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Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity

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In both rats and mice a single large dose of methylenedioxymethylamphetamine (MDMA; 25 mg/kg i.p.) caused a fall 3 h after injection in the content of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in cortex, a fall in noradrenaline in hippocampus and cerebellum, and a rise in dopamine (DA) but fall in dihydroxyphenylacetic acid (DOPAC) in striatum. These effects were transient, levels being essentially back to normal by 24 h after injection. Repeated large doses (3×25 mg/kg in 24 h) of MDMA caused a large long-lasting fall in the content of 5-HT and 5-HIAA in cortex in rats but had only a slight effect in mice. Increasing the dose to 3×50 mg/kg in mice produced a large long-lasting fall in striatal DA. The analogue MDEA(3,4-methylenedioxyethylamphetamine) caused a similar slight fall in 5-HT but in contrast to MDMA caused a slight rise in DA content in mice. The nature and degree of neurotoxicity with methylenedioxyamphetamines appears to be drug and species-specific.

Methylenedioxyamphetamines; Catecholamines; 5-Hydroxytryptamine; (Neurotoxicity, Species and strain differences)

1. Introduction

The designer drug MDMA (3,4-methylenedioxymethylamphetamine) has already had a brief but chequered career. A period of use as a psychotherapeutic adjunct and as a street euphoriant (Shulgin, 1986) has been followed by its withdrawal from legal use through Schedule 1 classification in the United States. Its apparently beneficial affects are not well documented (Greer and Tolbert, 1986) so that the suggestion of permanent neurotoxicity (Schmidt et al., 1986) as a consequence of its use was sufficient to cause its classification.

In our earlier animal studies on rats and mice using MDMA we had difficulty in reproducing the reported neurotoxic effects of MDMA in rats (Logan and Laverty, 1987) even though obvious behavioural effects were observed in both species. In this paper we extend these studies by showing that higher and repeated doses of MDMA were required to reproduce the neurotoxic effects in our rats, and that, in mice, very high and repeated doses caused a neurotoxic effect involving the dopamine rather than the 5-hydroxytryptamine (5-HT)-containing neuronal system. Thus these neurotoxic effects of MDMA are species- and strain-dependent. The effect of MDMA in mice in reducing dopamine (DA) levels was not seen with the ethyl analogue, MDEA (3,4-methylenedioxyethylamphetamine).

2. Materials and methods

Animals used were male random-bred albino rats (200-300 g) and random bred mice (30-45 g)

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supplied by the University of Otago Animal Breeding Station. In experiments with rats each dose or time point is the result of the mean of at least seven brain samples, and with mice the mean of at least nine brain samples. Normally eight rats or 10 mice were used for each time point.

The MDMA and MDEA were synthesized in the Pharmacy Department, University of Otago; purity was established by IR spectra, elemental analysis and melting point (MDMA hydrochloride 150-153° C, MDEA hydrochloride 200-203° C).

Drug was injected i.p. in saline solution. Animals were killed by decapitation; the brains were removed, separated into regions by blunt dissection, and the tissue samples frozen over dry ice and stored at −80° C until assayed. Each rat brain was separated into frontal, parietal, occipital and cingulate cortex, hippocampus, striatum, hypothalamus and cerebellum. In some experiments right and left sides were stored and analysed separately. With mice the brain was dissected into cortex, hippocampus (sometimes analysed as a combined sample) striatum and cerebellum.

Tissues were analysed for amines and metabolites by reverse phase HPLC with electrochemical detection. The system included a 15 cm (i.d. 4.6 mm) column packed with Hypersil ODS 2, 5 μm packing, and an ESA Coulochem detector using the analytical cell 5010, detector 1 + 0.05 V, and detector 2 at +0.35 V. Preweighed tissues were sonicated in 0.2 M perchloric acid with the appropriate internal standard and centrifuged. The supernatant was used for the analysis of one amine and metabolite pair only.

