elicited in human subjects by stimulation of the larger arteries at the ventral surface of the brain[19]. Terminals of trigeminal origin are known to innervate the internal carotid artery within the cavernous sinus in monkeys[21] but quantitative data are lacking and it remains to be seen whether or not their density profile reflects that of the full innervation sleeve. If it does, it raises the possibility that the sleeve represents a locus of low nociceptive threshold.

Acknowledgements. Some of the animal material used in this study was generously supplied by Professor S. Zeki and Dr. J. Cooper.

REFERENCES

1 Alafaci, C., Cowen, T., Crockard H.A. and Burnstock, G., Cerebral perivascular serotonergic fibres have a peripheral origin in the gerbil, *Brain Res. Bull.*, 16 (1986) 303–304.
2 Dhall, U. and Burnstock, G., Distribution of serotonin-like immunoreactive nerves in major cerebral arteries of rabbit; an immunohistochemical study, *J. Histochem. Cytochem.*, 37 (1989) 1383–1386.
3 Edvinsson, L. and Ekman, R., Distribution and dilatory effect of vasoactive intestinal polypeptide (VIP) in human cerebral arteries, *Peptides*, 5 (1984) 329–331.
4 Edvinsson, L., Owman, C. and Sjoberg, N.O., Autonomic nerves, mast cells and amine receptors in human brain vessels. A histochemical and pharmacological study. *Brain Res.*, 115 (1976) 377–394.
5 Ekbom, K. and Greits, T., Carotid angiography in cluster headache, *Acta Radiol. Diagn.*, 10 (1970) 177–183.
6 Friberg, L., Olesen, S., Iversen, H.K. and Sperling, B., Migraine pain associated with middle cerebral artery dilatation: reversal by sumatriptan, *Lancet*, 338 (1991) 13–17.
7 Hanko, J., Hardebo, J.E., Kåhrström, J., Owman, C. and Sundler, F., Existence and co-existence of calcitonin gene-related peptide (CGRP) and substance P in cerebrovascular nerves and trigeminal ganglion cells, *Acta Physiol. Scand.*, 127 Suppl. 552 (1986) 29–32.
8 Hardebo, J.E., Arbab, M., Suzuki, N and Svengaard, N.A., Pathways of parasympathetic and sensory cerebrovascular nerves in monkeys, *Stroke*, 22 (1991) 331–342.
9 Hara, H., Hamill, G.S. and Jacobowitz, D.M., Origin of cholinergic nerves to the rat major cerebral arteries: coexistence with vasoactive intestinal polypeptide, *Brain Res. Bull.*, 14 (1985) 179–188.
10 Hara, H. and Wier, B., Different distributions of substance-P and vasoactive intestinal polypeptide in the cerebral arterial innervation in rat and guinea pig, *Anat. Anz.*, 163 (1987) 19–24.
11 Hernández-Pérez, M.J. and Stone, H.L., Sympathetic innervation of the circle of Willis in the macaque monkey, *Brain Res.*, 80 (1974) 507–511.
12 Itakura, T., Okuno, T., Nakakita, K., Kamei, I., Naka, Y., Nakai, K., Imai, H., Komai, N., Kimura, H. and Maeda, T., A light and electron microscopic immunohistochemical study of vasoactive intestinal polypeptide- and substance P-containing nerve fibers along the cerebral blood vessels: comparison with aminergic and cholinergic fibers, *J. Cereb. Blood Flow Metab.*, 4 (1984) 407–414.
13 Kawai, Y. and Ohhashi, T., Histochemical studies of the adrenergic innervation of canine cerebral arteries, *J. Autonom. Nerv. Syst.*, 15 (1986) 103–108.
14 Kobayashi, S., Tsukahara, S., Sugita, K. and Nagata, T., Adrenergic and cholinergic innervation of rat cerebral arteries. Consecutive demonstration of whole-mount preparations, *Histochemistry*, 70 (1981) 129–138.
15 Matsuyama, T., Shiosaka, S., Matsumoto, M., Yoneda, S., Kimura, K., Abe, H., Hayakawa, T., Inoue, H. and Tohyama, M., Overall distribution of vasoactive intestinal polypeptide-containing nerves on the wall of the cerebral arteries: an immunocytochemical study using whole-mounts, *Neuroscience*, 10 (1983) 89–96.
16 Mayberg, M., Langer, R.S., Zervas, N.T. and Moskowitz, M.A., Perivascular meningeal projections from cat trigeminal ganglia: possible pathway for vascular headaches in man, *Science*, 213 (1981) 228–230.
17 Moskowitz, M.A., The neurobiology of vascular head pain, *Ann. Neurol.*, 16 (1984) 157–168.
18 Nichols, F.T., Mawad, M., Mohr, M., Stein, B., Hilal, S. and Michelson, W.J., Focal headache during balloon inflation in the internal carotid and middle cerebral arteries, *Stroke*, 21 (1990) 555–559.
19 Ray, B.S. and Wolff, H.G., Experimental studies on headache: pain-sensitive structures of the head and their significance in headache, *Arch. Surg.*, 41 (1940) 813–856.
20 Ruskell, G.L., The orbital branches of the pterygopalatine ganglion and their relationship with internal carotid nerve branches in primates, *J. Anat.*, 106 (1970) 323–339.
21 Ruskell, G.L. and Simons, T., Trigeminal nerve pathways to the cerebral arteries in monkeys, *J. Anat.*, 155 (1987) 23–37.
22 Simons, T. and Ruskell, G.L., Distribution and termination of trigeminal nerves to the cerebral arteries in monkeys, *J. Anat.*, 159 (1988) 57–71.
23 Tsai, S.-H., Tew, J.M., McLean, J.H. and Shipley, M.T., Cerebral arterial innervation by nerve fibers containing calcitonin gene-related peptide (CGRP): 1. Distribution and origin of CGRP perivascular innervation in the rat, *J. Comp. Neurol.*, 271 (1988) 435–444.
24 Walsh, J.P. and O'Doherty, D.S., A possible explanation of the mechanism of ophthalmoplegic migraine, *Neurology*, 10 (1960) 1079–1084.
25 Walters, B.B., Gillespie, S.A. and Moskowitz, M.A., Cerebrovascular projections from the sphenopalatine and otic ganglia to the middle cerebral artery of the cat, *Stroke*, 17 (1986) 488–494.

