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Short communication

# The spin trap reagent α-phenyl-*N*-tert-butyl nitrone prevents 'ecstasy'-induced neurodegeneration of 5-hydroxytryptamine neurones

Maria Isabel Colado [1], A. Richard Green *

*Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ, UK*

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**Abstract**

Administration of a single dose (10 mg/kg i.p.) of 3,4-methylenedioxy-methamphetamine (MDMA or 'ecstasy') produced a 40% loss of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cortex and hippocampus of Dark Agouti rats 7 days later. Binding of [$^3$H]paroxetine to the presynaptic 5-HT nerve terminals in cortex was decreased by approximately 30%. Injection of the spin trap reagent α-phenyl-*N*-tert-butyl nitrone (PBN; 150 mg/kg i.p.) 10 min prior and 120 min post MDMA administration totally prevented the loss in [$^3$H]paroxetine binding in the cortex and attenuated the loss of 5-HT and 5-HIAA in both brain regions. PBN alone had no effect on [$^3$H]paroxetine binding or brain 5-HT content. These data suggest that MDMA produces neurodegeneration of 5-HT neurones because of reactive free radical formation.

*Keywords:* MDMA (3,4-methylenedioxymethamphetamine); Ecstasy; Neurodegeneration; PBN (α-phenyl-*N*-tert-butyl nitrone); Neuroprotection; Free radical

## 1. Introduction

There is substantial evidence that the commonly misused recreational drug 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') produces a long-term neurotoxic loss of 5-hydroxytryptamine (5-HT) content in several regions of rat and primate brain, this loss reflecting neurodegeneration of 5-HT nerve terminals (see Green et al., 1995 for review).

The mechanisms by which MDMA produces these neurodegenerative changes are not clear. It appears that neither the parent compound (Paris and Cunningham, 1992) nor the acute 5-HT release that occurs after MDMA injection (Hekmatpanah et al., 1989; Colado and Green, 1994) is responsible. It has therefore been suggested that toxicity probably results from the effects of MDMA metabolic products. Several compounds formed from MDMA and its demethylated product methylenedioxyamphetamine (MDA) have been implicated, including catechol compounds (Hiramatsu et al., 1990). Such compounds can be oxidized to quinones, and a quinone metabolite of MDMA has been detected in rat brain (Hiramatsu et al., 1990). Autoxidation of catechols and quinones can result in the formation of free radicals, which are known to produce neurodegeneration (Chiueh et al., 1993). It seemed possible therefore that free radical formation is responsible for the neurodegeneration of 5-HT terminals in the brain.

To examine this possibility we have administered to rats the spin trap reagent α-phenyl-*N*-tert-butyl nitrone (PBN) concurrently with MDMA to determine whether it would prevent the neurodegenerative loss of 5-HT that normally occurs after injection of the amphetamine derivative. The Dark Agouti strain of rats was used because we recently found that these animals are very sensitive to the long-term neurotoxic effects of MDMA, a single dose of 10 mg/kg producing significant loss of cortical 5-HT neurones (Colado et al., 1995).

* Corresponding author. Fax 0171-837-4347.

[1] Permanent address: Departmento de Farmacologia, Facultad de Medicina, Universidad, Complutense, Madrid 28040, Spain.

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## 2. Materials and methods

### 2.1. Animals and compounds

Male Dark Agouti strain rats (Harlan Olac, Bicester, UK), weighing 160–200 g were used. They were housed in groups in conditions of constant temperature (21° C) and a 12 h light/dark cycle (lights on: 07.00 h) and given free access to food and water. Four experimental groups were investigated. Two groups were given a single dose of MDMA (10 mg/kg i.p.; Sigma Chemical Co., Poole, Dorset, UK). One group was injected with PBN (150 mg/kg i.p., Sigma Chemical Co.) 10 min prior to MDMA and 2 h after the MDMA while the other received saline in place of the PBN. Two further groups were injected with saline in place of the MDMA, again one group receiving PBN and the other saline. Rectal temperature was measured in all four groups over the 24 h following the MDMA administration. Animals were killed 7 days later for analysis of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the cortex and hippocampus and for measurement of [$^3$H]paroxetine binding in cortex.

