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A study of the neurotoxic effect of MDMA ('ecstasy') on 5-HT neurones in the brains of mothers and neonates following administration of the drug during pregnancy

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1 It is well established that 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') is neurotoxic and produces long term degeneration of cerebral 5-hydroxytryptamine (5-HT) nerve terminals in many species. Since MDMA is used extensively as a recreational drug by young people, it is being ingested by many women of child bearing age. We have therefore examined the effect of administering high doses of MDMA to rats during pregnancy on the cerebral content of both the dams and the neonates.

2 MDMA (20 mg kg^{-1}, s.c.) was injected twice daily on days 14–17 of the gestation period. The initial dose produced a marked hyperthermic response in the dam which was progressively attenuated in both peak height and area under the curve following further doses of the drug. The body weight of the dams decreased during the period of treatment.

3 There was a modest decrease in litter size (−20%) of the MDMA-treated dams.

4 The concentration of 5-HT and its metabolite 5-HIAA was decreased by over 65% in the hippocampus and striatum and 40% in the cortex of the dams 1 week after parturition. In contrast, the content of 5-HT and 5-HIAA in the dorsal telencephalon of the pups of the MDMA-treated dams was the same as that seen in tissue from pups born to control animals.

5 Administration of MDMA (40 mg kg^{-1}, s.c.) to adult rats increased thiobarbituric acid reacting substances (TBARS) in cortical tissue 3 h and 6 h later, indicating increased lipid peroxidation. No increase in TBARS was seen in the cortical tissue of 7–10 day neonates injected with this dose of MDMA 3 h or 6 h earlier.

6 The data suggest that exposure to MDMA *in utero* during the maturation phase does not produce damage to 5-HT nerve terminals in the foetal rat brain, in contrast to the damage seen in the brains of the mothers. This may be due to MDMA being metabolized to free radical producing entities in the adult brain but not in the immature brain or, alternatively, to more effective or more active free radical scavenging mechanisms being present in the immature brain.

Keywords: Ecstasy; MDMA (3,4-methylenedioxymethamphetamine); 5-hydroxytryptamine; free radicals; lipid peroxidation; neurotoxicity; neonates; neurodegeneration

Introduction

The extensive recreational use of the drug 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') continues to give rise to concern because of the occasional cases of acute toxicity in young persons, some of which are fatal (see review of Green *et al*., 1995). Recently, the possibility of long term neurodegenerative changes in the brain of regular users has also been emphasized (Green & Goodwin, 1996), a possibility that has often been ignored, even though it has been known for several years that MDMA administration produces degeneration of 5-hydroxytryptamine (5-HT) neurones in the brains of rodents and primates (see Green *et al*., 1995).

We have recently shown that in the DA strain of rats, long term damage to 5-HT neurones occurs after a single low dose of MDMA (10 mg kg^{-1}, i.p.) and data indicated that the damage probably occurs because of neurotoxic metabolites of MDMA, which form free radicals on further metabolism (Colado *et al*., 1995; 1996; 1997b). The idea that free radicals are involved in the production of the damage is supported by the observation that the free radical scavenging agent α-phenyl-N-tert-butyl nitrone (PBN), when given to MDMA-treated rats, prevented the neurodegenerative loss of 5-HT (Colado & Green, 1995). The finding of increased lipid peroxidation in rat brain following MDMA also suggests a role for free radicals in MDMA-induced damage (Sprague & Nichols, 1995).

The fact that MDMA is primarily a recreational drug used by young people means that it is being ingested by many women of child bearing age. While there have apparently been no studies on the transfer of MDMA across the placenta, the fact that it is of low molecular weight and highly liposoluble means that it will readily cross the placenta (Reynolds, 1979; Sonaware & Jaffe, 1986). Furthermore, it has been demonstrated that the structurally related compounds methamphetamine (Nakamura *et al*., 1992; Stek *et al*., 1995) and fenfluramine (Gilbert *et al*., 1971) appear in both foetal tissue and amniotic fluid following their administration to the mother. There is every reason therefore to believe that ingestion of MDMA by the mother will result in MDMA being present in foetal tissues including the brain. This in turn gives rise to concern that the foetus may be at risk when carried by an MDMA user unaware of being pregnant.