Brain Research, 595 (1992) 121–127

BRES 18225

Farfel 1992

The *N*-methyl-D-aspartate antagonist MK-801 protects against serotonin depletions induced by methamphetamine, 3,4-methylenedioxymethamphetamine and *p*-chloroamphetamine

Gail M. Farfel, Georgetta L. Vosmer and Lewis S. Seiden

The University of Chicago, Department of Pharmacological and Physiological Sciences, Chicago, IL 60637 (USA)

(Accepted 9 June 1992)

Key words: Serotonin; Dizocilpine; 3,4-Methylenedioxymethamphetamine; Neurotoxicity; Striatum; Hippocampus

The non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 blocks the ability of D-methamphetamine (MA) to deplete striatal dopamine (DA). We now report that MK-801 attenuates decreases in serotonin (5-HT) concentration induced by MA and two other amphetamine analogues, 3,4-methylenedioxymethamphetamine (MDMA) and *p*-chloroamphetamine (PCA). Rats were injected with saline (1.0 ml/kg) or MK-801 (0.5, 1.0 or 2.5 mg/kg) followed by either saline (1.0 ml/kg), MA (4, 2 or 1 injection(s); 10.0, 20.0 or 40.0 mg/kg), MDMA (20.0 or 40.0 mg/kg) or PCA (5.0 or 10.0 mg/kg). In some experiments, two injections of MK-801 or saline were used. Seventy-two hours after the last injection rats were sacrificed and concentrations of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and DA were determined in hippocampus and striatum. MA caused a depletion of 5-HT to 33% of control in hippocampus and to 50% of control in striatum after the 4×10.0 mg/kg dose regimen. When MK-801 (2.5 mg/kg) was co-administered with MA, concentrations of 5-HT did not differ from control levels in either brain region. MDMA depleted 5-HT to approximately 58% of control in hippocampus and 66% of control in striatum at the 40 mg/kg dose. Co-administration of 0.5, 1.0 or 2.5 mg/kg MK-801 with MDMA caused a dose-related attenuation of the 5-HT depletions. PCA caused dose-dependent depletions of 5-HT to approximately 20% of control levels in both hippocampus and striatum at the highest dose. Co-administration of one or two injections of MK-801 (2.5 mg/kg) caused small attenuations of 5-HT depletions in all groups, although statistical significance was reached only when experiments were pooled to increase the sample size. Changes in 5-HIAA concentration were similar to changes in 5-HT concentration in all experiments. Striatal DA was depleted by MA but not MDMA or PCA, and co-administration of MK-801 attenuated the MA-induced DA depletion. These results are discussed in terms of possible mechanisms of serotonergic neurotoxicity.

