA is attenuated by pretreatment with L-cysteine, a free radical scavenger (Steranka and Rehind, 1987). Both these observations provide evidence to support our current findings implicating free radical formation to be key in PCA- and MDMA-induced neurodegeneration.

Our data argue against the proposals of others (e.g. Berger *et al*., 1992) that the degeneration of 5-HT nerve terminals induced by fenfluramine, MDMA, PCA and methamphetamine is through a common mechanism of action, since the current data indicate that fenfluramine-induced damage does not involve free radical formation.

What can probably be discounted as being fundamental to the neurodegenerative process following either fenfluramine or PCA is any effect of hyperthermia. Whilst there is evidence that significant hyperthermia enhances MDMA-induced neurodegeneration (Broening *et al*., 1995), neither PCA nor fenfluramine raised rectal temperature by more than 1°C in the current study. Furthermore, our earlier study observed neurodegeneration following a low dose of PCA which was not accompanied by any hyperthermic response (Colado *et al*., 1993). It is established that hypothermia attenuates neurodegeneration produced by either cerebral ischaemia (Busto *et al*., 1987) or administration of substituted amphetamines (e.g. Farfel and Seiden, 1995; Broening *et al*., 1995). Nevertheless, administration of PBN to the PCA-treated rats did not result in their body temperature generally decreasing by more than 1°C below control values, so we feel that hypothermia cannot be evoked to explain the protective action of PBN. We acknowledge that rectal temperature need not reflect brain temperature changes. Nevertheless, previous studies have used rectal temperature when determining the relationship between body temperature and neuroprotection (e.g. Farfel and Seiden, 1995; Broening *et al*., 1995).

In conclusion, therefore, we suggest that PCA, like methamphetamine and MDMA, induces neurodegeneration of 5-HT nerve terminals by producing metabolic products which form free radicals on further metabolism. This conclusion is supported by our most recent studies in which we examined free radical formation in the hippocampus by the use of *in vivo* microdialysis. MDMA, but not fenfluramine, increased the conversion of salicylic acid to 2,3-dihydroxybenzoic acid in the dialysate, a clear indication that free radicals were formed after injection of MDMA, but not fenfluramine (Colado *et al*., 1996). The mechanism(s) by which fenfluramine induces neurodegeneration remains unclear.

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REFERENCES

Berger U. V., Gu X. F., Vandange J. W. L. and Azmitia E. C. (1992) Evidence for a common mechanism of serotonin release induced by substituted amphetamines *in vitro*. *Ann. N.Y. Acad. Sci.* **648:** 358–360.

Broening H. W., Bowyer J. F. and Slikker W. J. (1995) 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.* **275:** 325–333.

Busto R., Dietrich W. D., Globus M. Y., Valdes I., Steinberg P. and Ginsberg W. D. (1987) Small differences in intraischemic brain temperature cortically determine the extent of ischemic brain injury. *J. Cereb. Blood Flow Metab.* **7:** 729–738.

Caccia S., Ballabio M., Guiso G., Rochetti M. and Garattini S. (1982) Species differences in the kinetics and metabolism of fenfluramine isomers. *Ann. Int. Pharmacodyn. Ther.* **258:** 15–28.

Campbell D. B. (1971) Plasma concentration of fenfluramine and its metabolite, norfenfluramine, after a single and repeated oral administration. *Br. J. Pharmacol.* **43:** 465P–466P.

Carney J. M. and Floyd R. A. (1991) Protection against oxidative damage to CNS by α-phenyl-tert-butylnitrone (PBN) and other spin trapping agents: a novel series of non-lipid free radical scavengers. *J. Molec. Neurosci.* **3:** 47–57.

Chiueh C. C., Miyake H. and Peng M. (1993) Role of dopamine antoxidation, hydroxyl radical generation and calcium overload in underlying mechanisms involved in MPTP-induced parkinsonism. *Adv. Neurol.* **60:** 251–258.

Colado M. I. and Green A. R. (1995) The spin trap reagent α-phenyl-*N*-tert-butyl nitrone prevents "ecstasy"-induced neurodegeneration of 5-hydroxytryptamine neurones. *Eur. J. Pharmacol.* **280:** 343–346.

Colado M. I., Murray T. K. and Green A. R. (1993) 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), *p*-chloroamphetamine and fenfluramine administration and effects of chloramethiazole and dizocilpine. *Br. J. Pharmacol.* **108:** 583–589.

Colado M. I., Williams J. L. and Green A. R. (1995) The hyperthermic and neurotoxic effects of "Ecstasy" (MDMA) and 3,4-methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. *Br. J. Pharmacol.* **115:** 1281–1289.

Colado M. I., O'Shea E., Granados R., Murray T. K., Williams J. L. and Green A. R. (1996) Effect of MDMA and fenfluramine on the generation of free radicals in the rat hippocampus. Abstract, *8th Biennial Meet. Int. Soc. Free Radical Research*, Barcelona.