There only appears to have been one study on the behavioural and neurochemical effects of prenatal exposure to MDMA (St. Omer *et al*., 1991). This showed that when the drug was administered to the mother on alternative days from day 6–18 of the pregnancy, it had no significant effect on the

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cerebral 5-HT content of the pups when measured 27 days after parturition. However the doses of MDMA used were relatively low (5 and 10 mg kg^{-1}), only the higher dose produced a loss in 5-HT content in the brain of the mothers and even that loss was modest. The authors admitted therefore that the concentration of MDMA or its metabolites might not have attained toxic concentrations in the foetal brain. We have therefore now administered high doses of MDMA repeatedly to pregnant rats, over a 4-day period during the maturation phase of the foetus, and subsequently examined cerebral monoamine content of both the dams and pups.

Since some of our previous studies (Colado & Green, 1995; Colado *et al*., 1996; 1997b; Murray *et al*., 1996) have suggested that a critical factor in the MDMA-induced neurodegenerative process is the production of free radicals, possibly formed from the degradation of MDMA metabolites (Hiramatsu *et al*., 1990), we also undertook a brief study on the effect of MDMA administration on lipid peroxidation in cerebral tissue taken from adult and neonate rats.

Some of this work has been presented to a meeting of the British Pharmacological Society (Colado *et al*., 1997a).

Methods

Animals, drug administration and experimental protocol

Ten pregnant female Wistar rats (Interfauna, Barcelona) weighing approximately 250 g were obtained at day 1 of the gestation period. They were housed individually in conditions of constant temperature (21° ± 2°C) and a 12 h light-dark cycle (lights on: 07h 00min) and given free access to food and water. The animals were monitored daily until delivery to determine weight gain.

At day 14 of the gestation period the animals were randomly assigned to 2 groups. Group 1 (control) were injected with saline (0.9% w/v NaCl) and group 2 (treatment group) were injected with MDMA (20 mg kg^{-1}, s.c.). Injections were given at 09h 00min and 17h 00min on days 14–17 of the gestational period. Rectal temperature was measured for 6 h following the first of each daily injection. One female in the experimental group died on day 15. The pups were sexed and counted within 24 h of the birth (day 1) and each litter then randomly reduced to 10 pups. Pup weights were recorded 7 days post-partum.

(±)-3,4-methylenedioxymethamphetamine HCl was obtained from the Ministry of Health (Spain). It was dissolved in saline and injected s.c. (1 mg kg^{-1}) with doses being quoted in terms of the base.

Preparation of cerebral tissue for monoamine analysis

On day 7 post-partum (day 11 after cessation of MDMA treatment) both dams and pups were killed. Brains of the mothers were rapidly removed and dissected on ice into cortex, hippocampus and striatum for subsequent monoamine analysis. The pup brain was also placed on ice and hindbrain (consisting of cerebellum and spinal cord) was removed. The remaining brain was turned over (resting on cortex) and pituitary and white matter removed with fine forceps. The