INTRODUCTION

Administration of D-methamphetamine (MA) to rats in a variety of dose regimens has been shown to be toxic to serotonin (5-HT) neurons, as evidenced by long-lasting changes in several neuronal markers. MA causes a long-lasting decrease in concentration of 5-HT[23,24,45], a decrease in tryptophan hydroxylase (TPH) activity[2,11], and a decrease in the number of 5-HT transporters[19,24,45]. 3,4-Methylenedioxymethamphetamine (MDMA) and *p*-chloroamphetamine (PCA) have also been shown to engender changes in 5-HT neurons similar to those of MA. Both MDMA and PCA cause a long-lasting decrease in the concentration of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA)[6,7,26,33,40], a decrease in TPH activity[26,31], a decrease in the number of 5-HT transporters[3,4,27,35,42], and terminal degeneration as determined by histological markers[8,21,22].

Several studies have provided evidence that dopamine (DA) release is necessary to induce serotonergic toxicity by substituted amphetamines. Inhibition of tyrosine hydroxylase (TH) by α-methyl-*p*-tyrosine prior to administration of MA[10,30], MDMA[28,39] or PCA[1] attenuates the depletion of 5-HT and/or the decrease in TPH activity. Lesioning the substantia nigra with 6-hydroxydopamine attenuates MDMA-induced 5-HT depletions in hippocampus and cortex[28]. Blocking DA receptors with haloperidol or the D1 antagonist SCH 23390 has also been shown to partially block the serotonergic toxicity of MA[36]. Also, the S-(+) stereoisomer of MDMA, which releases striatal DA in vivo[9], induces

Correspondence: L.S. Seiden, Dept. of Pharmacological and Physiological Sciences, The University of Chicago, 947 E. 58th Street, Chicago, IL 60637, USA.

serotonergic toxicity at doses equivalent with the racemate[27]. The R-(−) isomer does not release striatal DA in vivo[9], nor does it appear to induce serotonergic toxicity[27]. These data suggest that synaptic DA release is involved in the mechanism of serotonergic neurotoxicity, and MA, MDMA and PCA have all been shown to cause DA release[29].

MA is toxic to DA neurons in the same doses that cause 5-HT neurotoxicity[24]. The non-competitive NMDA receptor antagonist MK-801 blocks decreases in both DA concentration and TH activity after MA administration[37]. It has been suggested that MK-801 protects against MA-induced dopaminergic toxicity either by attenuating basal DA release[15], attenuating MA-induced DA release[43], or by attenuating DA release evoked by glutamate[5]. Since there is strong evidence that synaptic DA is required to mediate serotonergic toxicity, this study was designed to determine whether the protective effect of MK-801 extends to 5-HT neurons. We report that co-administration of MK-801 with MA, MDMA or PCA attenuates 5-HT depletions in rat hippocampus and striatum.

MATERIALS AND METHODS

Male Long–Evans rats (200–225 g; Harlan, Indianapolis, IN) were housed individually in plexiglass cages (21×42×20 cm) in a room maintained at 21–23°C on a 12-h light-dark cycle (lights on from 07.00–19.00 h). Food (Teklad Rat and Mouse Diet, Teklad, Winfield, IA) and water were available ad libitum, except during the injection regimen.

Rats were injected with either 0.9% saline (SAL; 1.0 ml/kg) or MK-801 (MK; 0.5, 1.0 or 2.5 mg/kg), followed by injections of either SAL, MA (10.0, 20.0 or 40.0 mg/kg), MDMA (20.0 or 40.0 mg/kg) or PCA (5.0 or 10.0 mg/kg) at least 15 min later. See figure legends for exact dose regimens.