Noradrenaline (NA) and dihydroxyphenylglycol (DHPG) in rat tissues were prepared following the method of Ehrenstrom and Johansson (1985), with dihydroxybenzylamine (DHBA) as the internal standard. Samples were incubated with sulphatase to cleave the conjugates and extracted with alumina. For mouse samples the sulphatase step was omitted. The mobile phase used for NA and DHPG contained 100 mM phosphate, 0.011% EDTA, 0.16% sodium acetate, 0.015% heptane sulphonic acid, 2.5% methanol, at pH 6.4. Dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were extracted with alumina with DHBA as the internal standard and injected onto the HPLC. The mobile phase consisted of 100 mM phosphate, 0.16% sodium acetate, 0.011% EDTA, 0.02% heptane sulphonic acid, and 10% methanol, pH 4.4. For 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) the supernatant with isoprenaline as the internal standard was filtered and injected directly onto the HPLC. The mobile phase was 100 mM phosphate, 0.04% octyl sulphonic acid and 11% acetonitrile, pH 4. For all methods contents were estimated from peak heights and against internal standard, using pure samples carried through the extraction process as reference standard curves.

Results were analysed using one-way analysis of variance and are expressed as the mean (± S.E.M.) of the treated group expressed as a percentage of the mean of the control group.

3. Results

3.1. Single dose of MDMA

A single dose of MDMA (10 or 25 mg/kg i.p.) into rats caused a large fall 3 h after injection in the contents of both 5-HT and 5-HIAA in cortex samples, a rise in striatal DA accompanied by a fall in DOPAC, and a slight fall in NA accompanied by larger falls in DHPG in cerebellum, hippocampus and hypothalamus. Representative results following 25 mg/kg are shown in fig. 1; other tissues at 25 mg/kg and all tissues at 10 mg/kg showed similar results. In mice the pattern was similar. The fall in cortical 5-HT was much less pronounced; the fall in NA appeared to be larger (fig. 1) than in rats.

By 24 h after injection all values had returned to the control range and remained at control levels at 3 days after injection. The raised value of cortical 5-HT 3 days after injection of 25 mg/kg in mice was not seen with 10 mg/kg and is not thought to be biologically significant.

3.2. Repeated doses of MDMA

In view of the absence of any permanent effect of a single dose of MDMA on brain amine systems, the treatment was increased by giving three

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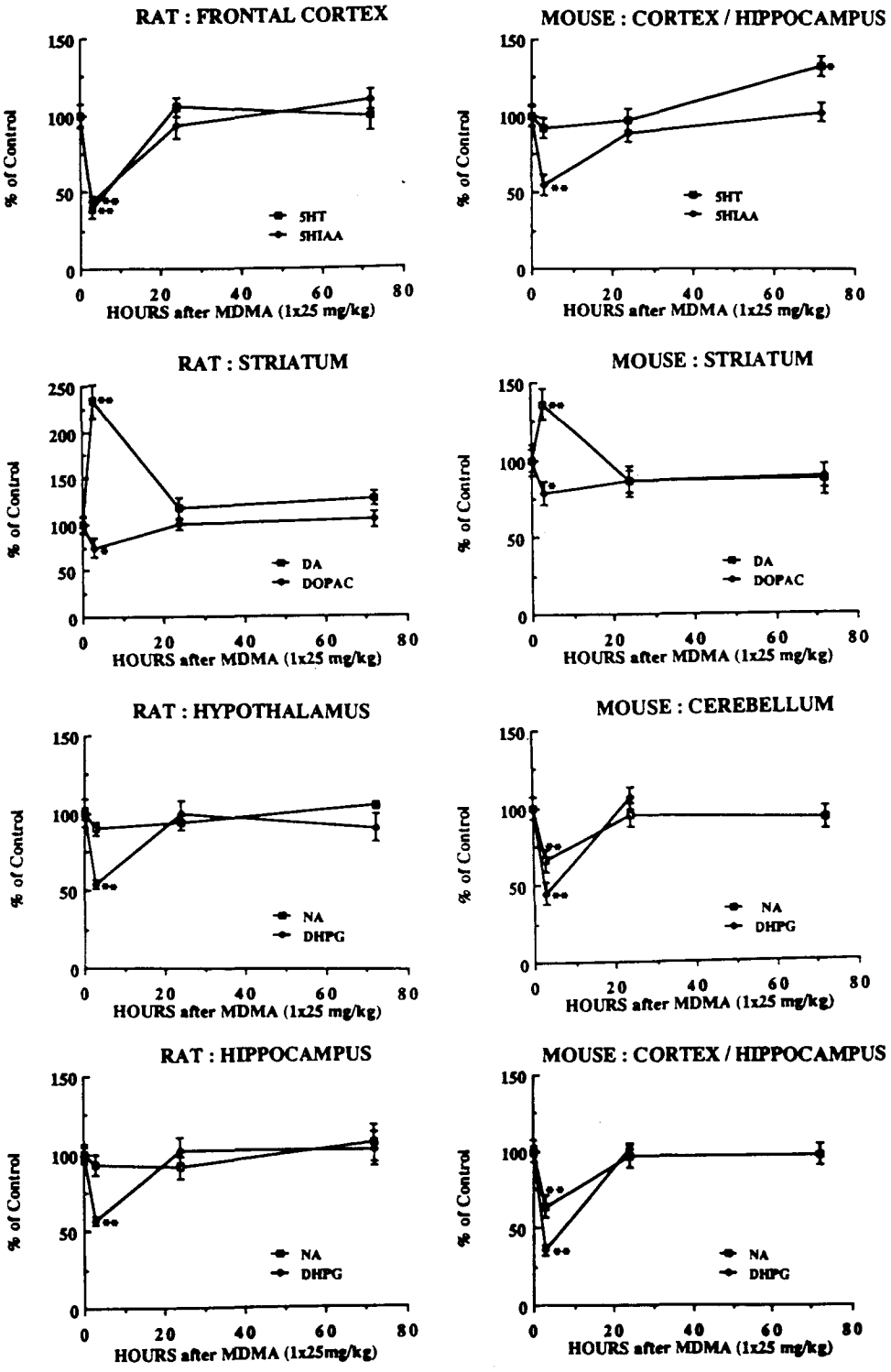