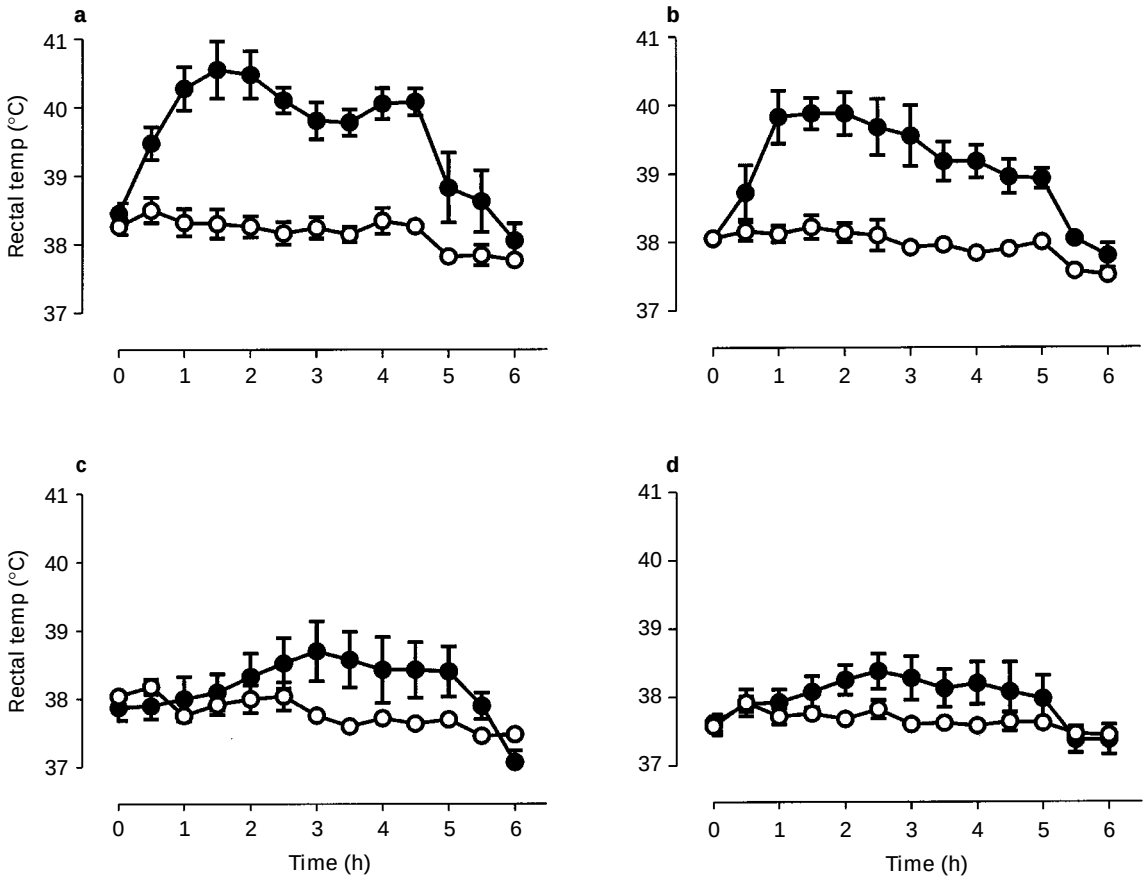

Figure 1 Rectal temperature of rats injected with (○) saline ($n=5$) or (●) MDMA (20 mg kg^{-1}, s.c., $n=4$) at 09h 00 min and 17h 00min during days 14–17 (a, b, c and d, respectively) of gestation. Results show changes in temperature in the 6 h following the first injection each day. Symbols show mean and vertical lines indicate s.e.mean. There was an overall effect of treatment ($F(1,7)=17.84$, $P<0.004$), MDMA producing a significant increase in temperature. There was also a significant interaction of day × group ($F(2,13)=17.82$, $P<0.001$), indicating that the effect of MDMA differed for the 2 groups across days, and a day × time interaction ($F(4,26)=4.64$, $P<0.01$), indicating that the temperature response differed across days. Finally there was a significant interaction of day × time × group ($F(4,26)=3.01$, $P<0.05$) indicating that MDMA produced an effect in temperature which had a different response on each day of treatment.

remaining tissue consisting mainly of hippocampus and cortex was designated as dorsal telencephalon.

Measurement of monoamines and their metabolites

Tissue obtained from the dams and neonates was homogenized and 5-HT, 5-hydroxyindole acetic acid (5-HIAA) and dopamine measured by high performance liquid chromatography (h.p.l.c.) with electrochemical detection. Briefly, the mobile phase, consisting of KH_2PO_4 (0.05 M), octanesulphonic acid (0.8 mM), EDTA (0.1 mM) and methanol (16%), was adjusted to pH 3 with phosphoric acid, filtered and degassed. The flow rate was 1 ml min^{-1} and the working electrode potential was set at 0.8 V.

The h.p.l.c. system consisted of a pump (Waters 510) linked to an automatic sample injector (Loop 200 μl, Waters 712 WISP), a stainless steel reversed phase column (Spherisorb ODS 2, 5 μm, 150 × 3.9 mm) with a precolumn and amperometric detector (Waters M460). The current was monitored by using an integrator (Waters M745).

Measurement of tissue lipid peroxidation

Lipid peroxidation was measured by a modification of the method of Das & Ratty (1987), whereby the thiobarbituric acid reacting substances (TBARS), predominantly malondialdehyde, produced as a secondary product were quantified by use of the 2-thiobarbituric acid colour reaction.

Adult (160–180 g) and neonate (7–10 days, 15–20 g) rats were injected with MDMA (40 mg kg^{-1}, i.p.) and killed by cervical dislocation and decapitation 3 h or 6 h later. The cortex and hippocampus of adult rats and forebrain of neonate rats was dissected out as described earlier, except that hippocampal tissue was removed from the neonate brain together with the pituitary and white matter.

The tissue was homogenized with an Ultra Turrax in 10 volumes (w/v) of sodium phosphate buffer (pH 7.4). Assays contained tissue homogenate (500 μl), trichloroacetic acid (30% w/v, 300 μl), HCl (5 M, 150 μl) and 2-thiobarbituric acid (2% w/v, 300 μl). Samples were heated for 15 min at 90°C and centrifuged at 12,000 *g* for 10 min. The pink colour of the supernatant was measured spectrophotometrically (532 nm) and the malondialdehyde concentration calculated by use of a standard curve prepared with malondialdehyde tetrabutylammonium salt.