Drugs were administered in a volume of 1.0 ml/kg in 0.9% saline vehicle except for MK, which was dissolved in water. All injections were given IP, except MDMA and its control injections which were given SC. Doses of drugs were calculated for the salt forms. D-Methamphetamine HCl and DL-*p*-chloramphetamine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). (+) MK-801 hydrogen maleate was purchased from Research Biochemicals, Inc. (Natick, MA), and 3,4-methylenedioxymethamphetamine HCl was generously provided by the National Institute on Drug Abuse.

Seventy-two hours after the last injection, rats were sacrificed by decapitation and their brains dissected over ice. Tissue from striatum and hippocampus was stored in liquid nitrogen until assayed for 5-HT, 5-HIAA and DA using HPLC with electrochemical detection[1,18]. Briefly, samples were eluted on stainless-steel RSIL 5 μm C18-HL ODS reverse-phase columns at a flow rate of 1 ml/min. The aqueous mobile phase contained 0.27 mM EDTA, 0.43 mM octylsodium sulfate, 7.9 mM Na_2HPO_4, 62.5 mM citric acid and 19.5% methanol. Compounds were detected electrochemically by oxidation over a glassy carbon working electrode at a potential of +0.8 V relative to the Ag/AgCl reference electrode. Output from the detector (ESA Coulochem 5200, ESA Inc., or EG&G Model 400, Princeton Applied Research) was channeled to an integrator (Shimadzu CR5-A) which monitored peak retention times and calculated peak areas and values based on a two point calibration of standards.

Significant differences in monoamine concentrations between treatment groups in a single experiment were determined by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls post-hoc comparisons[17] using SuperANOVA software (Abacus Concepts, Berkeley, CA) for Apple Macintosh computers. $P \leq 0.05$ was considered to be statistically significant. All results are reported as mean ± S.E.M. in ng/mg wet weight tissue.

RESULTS

The SAL/MA (10.0 mg/kg × 4 inj) group showed 5-HT depletions when compared to the SAL/SAL group in hippocampus and striatum (Fig. 1). Mean 5-HT concentration (in ng/mg wet weight tissue) was reduced to 33% of control ($F = 31.79$, $P \leq 0.0001$) in hippocampus and 50% of control ($F = 8.02$, $P \leq 0.001$) in striatum. In the MK/MA (10.0 mg/kg × 4 inj) group, 5-HT concentration did not differ from the SAL/SAL group in either region. The other doses of MA (40.0 × 1 inj and 20.0 × 2 inj) did not affect 5-HT concentration. (Data for the 20.0 mg/kg × 2 injection experiment is pooled from two separate experiments, one in which 3 of 8 MA-treated rats survived and another in which 15 out of 15 survived. The data therefore may be weighted in favor of animals who were resistant to the effects of MA.)

In the SAL/MDMA-treated rats, hippocampal 5-HT concentration was 58% of control after the 20.0 mg/kg dose ($F = 12.57$, $P \leq 0.0001$), while the 40.0 mg/kg dose reduced 5-HT to 40% ($F = 13.51$, $P \leq 0.0001$), 70% ($F = 9.81$, $P \leq 0.0001$) and 65% ($F = 14.63$, $P \leq 0.0001$) of control (Fig. 2). Co-administration of MK-801 (2.5 mg/kg) with MDMA (20.0 or 40.0 mg/kg) attenuated 5-HT depletions in hippocampus when compared to the SAL/MDMA groups, although 5-HT levels were still significantly lower than the SAL/SAL groups. MK-801 (1.0 mg/kg) combined with MDMA (40.0 mg/kg) also attenuated 5-HT depletion in hippocampus compared to SAL/MDMA ($F = 9.81$, $P \leq 0.0001$), while MK-801 (0.5 mg/kg) did not afford protection against 5-HT depletion.

In striatum, MDMA (40.0 mg/kg) caused depletion of 5-HT (Fig. 2) to 60% ($F = 2.90$, $P \leq 0.05$) and 72% ($F = 5.71$, $P \leq 0.005$) of control values. In a third experiment using this dose, MDMA did not cause significant 5-HT depletion. When MK-801 (2.5 and 0.5 mg/kg) was co-administered with MDMA, 5-HT levels did not differ from the SAL/SAL-treated rats.