Fig. 1. Comparison between rats and mice of the effects of a single dose of MDMA (25 mg/kg) on the concentration of amines and their metabolites in brain regions. Significance of difference from controls: * $P < 0.05$, ** $P < 0.01$.

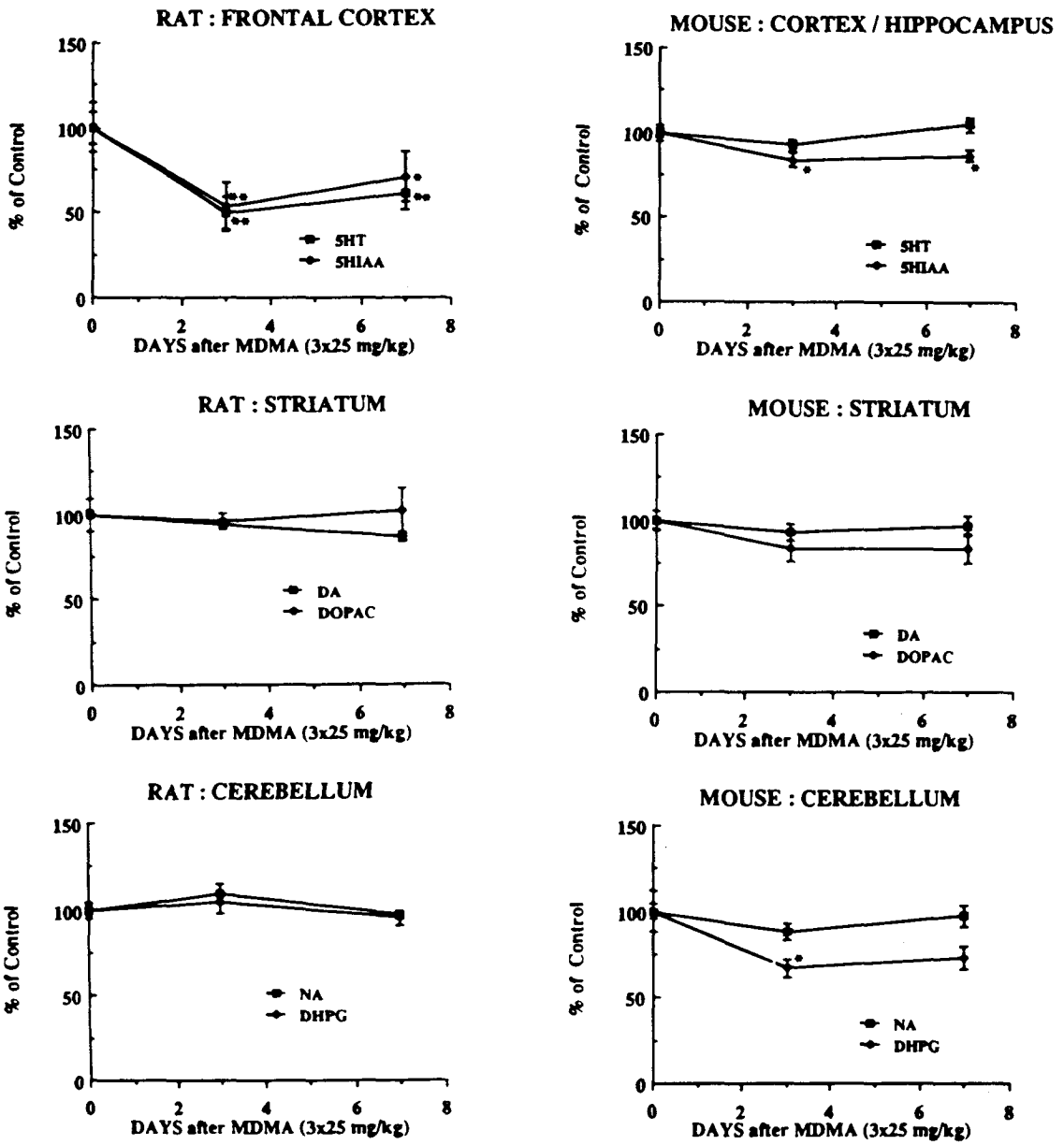

Fig. 2. Comparison between rats and mice of the effects of repeated doses of MDMA (3 doses of 25 mg/kg in 24 h) on brain amine and metabolite levels of rat and mouse brain regions.

doses of MDMA within a 24 h period, i.e. at approximately 12 h intervals.

In rats 3×25 mg/kg MDMA caused for at least 7 days after the last injection a large and prolonged fall in 5 HT and 5 HIAA in all cortical samples but had no significant effect on the DA or NA systems (fig. 2). In mice there was a small but persisting fall in 5-HIAA in cortex/hippocampus and falls in DOPAC and DHPG, but no changes in the content of the catecholamines themselves (fig. 2).

Increasing the dose in mice to 3×50 mg/kg again produced for up to 14 days a persisting fall in 5-HIAA with a slight but non-significant fall in

5-HT, a profound and persisting fall in both DA and DOPAC, but no significant change in NA (fig. 3). This dose is comparable with that used in rats, with respect to their relative toxicities (Hardman et al., 1973).

3.3. Comparison of effects of MDEA with MDMA

In a further experiment mice were treated with injections (3×50 mg/kg i.p.) of either MDMA or MDEA, and the brain amines and metabolites were analysed 1, 3 and 7 days after injection (table 1). The results with MDMA were similar to those shown in fig. 3 though in this experiment the small fall in 5-HT and 5-HIAA was statistically significant. MDEA, while causing a similar small fall in cortical 5-HT and 5-HIAA, failed to produce the fall in DA and DOPAC caused by MDMA, but instead caused a rise in striatal DA and DOPAC. There was no change in cerebellar NA.