Measurement of rectal temperature

Temperature was measured by insertion of a thermocouple probe (connected to a digital readout) inserted 2.5 cm into the rectum, the rat being lightly restrained by being held in the hand. A steady readout was obtained within 10 s.

Statistical analysis

The monoamine concentrations were measured in the individual male and female pups from each litter. A mean value of these values was then obtained to give a single datum for each litter. Results are therefore expressed as mean ± s.e.mean of the results of all control or experimental litters.

Statistical analysis of the temperature changes was performed by use of the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Cork, Eire). The data were analysed by analysis of variance (ANOVA) with repeated measures with treatment as the between subjects factor and both day and time as the repeated measures. Symmetry of the orthogonal polynomials was tested. Where there was a failure to meet the symmetry assumption the Greenhouse-Geisser adjusted *P*-values provided conservative tests of the repeated measures factor. Direct comparison data were analysed by use of Student's *t* test (unpaired) and Newman-Keuls test where shown.

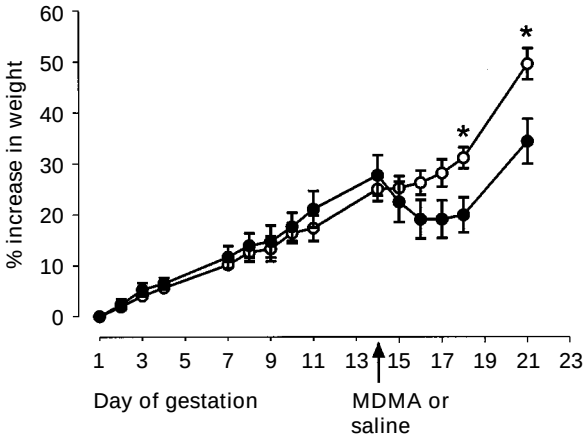

Figure 2 Effect of MDMA during pregnancy on weight gain of mother rats administered (●) MDMA, 20 mg kg^{-1}, s.c. ($n = 4$) or (○) saline ($n = 5$) twice daily during 4 consecutive days (14–17 of gestation). Results show percentage increase in weight per day during gestational period with the time of treatment marked by the arrow. Symbols show mean and vertical lines indicate s.e.mean. $^*P < 0.05$ versus the corresponding point of saline group (Student's unpaired *t* test).

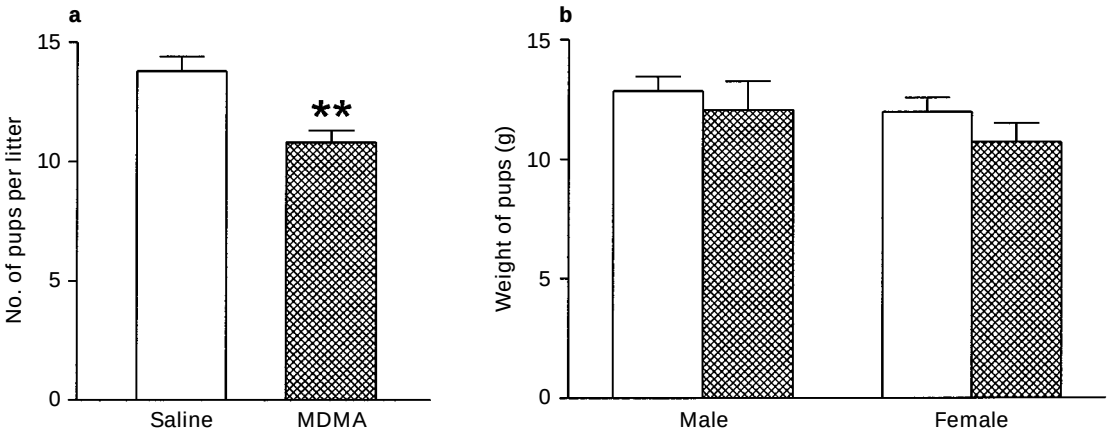

Figure 3 Effect of MDMA during pregnancy on (a) litter size and (b) weight of pups on postnatal day 7. Hatched columns: MDMA (20 mg kg^{-1}, s.c.) was administered twice daily during days 14–17 of gestation. Open columns: saline treated (male, $n = 21$; female, $n = 29$). MDMA treated (male, $n = 13$; female, $n = 17$). Each column shows the mean ± s.e.mean. Significantly different from saline injected control group, $^{**}P < 0.01$ (Student's unpaired *t* test).