The SAL/PCA-treated rats showed 5-HT depletions at both doses (5.0 and 10.0 mg/kg) in hippocampus compared to the SAL/SAL rats (Fig. 3). 5-HT concentration in hippocampus was 50% of control after the 5.0 mg/kg dose ($F = 23.68$, $P \leq 0.0001$), while the 10.0 mg/kg dose reduced 5-HT to 17% ($F = 82.33$ $P \leq 0.0001$) and 21% ($F = 59.42$, $P \leq 0.0001$) of control. Co-administration of MK-801 (2.5 mg/kg, one or two injections) with PCA (5.0 or 10.0 mg/kg) caused

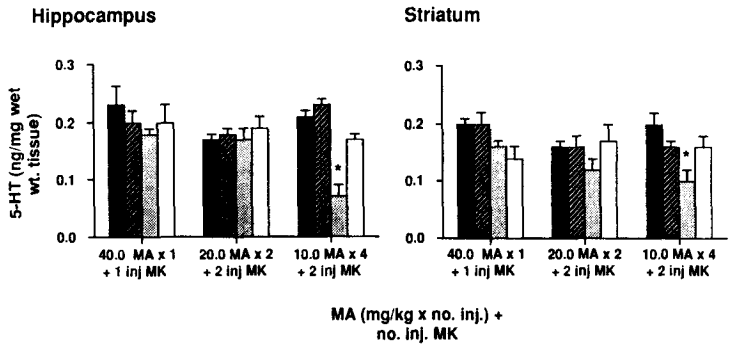

Fig. 1. Effects of MA and MK on 5-HT in hippocampus and striatum. Rats were administered one of the following regimens: (a) MK (2.5 mg/kg) or SAL (1.0 ml/kg) followed 15 min later by one injection of MA (40.0 mg/kg) or SAL (1.0 ml/kg); (b) MK (2.5 mg/kg) or SAL (1.0 ml/kg) followed by two injections of MA (20.0 mg/kg) or SAL (1.0 ml/kg) beginning 15 min later and spaced 1 h apart. Concurrent with the last MA or SAL injection, a second injection of MK or SAL was administered; (c) MK (2.5 mg/kg) or SAL (1.0 ml/kg) followed by 4 injections of MA (10.0 mg/kg) or SAL (1.0 ml/kg) beginning 15 min later and spaced 1 h apart. Concurrent with the last MA or SAL injection, a second injection of MK or SAL was administered. Values represent mean ± S.E.M. expressed in ng/mg wet weight tissue. Solid bars represent SAL/SAL-treated groups ($n = 7$–13); striped bars represent MK/SAL-treated groups ($n = 8$–14); gray bars represent SAL/MA-treated groups ($n = 9$–18), and open bars represent MK/MA-treated groups ($n = 8$–14). * Indicates significantly different from SAL/SAL ($P \leq 0.05$).

small attenuations of hippocampal 5-HT depletions in all experiments. However, this attenuation was statistically significant for only one dose combination, MK-801 (2.5 mg/kg × 1 inj)/PCA (10.0 mg/kg). In this case the data were pooled from two separate experiments, increasing the sample size. (Sample n's were 12, 14, 22 and 20 for SAL/SAL, MK/SAL, SAL/PCA and MK/PCA, respectively. There was no significant difference between the values obtained in the first experiment and the replication.) In striatum, PCA (10.0