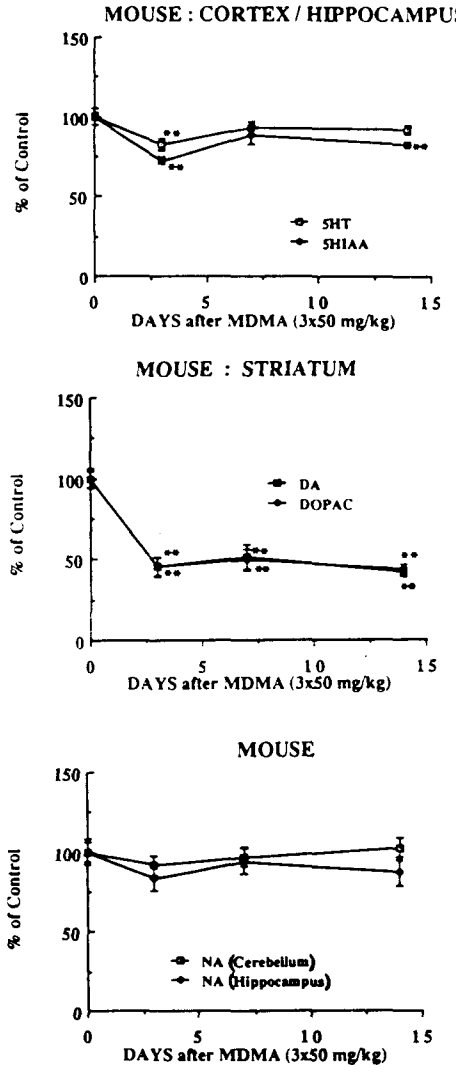

Fig. 3. Effects of repeated large doses of MDMA (3 doses of 50 mg/kg in 24 h) on mouse brain amines and metabolites.

4. Discussion

The results of these experiments are consistent with earlier reports in the literature on the effects of MDMA and other amphetamine analogues on brain amine levels in the rat, though there may be differences in the sensitivity of different rat strains to the drugs. Single doses of MDMA (fig. 1) cause a fall in 5-HT and a rise in DA at 3 h after injection (Schmidt et al., 1986; 1987; Schmidt, 1987a,b); in our experiments in both rats and mice these levels recovered by 24 h after the injection, whereas in the reported experiments, rats treated with smaller doses (10 or 20 mg/kg) showed levels of 5-HT remaining low at 7 or 14 days after injection (Commins et al., 1987; Ricaurte et al., 1987). Thus our rats appear to be more resistant to these long-term effects of MDMA (Logan and Laverty, 1987). In mice, the acute effects of a single dose of MDMA are similar to those in rats, though the effects of 5-HT appear less dramatic while the effects of NA are more obvious. Thus even in these acute effects there could be species differences.

The rise in DA content following an acute injection of MDMA suggests that the mechanism of action of MDMA differs from that of other amphetamines. In most species amphetamine does not cause a large increase in brain DA (Moore and Lariviere, 1963; Laverty and Sharman, 1965) though increases in DA due to amphetamines have been reported in mice (Smith, 1965). Amphetamines cause a release of DA (Kuczenski, 1986) and since the DA metabolite, homovanillic acid, is increased by MDMA (Schmidt et al., 1986) it is probable that MDMA is also causing DA release (Johnson et al., 1986). Why release

TABLE 1

Comparison of effects of MDMA and MDEA on the contents of mouse brain region amines and metabolites. Mice were treated with three doses of 50 mg/kg of either agent in a 24 h period, and killed at the respective times after the last injection. Results are the mean tissue contents in μg/g (± S.E.M.) from 10 mice. [a] $P < 0.05$ [b] $P < 0.01$

	Cortex/hippocampus				Striatum				Cerebellum	
	5-HT 0.63 ± 0.04 [a]		5-HIAA 0.17 ± 0.01 [a]		DA 10.3 ± 0.5 [a]		DOPAC 0.88 ± 0.07 [a]		NA 0.23 ± 0.01 [a]	
	MDMA	MDEA	MDMA	MDEA	MDMA	MDEA	MDMA	MDEA	MDMA	MDEA
Days after injection										
1	0.51 [b] ± 0.02	0.49 [b] ± 0.02	0.16 ± 0.01	0.16 ± 0.01	9.2 ± 0.6	11.7 ± 0.4	0.9 ± 0.11	1.60 [b] ± 0.16	0.23 ± 0.01	0.24 ± 0.02
3	0.56 [a] ± 0.03	0.54 [b] ± 0.02	0.15 [a] ± 0.01	0.15 [a] ± 0.01	9.6 ± 1.0	9.7 ± 0.4	0.82 ± 0.08	0.97 ± 0.08	0.24 ± 0.01	0.25 ± 0.01
7	0.55 [a] ± 0.03	0.55 [b] ± 0.02	0.14 [b] ± 0.01	0.14 [b] ± 0.01	6.3 [b] ± 0.9	12.1 [a] ± 0.4	0.59 [a] ± 0.1	1.00 ± 0.07	0.23 ± 0.01	0.22 ± 0.02