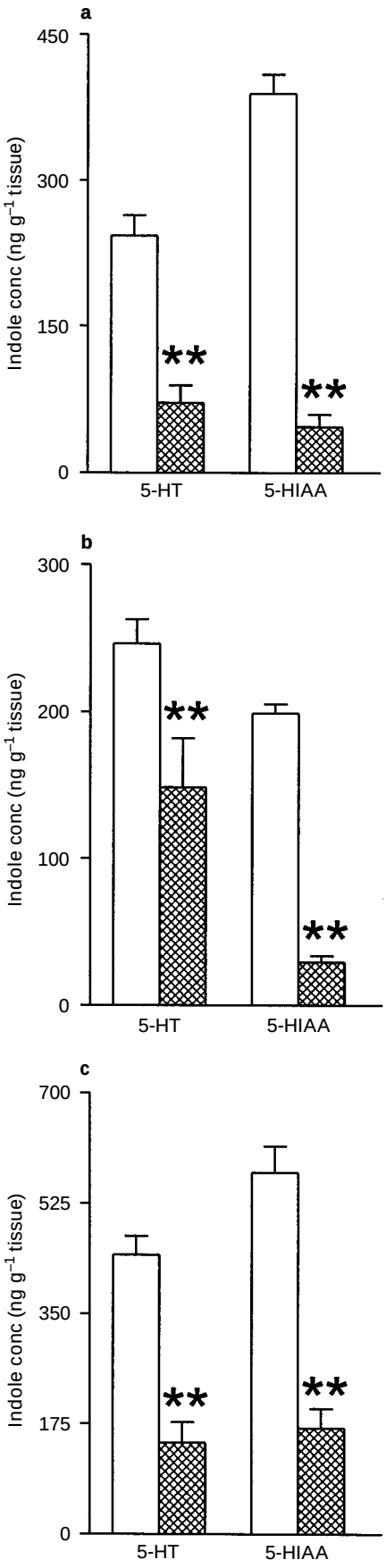

Figure 4 Effect of MDMA (20 mg kg^{-1}, s.c., hatched columns) given to pregnant rats twice daily during days 14–17 of gestation on the indole content in hippocampus (a), cortex (b) and striatum (c) 7 days after parturition. Saline (open columns, $n=5$), MDMA ($n=4$). Each column is the mean$\pm$s.e.mean. **Significantly different from saline injected control group, $P<0.01$ (Newman-Keuls test).

Results

The effect of repeated MDMA administration on the rectal temperature of the pregnant rats

The first injection of MDMA (20 mg kg^{-1}) to the pregnant dams produced a marked and long lasting hyperthermia (Figure 1). The subsequent injections on days 2–4 of the treatment period produced progressively attenuated responses in terms of both peak height and the area under the response/time curve, so that the seventh administration produced almost no hyperthermia (Figure 1). The decrease in the size of the hyperthermic response, both peak height and area, appeared linearly related to the number of doses of the drug given (data not shown).

The effect of repeated MDMA administration on the body weight of the dam

The dams injected with MDMA showed a decrease in body weight during the period of treatment, in contrast to the saline injected controls which continued to gain weight (Figure 2). Following cessation of treatment the rats started to regain weight, but on Day 21, just before parturition, the drug treated group still had a decreased body weight in comparison with saline treated control animals (Figure 2).

The effect of repeated MDMA administration to the dams on litter size and body weight of pups

There was a modest, but statistically significant, decrease in litter size from the MDMA treated dams (Figure 3a). There was no difference in the body weight of the male or female pups delivered by MDMA treated mothers (Figure 3b).

The effect of MDMA on cerebral 5-HT, 5-HIAA and dopamine content of the dams and pups

Eleven days after cessation of MDMA treatment, that is on Day 28, one week after parturition, the concentration of 5-HT decreased by over 65% in the hippocampus and striatum of the dams, while cortical 5-HT content was decreased by 45% (Figure 4). The concentration of the 5-HT metabolite 5-HIAA decreased in all three regions by over 65% (Figure 4). No change in striatal dopamine content was observed following repeated administration of MDMA (control: 4.32 ± 0.26 ($n=5$); MDMA treated: 4.04 ± 0.58 ($n=4$), results expressed in μg g^{-1} brain wet weight).

In contrast, neither male nor female neonates showed any loss in 5-HT, 5-HIAA or dopamine in the dorsal telencephalon 7 days after parturition or 11 days after cessation of treatment (Figure 5).