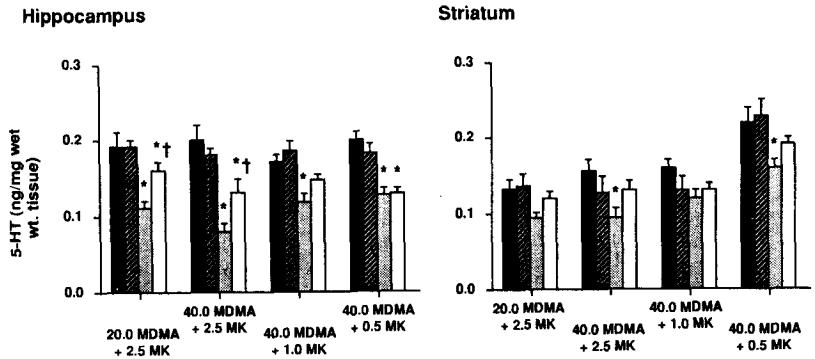

Fig. 2. Effects of MDMA and MK on 5-HT in hippocampus and striatum. Rats were administered MK (0.5, 1.0 or 2.5 mg/kg) or SAL (1.0 ml/kg) followed 15 min later by one injection of MDMA (20.0 or 40.0 mg/kg) or SAL (1.0 ml/kg). Values represent mean ± S.E.M. expressed in ng/mg wet weight tissue. Solid bars represent SAL/SAL-treated groups ($n = 6$–8); striped bars represent MK/SAL-treated groups ($n = 7$–8); gray bars represent SAL/MDMA-treated groups ($n = 9$–12), and open bars represent MK/MDMA-treated groups ($n = 7$–12). * Indicates significantly different from SAL/SAL ($P \leq 0.05$). † Indicates significantly different from SAL/MDMA ($P \leq 0.05$). HPLC columns were changed for the 40.0 MDMA/0.5 MK experiment which resulted in higher mean values for all groups in this experiment.

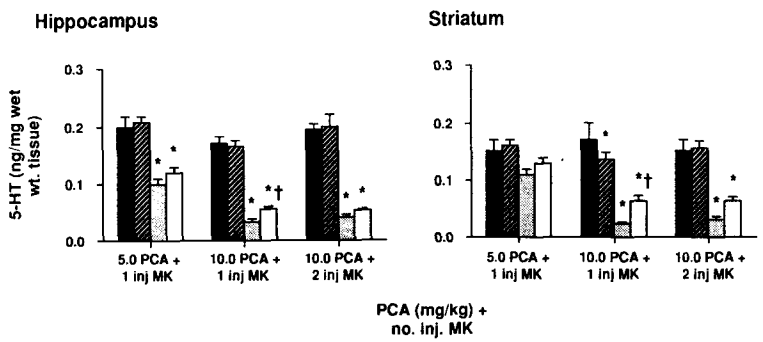

Fig. 3. Effects of PCA and MK on 5-HT in hippocampus and striatum. Rats were administered MK (2.5 mg/kg) or SAL (1.0 ml/kg) followed 15 min later by one injection of PCA (5.0 or 10.0 mg/kg) or SAL (1.0 ml/kg). Some rats received a second injection of MK or SAL 30 min after the PCA or SAL injection. Values represent mean ± S.E.M. expressed in ng/mg wet weight tissue. Solid bars represent SAL/SAL-treated groups (n = 7–13); striped bars represent MK/SAL-treated groups (n = 8–14); gray bars represent SAL/PCA-treated groups (n = 8–22), and open bars represent MK/PCA-treated groups (n = 8–20). * Indicates significantly different from SAL/SAL ($P \leq 0.05$). † Indicates significantly different from SAL/PCA ($P \leq 0.05$).

mg/kg) caused significant depletions of 5-HT to 14% ($F = 28.90$, $P \leq 0.0001$) and 21% ($F = 27.43$, $P \leq 0.0001$) of control values, and co-administration of MK-801 (2.5 mg/kg, one or two injections) attenuated both depletions but reached statistical significance only in the MK-801 (2.5 mg/kg × 1 inj)/PCA (10.0 mg/kg) experiment.

The effects of SAL/MA on 5-HIAA were similar to the effects on 5-HT. SAL/MA (10.0 mg/kg × 4 inj) caused depletion of 5-HIAA in hippocampus (0.10 ± 0.01 vs. 0.21 ± 0.02; mean ± S.E.M. in ng/mg tissue; $F = 8.38$, $P \leq 0.001$) while co-administration of MK-801 (2.5 mg/kg × 2 inj) attenuated the depletion (0.10 ± 0.01 for SAL/MA vs. 0.16 ± 0.02 for MK/MA). SAL/MA (10.0 mg/kg × 4 inj) did not deplete 5-HIAA in striatum (0.23 ± 0.04 for SAL/MA vs. 0.25 ± 0.01 for SAL/SAL), although it should be noted there was a large amount of variability in the MA-treated animals (values ranged from 0.07–0.39 ng/mg tissue). There were no depletions of 5-HIAA by SAL/MA in either region at the 40.0 mg/kg (×1 inj) dose, although the 20.0 mg/kg (×2 inj) dose engendered a small (0.14 ± 0.01 vs. 0.16 ± 0.01) but significant ($F = 4.01$, $P \leq 0.05$) depletion in hippocampus.