### 2.2. Analytical methods

Rats were killed by cervical dislocation and decapitation, the brain removed and cortex and hippocampus dissected out. 5-HT and 5-HIAA were measured by high-performance liquid chromatography with electrochemical detection by the method previously reported in detail elsewhere (Colado et al., 1993). The binding of [$^3$H]paroxetine was performed as described by Hewitt and Green (1994).

### 2.3. Statistical methods

Statistical analysis was performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Software, Cork, Eire). Temperature data were analysed by repeated measures analysis of variance (ANOVA, program 2V) with treatment as the between-subject factor and time as the repeated measure. Neurochemical data were analysed by analysis of variance (one-way) followed by post-hoc 2-tailed $t$-tests.

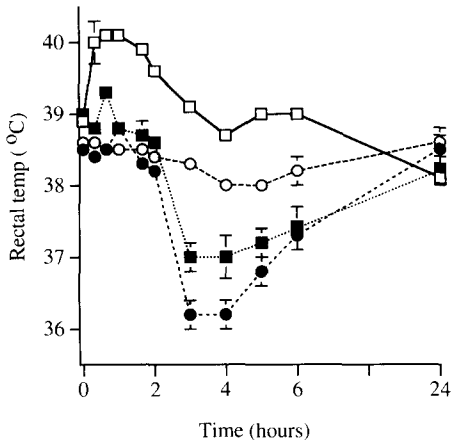


Fig. 1. The graph shows the effect on rectal temperature of rats injected at −10 min and +120 min with PBN (150 mg/kg i.p.) in animals either given saline (●) or MDMA, 10 mg/kg i.p. (■). Also shown are the rectal temperatures of animals injected with saline instead of PBN and administered either saline (○) or MDMA, 10 mg/kg i.p. (□) at time 0 h. The temperature of PBN-treated rats did not differ from saline-treated rats in the period 0–2 h, but was lower in the period 2–6 h, $F(1,10)$ 86.26, $P < 0.001$. Administration of MDMA resulted in a significant hyperthermia in the period 0–2 h, $F(1,7)$ 281.25, $P < 0.001$. Administration of PBN to MDMA-treated rats prevented the MDMA-induced hyperthermia in the period 0–2 h, $F(1,9)$ 13.2, $P < 0.01$.

## 3. Results

Administration of MDMA (10 mg/kg i.p.) resulted in significant but short-lasting hyperthermia, which was attenuated in rats given PBN (150 mg/kg i.p.) 10 min prior to the MDMA injection (Fig. 1). PBN treatment

Table 1
The effect of PBN on the neurotoxic loss of 5-HT neurochemical markers 7 days following MDMA administration

| Measure | Saline | MDMA | | MDMA/PBN | | PBN | |
|---|---|---|---|---|---|---|---|
| *Cortex* | | | | | | | |
| [$^3$H]Paroxetine | 7.44 ± 0.16 | 5.41 ± 0.34 | [73] [b] | 7.23 ± 0.22 | [97] [d] | 8.04 ± 0.30 | [108] |
| 5-HT | 377 ± 13 | 228 ± 22 | [60] [b] | 317 ± 11 | [84] [a,d] | 378 ± 20 | [100] |
| 5-HIAA | 141 ± 5 | 84 ± 4 | [60] [b] | 112 ± 4 | [80] [b,d] | 172 ± 5 | [121] [a] |
| *Hippocampus* | | | | | | | |
| 5-HT | 401 ± 23 | 237 ± 20 | [59] [b] | 351 ± 19 | [88] [d] | 358 ± 9 | [90] |
| 5-HIAA | 429 ± 14 | 299 ± 9 | [70] [b] | 366 ± 15 | [86] [b,c] | 455 ± 11 | [106] |

The table shows the values of [$^3$H]paroxetine binding (in fmol/mg tissue) and 5-HT and 5-HT content (in ng/g tissue) following saline, MDMA, MDMA + PBN and PBN alone (for experimental details, see Materials and methods) with values expressed as mean ± S.E.M., $n = 5$–10. The percent value of the measure compared to the saline-injected controls (100%) is also shown in square brackets. Results shown different from saline-treated group: [a] $P < 0.05$; [b] $P < 0.01$ and from MDMA-treated group [c] $P < 0.05$; [d] $P < 0.01$.

also resulted in the temperature of the MDMA-treated rats being lower than saline-injected controls in the period 3–6 h post-MDMA injection. However at no time did their rectal temperature fall below 37° C, in contrast to animals injected only with PBN. In that situation the second PBN injection resulted in a mean rectal temperature of 36.2° C in the succeeding 2 h (Fig. 1).