The effect of MDMA on lipid peroxidation in the cerebral tissue of adult and neonate rats

Three hours after a single injection of MDMA (40 mg kg^{-1}, i.p.) there was a 94% increase in TBARS in the homogenate prepared from the cortex of adult rats. At 6 h the increase was 41% (Figure 6). In tissue prepared from the hippocampus there was a 16% increase in the mean value 3 h after treatment albeit this increase failed to reach statistical significance (Figure 6). No change in the mean value was seen in this tissue 6 h later.

Because little change in lipid peroxidation was seen in the adult hippocampus the neonate brain was dissected to try and obtain mainly cortical tissue (see Methods). In neonate cortex no change in TBARS formation was seen at either 3 h or 6 h, the change at 3 h being less than 5% (Figure 6).

Discussion

Large doses of MDMA were given to the pregnant rat dams in order to examine whether offspring would be at risk from this

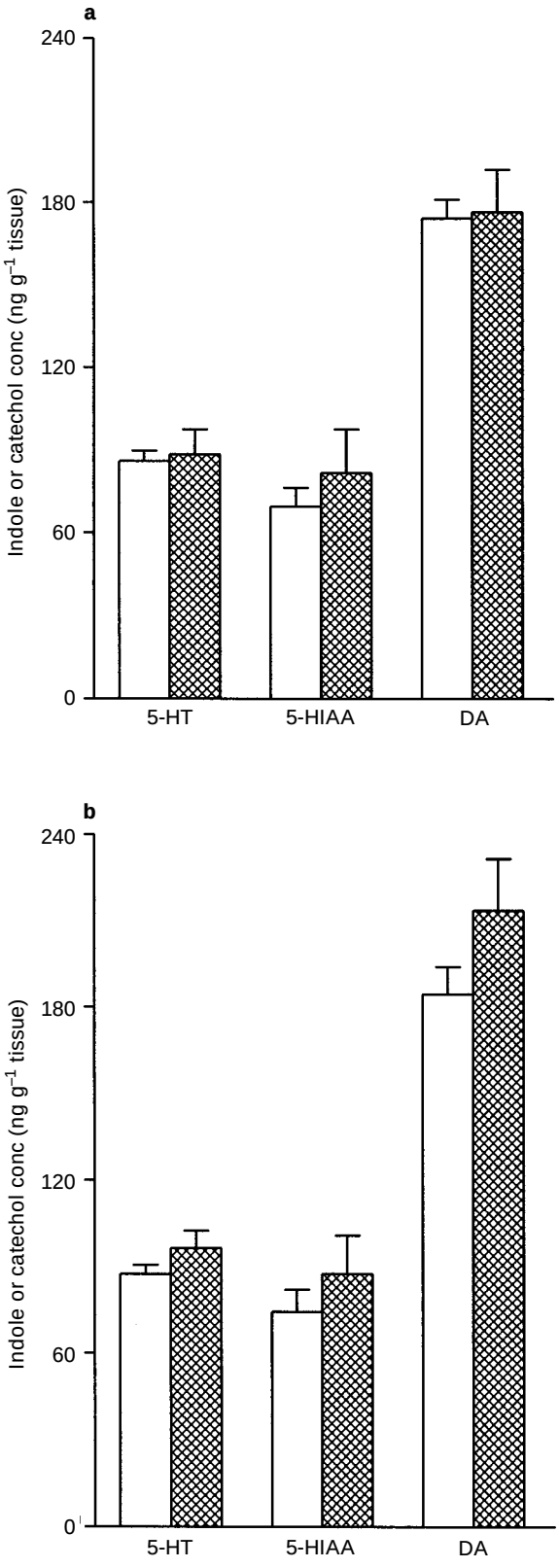

Figure 5 Effect of prenatal exposure to MDMA on the indole and catechol (DA, dopamine) content in dorsal telencephalon of male (a) and female (b) pups on postnatal day 7. MDMA (20 mg kg^{-1}, s.c., hatched columns) was administered twice daily during days 14–17 of gestation. Saline-treated dam litter group (open columns), $n=5$; MDMA-treated dam litter group, $n=4$. All male and female pups in litter were analysed separately and mean value shown calculated by use of pooled value for each litter (see statistical analysis in Methods for details). Each column is the mean ± s.e.mean.