SAL/MDMA (20.0 mg/kg) depleted 5-HIAA in hippocampus compared to SAL/SAL (0.12 ± 0.01 vs. 0.15 ± 0.01, $F = 5.31$, $P \leq 0.005$) as did the 40.0 mg/kg dose (0.07 ± 0.01 vs. 0.14 ± 0.00, $F = 17.11$, $P \leq 0.0001$). Similar results were obtained with the 40.0 mg/kg dose in 2 additional experiments. When MK-801 (1.0 or 2.5 mg/kg) was co-administered with MDMA (20.0 or 40.0 mg/kg), 5-HIAA concentration in hippocampus did not differ from the SAL/SAL rats. MK-801 (0.5 mg/kg) did not protect against the MDMA (40.0 mg/kg)-induced 5-HIAA depletion. In striatum, MDMA (40.0 mg/kg) engendered a depletion of 5-HIAA in two of four experiments, and co-administration of MK-801 (0.5; or 2.5 mg/kg × 2 inj) attenuated these depletions.

SAL/PCA caused depletions of 5-HIAA in hippocampus at both the 5.0 mg/kg (0.11 ± 0.01 vs. 0.16 ± 0.01, $F = 14.73$, $P \leq 0.0001$) and 10.0 mg/kg (0.03 ± 0.00 vs. 0.15 ± 0.01, $F = 99.52$, $P \leq 0.0001$) doses. Analogous to the effects on 5-HT, co-administration of MK-801 (1.0 or 2.5 mg/kg, or 2.5 mg/kg × 2 inj) provided small attenuations of 5-HIAA depletions which only reached statistical significance in the MK-801 (2.5 mg/kg × 1)/PCA (10.0 mg/kg) group. In striatum, only the 10.0 mg/kg dose significantly depleted 5-HIAA (0.05 ± 0.01 vs. 0.20 ± 0.02, $F = 21.32$, $P \leq 0.0001$) and co-administration of MK-801 (1.0 or 2.5 mg/kg, or 2.5 mg/kg × 2 inj) attenuated the depletions. However, the attenuation was statistically significant (0.04 ± 0.01 vs. 0.09 ± 0.01, $F = 34.09$, $P \leq 0.0001$) in only one experiment.

Concentration of DA was measured in striatum in these rats in order to compare our results with published work. SAL/MA (10.0 mg/kg × 4 inj) engendered significant depletion of DA (4.53 ± 1.1 vs. 9.54 + 0.3, $F = 7.15$, $P \leq 0.0005$), and co-administration of MK-801 (2.5 mg/kg × 2 inj) partially protected against this depletion (7.28 ± 0.83 for MK/MA) which is in agreement with the previously published study[37]. Neither MDMA nor PCA significantly affected DA concentration at any dose used.

MK/SAL (2.5 mg/kg × 1 or 2 inj, or 1.0 mg/kg × 1 inj) had no significant effect on 5-HT, 5-HIAA or DA concentration in hippocampus or striatum.

DISCUSSION

The results of the present study show that MA, MDMA and PCA cause depletions of 5-HT in rat hippocampus and striatum which can be attenuated by co-administration of the non-competitive NMDA receptor antagonist MK-801. 5-HT depletions caused by all three of these amphetamines have been correlated with toxicity of serotonergic nerve terminals[20,24,44]. The results of this study suggest that co-administration of MK-801 protects against serotonergic toxicity engendered by MA, MDMA and PCA.

Co-administration of MK-801 with MA (10.0 mg/kg × 4 injections) completely protected against decreases in hippocampal and striatal 5-HT in this study, and these results agree with what has been seen in another lab (P. Sonsalla, personal communication). The dose of MA used here also causes long-lasting decreases in DA concentration and TH activity[10,24], and MK-801 (2.5 mg/kg) has been shown to attenuate these effects[37,38] as well. Since the mechanism of MA-induced neuronal toxicity is not well-understood, it is difficult to understand the role of MK-801 in neuroprotection. Nevertheless, these data suggest that MK-801 may work through a common mechanism to protect against serotonergic and dopaminergic toxicity.