Farfel G. M. and Seiden L. S. (1995) 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.* **272:** 868–875.

Fuller R. W., Perry K. W. and Molloy B. B. (1975) Reversible and irreversible phase of serotonin depletion by 4-chloroamphetamine. *Eur. J. Pharmacol.* **33:** 119–124.

Green A. R., Cross A. J. and Goodwin G. W. (1995) Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). *Psychopharmacology* **119:** 247–260.

Harvey J. A. and McMaster S. E. (1975) Fenfluramine: evidence for a neurotoxic action in midbrain and long term depletion of serotonin. *Pharmacol. Commun.* **1:** 217–228.

Henderson M. G., Hemrick-Luecke S. and Fuller R. W. (1992) MK801 protects against amphetamine-induced striatal dopamine depletion in iprindole-treated rats, but not against brain serotonin depletion after *p*-chloroamphetamine administration. *Ann. N.Y. Acad. Sci.* **648:** 286–288.

Hewitt K. E. and Green A. R. (1994) Chlormethiazole, dizocilpine and haloperidol prevent the degeneration of serotonergic nerve terminals induced by administration of MDMA ("ecstasy"). *Neuropharmacology* **33:** 1589–1595.

Hiramatsu M., Kumagai Y., Unger S. E. and Cho A. K. (1990) Metabolism of methylenedioxymethamphetamine: formation of dihydroxymethamphetamine and a quinone identified

as its glutathione addict. *J. Pharmacol. Exp. Ther*. **254:** 521–527.

Jerina D. M. and Daly J. W. (1974) Arene oxides: a new aspect of drug metabolism. *Science (Washington)* **185:** 573–582.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

Miller K. J., Anderholm D. C. and Ames M. M. (1986) Metabolic activation of the serotonergic neurotoxin para-chloroamphetamine to chemically reactive intermediates by hepatic and brain microsomal preparations. *Biochem. Pharmacol.* **35:** 1737–1742.

Molliver M. E., Berger U. V., Mamounas L. A., Molliver D. L., O'Hearn E. and Wilson M. A. (1990) Neurotoxicity of MDMA and related compounds: anatomic studies. *Ann. N.Y. Acad. Sci.* **600:** 640–661.

Murray T. K., Misra A., Williams J. L., Colado M. I. and Green A. R. (1996) Effects of the spin trap reagent PBN on degeneration of 5-HT neurones induced by *p*-chloroamphetamine and fenfluramine administration. *Br. J. Pharmacol.* **118:** 123P.

Neckers L. M., Bertilsson L. and Costa E. (1976) The action of fenfluramine and *p*-chloroamphetamine on serotonergic mechanisms: a comparative study in rat brain nuclei. *Neurochem. Res.* **1:** 29–35.

Parli C. J. and Schmidt B. (1975) Metabolism of 4-chloroamphetamine to 3-chloro-4-hydroxyamphetamine in rats: evidence for an *in vivo* "NIH Shift" of chlorine. *Res. Commun. Chem. Pathol. Pharmacol.* **10:** 601–610.

Sabol K. E., Richards J. B. and Seiden L. S. (1992) The NMDA receptor antagonist MK801 does not protect against serotonin depletion caused by high doses of DL-fenfluramine. *Brain Res.* **582:** 129–133.

Sanders-Bush E., Bushing J. A. and Sulser F. (1972) Long term effects of *p*-chloroamphetamine on tryptophan hydroxylase activity and on the levels of 5-hydroxytryptamine and 5-hydroxyiadole acetic acid in brain. *Eur. J. Pharmacol.* **20:** 385–388.

Schmidt C. J. (1987) Neurotoxicity of the psychedelic amphetamine methylenedioxymethamphetamine. *J. Pharmacol. Exp. Ther.* **240:** 1–7.

Schmidt C. J., Black C. K. and Taylor V. L. (1991) L-DOPA potentiation of the serotonergic deficits due to a single administration of 3,4-methylenedioxymethamphetamine, *p*-chloroamphetamine or methamphetamine to rats. *Eur. J. Pharmacol.* **203:** 41–49.

Schmidt C. J. and Taylor V. L. (1987) Depression of rat brain typtophan hydroxylase following acute administration of methylenedioxymethamphetamine. *Biochem. Pharmacol.* **36:** 4095–4102.

Sprague J. E. and Nichols D. E. (1995) The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. *J. Pharmacol. Exp. Ther.* **273:** 667–673.

Steranka L. R. and Rehind A. W. (1987) Effect of cysteine on the persistent depletion of brain monoamines by amphetamine, *p*-chloroamphetamine and MPTP. *Eur. J. Pharmacol.* **133:** 191–197.