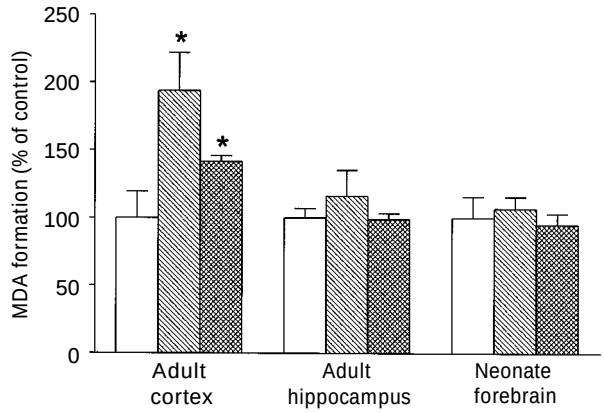

Figure 6 Formation of thiobarbituric acid reacting substances (TBARS) in the hippocampus and cortex of adult rats and cortex of neonate rats 3 h (hatched columns) and 6 h (cross-hatched columns) after MDMA (40 mg kg^{-1}, i.p.) administration. Results show % change in brain malondialdehyde (MDA) formation compared to saline-injected control animals (open columns), as mean ± s.e.mean, $n=8-10$. *Significantly different from saline; $P<0.05$ (Student's unpaired t test).

recreationally used drug. The first dose produced a substantial hyperthermic response, a well established effect of MDMA (Nash *et al*., 1988; Colado *et al*., 1993; Dafters, 1994) and one which is generally acknowledged to be due to the substantial release of 5-HT from nerve endings induced by the drug (Schmidt *et al*., 1986; Stone *et al*., 1986; 1987; Colado & Green, 1994; Green *et al*., 1995). Subsequent doses of MDMA produced an increasingly attenuated hyperthermic response, presumably because progressively smaller amounts of 5-HT were available for release. The initial dose of MDMA probably induced release of almost 80% of the 5-HT present (Colado & Green, 1994). This effect, together with both the subsequent inhibition by MDMA of the synthetic enzyme tryptophan hydroxylase (Schmidt & Taylor, 1987) and the neurodegeneration of 5-HT neurones that also occurs (see review by Green *et al*., 1995), must result in almost no 5-HT being available for release by the fourth day of drug administration. This would result in MDMA producing little temperature change.

In the study by St. Omer *et al*. (1991), the pregnant dams given MDMA (10 mg kg^{-1} × 7 over 12 days) gained 6% less weight over the gestation period than during the control period. In our study the dams given MDMA (20 mg kg^{-1} × 8 over 4 days) gained 15% less weight than controls, the larger effect presumably reflecting the higher dose of the drug given since an analysis of total dose versus reduction in weight gain by use of the data of St. Omer *et al*. (1991) and the current study suggested a linear relationship ($r^2=0.98$).

A reduced litter size in the MDMA-treated dams was also observed. This could reflect *in utero* deaths resulting in adsorbtion or, given the greater than average litter size of the saline-treated rats which was observed in the current study, it could be a chance finding. A study with substantial population sizes would be necessary before it could be stated with any authority that MDMA-administration to rats increased *in utero* deaths.

The neurodegeneration of 5-HT nerve terminals that follows MDMA injection has been demonstrated both histologically (O'Hearn *et al*., 1988; Molliver *et al*., 1990) and biochemically (Battaglia *et al*., 1987; Sharkey *et al*., 1991; Nash *et al*., 1991; Hewitt & Green, 1994; Colado *et al*., 1995) and is reflected in the long term loss in 5-HT content. This loss was observed 11 days after the last dose of MDMA in the hippocampus, cortex and striatum of the dams. The relative selectivity of this damage is suggested by the fact that striatal dopamine content was essentially unchanged, in agreement with observations of others (Schmidt & Kehne, 1990).

Despite this evidence of a substantial loss of 5-HT nerve terminals occurring in the brains of dams, the cerebral 5-HT content of their neonate pups was normal. This failure of MDMA to affect the cerebral 5-HT content of the offspring

has been shown by St. Omer *et al*. (1991), although lower doses of MDMA were used in that study and 5-HT concentrations were measured in much older pups. The present data therefore emphasize the apparent lack of vulnerability of the foetal rat brain to damage by MDMA.