Seven days after MDMA injection there was a 40% loss of both 5-HT and 5-HIAA content in the cortex and hippocampus (Table 1). A loss in [$^3$H]paroxetine binding of about 30% was observed in the same cortical tissue (Table 1). Administration of PBN (150 mg/kg) 10 min prior and 120 min post-MDMA injection totally prevented the loss of [$^3$H]paroxetine binding in the cortex and attenuated the 5-HT and 5-HIAA decrease. The 5-HT and 5-HIAA loss in the hippocampus which followed MDMA injection was also almost completely prevented by PBN administration (Table 1). Administration of PBN alone was without effect on either [$^3$H]paroxetine binding or brain amine concentration (Table 1).

## 4. Discussion

[$^3$H]Paroxetine binds to the presynaptic 5-HT nerve terminal and binding is decreased following administration of compounds shown histologically to produce damage, including, for example, selective 5-HT neurotoxins such as 5,7-dihydroxytryptamine (Habert et al., 1985) and MDMA (Hewitt and Green, 1994). The fact that PBN administration prevented the MDMA-induced decrease in [$^3$H]paroxetine binding means therefore indicates that this compound has almost certainly protected the brain from neurodegeneration caused by MDMA. Since the spin trap reagent PBN has previously been shown to protect against oxidative damage to the central nervous system by a free radical scavenger action (Carney and Floyd, 1991) it is reasonable to propose that this compound prevents neurodegeneration following MDMA by the same action. This view is reinforced by the evidence that metabolic products of MDMA and MDA include catechols and quinones which can be oxidized to products giving rise to free radicals (Chiueh et al., 1993; Hiramatsu et al., 1990). Damage would presumably occur when the ability of cerebral tissue mechanisms to trap the free radicals being formed was exhausted. This might explain why L-dopa potentiates the toxic action of MDMA (Schmidt et al., 1991) since administration of this amino acid would increase dopamine formation non-selectively in 5-HT nerve terminals and dopamine is also capable of being metabolised by mechanisms which result in free radical formation (Chiueh et al., 1993).

It might be argued that the dose of PBN employed in this study is high. However, earlier studies on the neuroprotective effect of PBN against ischaemia-induced damage utilised multiple doses of 100 mg/kg (Cao and Phillis, 1994; Clough-Helfman and Phillis, 1991). Two doses of 150 mg/kg can therefore be considered reasonable.

It should be noted that 5-HT and 5-HIAA concentrations were still somewhat diminished in the PBN/MDMA-treated rats. MDMA produces a long-term inhibition of tryptophan hydroxylase and may do so because the enzyme forms a sulphydryl complex with MDMA metabolites (Stone et al., 1989). Such a mechanism might not be altered by PBN administration and 5-HT synthesis would therefore continue to be impaired. Why PBN administration alone should have resulted in a small increase in 5-HIAA concentration is unclear.

The second injection of PBN did induce a significant lowering of body temperature in the control animals. This compound also attenuated the hyperthermia which follows MDMA injection. It did not, however, produce unequivocal hypothermia since the temperature of the rats never dropped below 37° C. We do not believe that either the prevention of the MDMA-induced hyperthermia or the subsequent modest lowering of body temperature can explain the mechanism by which PBN was neuroprotective. In the first place our recent study on the effects of MDMA in male and female Dark Agouti rats demonstrated that the degree of degeneration of 5-HT neurones was dependent on the dose of MDMA, not the severity of the hyperthermic response (Colado et al., 1995). Secondly, preventing hyperthermia did not prevent the neurodegeneration that occurred after administration of some other substituted amphetamine derivatives (Colado et al., 1993). Finally, whilst there is some evidence to support the notion that producing frank hypothermia can result in neuroprotection, body temperature did not fall below 37° C in the MDMA-treated rats given PBN.

In conclusion, we propose that the most plausible explanation for the results obtained is that MDMA produces neurodegeneration because of formation of free radicals in 5-HT nerve terminals and that PBN protects neurones from damage by a scavenger action.

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