[a] Control value.

should be accompanied by such a large increase in stored DA remains to be explained. The fall in DOPAC accompanying increased DA release and storage suggests that MDMA may, like other amphetamines, impair the re-uptake of released DA (Moore, 1978). The falls in NA, 5-HT and their metabolites following acute MDMA injection are consistent with an increased release, reduced re-uptake or inhibition of intraneuronal monoamine oxidase caused by MDMA, and parallel the effects of other amphetamines.

Long-lasting effects on brain amine content was only seen in our rats after intensive treatment with MDMA given as three doses in a 24 h period (fig. 2). Using 3 × 25 mg/kg doses, the content of brain 5-HT and 5-HIAA remained low for at least 7 days; this is consistent with earlier reports using multiple doses (Stone et al., 1986; 1987; Commins et al., 1987; Mokler et al., 1987). This dose regime produced falls in metabolite contents in mice, but no significant effect on the amines. However, increasing the dose to 3 × 50 mg/kg in mice produced a small prolonged fall in 5-HT and 5-HIAA (fig. 3, table 1) but marked falls in striatal DA and DOPAC. The doses used of 25 mg/kg in rats and 50 mg/kg in mice represent about half the LD_{50}s, respectively (Hardman et al., 1973). Chronic high-dose methamphetamine treatment causes large falls in 5-HT and lesser falls in DA content in rats (Ricaurte et al., 1980; Schmidt et al., 1985) suggesting that in the rat the 5-HT-containing pathways are the more susceptible. In mice, chronic amphetamine treatment (Steranka and Sanders-Bush, 1980) caused a large fall in brain DA with less effect on brain 5-HT, again suggesting there may be a species difference in susceptibility.

Possible explanations of these species or strain differences require elucidation. In rats amphetamines are predominantly metabolized by ring hydroxylation; in mice (and man) a greater proportion of unchanged amines and aliphatic deaminated metabolites are excreted (Smith and Dring, 1970). Thus the production of neurotoxic metabolites (Commins et al., 1987) from MDMA may differ between strains or species; MDMA, being resistant to ring hydroxylation, may persist longer in an unchanged form in rats. However guinea-pigs also metabolize amphetamines predominantly by side-chain oxidation, yet MDMA has a similar neurotoxic profile in guinea-pigs as in rats (Commins et al., 1987). It is also likely that the neurotoxic effects of amphetamines arise not solely from actions on single neurons but may also involve trans-synaptic events. Blockade of DA synthesis with α-methyl-p-tyrosine or blockade of DA receptors with haloperidol (Schmidt et al., 1985) reduce the toxic effects of methamphetamine on 5-HT systems, as also do inhibitors of DA uptake or release. Hence there are a number of possible sites for neurotoxic effects that may differ between strains and species.

MDEA, the N-ethyl analogue of MDMA, also caused in mice a small persisting fall in 5-HT and 5-HIAA of similar magnitude to that produced by MDMA (table 1). This differs from the reported effects in rats (Stone et al.,1987; Ricaurte et al., 1987; Schmidt, 1987b) in which MDEA is less than one quarter as potent as MDMA as a neurotoxic agent on the 5-HT system. MDEA also appeared to have no effect on the DA system in rats. In mice MDEA increased DA and DOPAC rather than the decrease produced by MDMA. The effect on DOPAC may indicate that MDEA is less potent as an inhibitor of monoamine oxidase than MDMA.

It appears then that the neurotoxic effects of methylenedioxyamphetamines may differ in different strains and species. It is not necessarily valid to assume that toxic effects found in some animals will also be found in man.

Acknowledgements

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