The 5-hydroxytryptaminergic innervation of the rat brain continues to develop rapidly immediately before birth and in the first few weeks thereafter; brain 5-HT content, for example, can be calculated to increase around 300% in the first 70 days post partum (Broening *et al*., 1994). It might therefore be argued that the apparent lack of damage in neonate brain following MDMA is due to the fact that re-growth of 5-HT nerve projections has occurred in the 11 days between the last day of treatment and 5-HT measurement. However, it seems extremely unlikely that this would explain the present results, since one would have to propose that the brains of the MDMA-treated pups were able not only to 'make up' a possible 50% drug induced loss, but additionally produce the expected normal increase in 5-HT content due to development. Furthermore, data from Broening *et al*. (1994) strongly support our contention that MDMA does not induce neurodegenerative damage in the brain of foetal or neonate rats, since they observed that when MDMA was given to neonates at postnatal day 10, the drug caused an acute release of 5-HT, but not the longer term neurodegeneration. The fact fact that the acute release of 5-HT occurred indicates that MDMA is being taken up by the 5-HT nerve endings. The failure to produce neurotoxicity cannot therefore be due to the drug not being transported into the nerve terminals.

Broening *et al*. (1994) further showed that even 40 days post-partum, MDMA administration only produced a modest (approximate 25%) long term neurotoxic loss of 5-HT and that it was only at 70 days post-partum that the normal 50–60% loss in 5-HT content was observed. These data together with those of St. Omer *et al*. (1991) and our present observations suggest that MDMA is unable, for some reason, to produce the damage in the foetal and neonate rat brain that it produces when given to adult rats. We therefore investigated one possible mechanism for this finding, namely that MDMA administration does not result in increased free radical formation in the neonate brain.

There is now a reasonable body of evidence suggesting that increased free radical production may be the mechanism underlying the damage induced by MDMA. For example, administration of the free radical scavenging spin trap compound α-phenyl-N-tert-butyl nitrone (PBN) prevents MDMA-induced damage (Colado & Green, 1995; Yeh, 1996). Furthermore, following MDMA, increased free radical production has been detected *in vivo* by use of microdialysis probes (Colado *et al*., 1996; 1997b), while transgenic mice over expressing CuZn-superoxide dismutase have been shown to be resistant to the lethal effects of MDMA (Cadet *et al*, 1994).

Recently, Sprague and Nichols demonstrated that following MDMA administration to rats cerebral tissue showed increased lipid peroxidation, a change that reflects increased free radical production. We therefore attempted to confirm this finding and also to see whether such a change occurred in neonate tissue after MDMA administration.

Sprague and Nichols (1994) obtained increased lipid peroxidation, as measured by an increase in TBARS, 12 h after MDMA (but not 8 h or 16 h later). We were unable to confirm an increase at any of these times, but did find a marked increase in TBARS formation in cortical tissue 3 h after injection, with a smaller change at 6 h – times at which we have shown increased free radical formation *in vivo* (Colado *et al*., 1997b). A small but statistically non-significant increase was seen in the hippocampus at 3 h. This lack of change in the hippocampus was surprising given the fact that this region is vulnerable to MDMA-induced damage (e.g. Colado *et al*., 1995). However, it is noteworthy that Sprague and Nichols (1994) found increased TBARS formation in hippocampal tissue following L-deprenyl administration, even though this compound protected against MDMA-induced injury and blocked TBARS formation in the cortex. This suggests that there may be problems in extrapolating from *in vitro* lipid peroxidation data in the hippocampus to *in vivo* events.

What was striking in our study was the total absence of increased TBARS formation in neonate tissue taken from the forebrain. While the tissue examined was mainly frontal cortex (see Methods), we cannot fully rule out that other tissue was present, thereby 'diluting' any effect occurring. However the correlation between the lack of damage and lack of increased lipid peroxidation was clear.

Finally, it is also worth noting that *p*-chloroamphetamine administration, which has also been shown to produce neurodegenerative loss of 5-HT in the adult brain (e.g. Harvey & McMaster, 1975; Murray *et al*., 1996), fails to induce damage when given to neonates (Clemens *et al*., 1978; Lucot *et al*., 1981). Since our studies with PBN indicate that this compound also induces damage by increasing free radical production (Murray *et al*., 1996), these data all suggest that the foetal and neonate brain is protected from damage by these substituted amphetamines because either (1) their brains fail to form neurotoxic metabolites from the parent amphetamine or (2) the neonate brain has a much higher capacity to scavenge and inactivate free radicals than the adult brain. Support for this latter proposal comes from the study of Floyd (1990), who showed young gerbils to be more resistant to the lethal effects of global cerebral ischaemia (a situation where oxidative damage occurs), than old animals.

In conclusion, it should be added that these data can only be used to illustrate the finding that when MDMA is given to the mother during one defined period in the foetal development process it does not appear to induce overt central teratogenic damage. Administration at other periods might give different results. Furthermore, without further studies of this type at different periods and in different species, the data cannot be taken to indicate that the human foetus is not at risk when carried by an 'ecstasy'-using mother.

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