MDMA caused depletions of 5-HT in hippocampus at both doses tested. Depletions in striatum were inconsistent, although a significant long-lasting depletion in striatal 5-HT has been shown previously[6] with the 40.0 mg/kg dose. The protective effect of MK-801 was dose-related in terms of the difference in 5-HT concentration between the SAL/MDMA- and MK/MDMA-treated groups. However, the degree of protection (as defined by whether or not the mean 5-HT concentration of the MK/MDMA group was significantly lower than the SAL/SAL group) appears to have been determined by the extent of the MDMA-induced depletion rather than the dose of MK-801. MDMA engendered larger depletions of 5-HT in the experiments using the higher dose of MK-801 (the left-hand histograms in Fig. 2), and thus the higher dose of MK-801 appeared to provide only partial protection while the lower dose appeared to afford complete protection. To assess whether additional doses of MK-801 would give better protection against 5-HT depletion, two injections of MK-801 (2.5 mg/kg) were administered at −15 and 90 min with MDMA (40.0 mg/kg). In hippocampus, 5-HT concentration was 37% of control in the SAL/MDMA group and 66% of control in the MK/MDMA group (data not shown), as compared to 40% and 64% with a single injection of MK-801. A second injection of MK-801 does not appear to increase the protective effect, although additional time points and doses should be examined.

Protection by MK-801 against MDMA-induced 5-HT depletions seen in this study was surprising in light of the report by Johnson and coworkers[12] in which MK-801 protected against decreases in TPH activity induced by MA but not MDMA 20 h after the last injection. If both TPH activity and tissue 5-HT concentration are accurate predictors of serotonergic toxicity, the critical difference between these two studies may be the time point at which TPH activity (18–20 h) and 5-HT concentration (72 h) were measured. Time course studies measuring both TPH activity and 5-HT concentration would help clarify this discrepancy.

Single injections of PCA caused dose-related depletions of 5-HT in hippocampus, while only the high dose (10.0 mg/kg) depleted 5-HT in striatum. Co-administration of MK-801 (2.5 mg/kg × one or two injections) appeared to provide an attenuation of the 5-HT depletion at each dose and region tested, and a lower dose of MK-801 (1.0 mg/kg) provided similar effects (data not shown). However, this attenuation was statistically significant for only one dose combination, MK (2.5 mg/kg × 1 inj)/PCA (10.0 mg/kg). In this case the data were pooled from two separate experiments which increased the sample size. (Sample n's were 12, 14, 22 and 20 for SAL/SAL, MK/SAL, SAL/PCA and MK/PCA, respectively. Sample sizes in other experiments ranged from 7–10/group. There was no significant difference between the values obtained in the first experiment and the replication.) These data suggest that MK-801 does provide protection against PCA-induced 5-HT depletions, since the effect is replicable and statistically significant with a large sample.

The effects of MA, MDMA, PCA and MK-801 on 5-HIAA were similar to the effects on 5-HT in all experiments. The 5-HIAA data are consistent with the hypothesis that MA, MDMA and PCA engender degeneration of serotonergic nerve terminals and co-administration of MK-801 protects against this terminal degeneration. MA-induced depletion of striatal DA and protection by co-administration of MK-801 are consistent with reports published previously in mice[37,38], and this study extends those findings to another species.

The different dosing regimens used in this study and the different toxic potencies make it difficult to com-

pare the effects across drugs, but the data suggest that the ability of MK-801 to protect against 5-HT depletions varies across drugs. The dosing regimens used in this study were chosen based on reports of neurotoxic regimens in the literature[1,33,37], and we selected doses which gave maximal 5-HT depletions with minimal mortality rates. Two injections of MK-801 (2.5 mg/kg) completely attenuated the effects of MA on 5-HT, while one or two injections of MK-801 only partially protected against MDMA-induced 5-HT depletions. Furthermore, co-administration of MK-801 (one or two injections) with MDMA increased hippocampal 5-HT approximately 62–78% over MDMA-induced depletion, while co-administration of MK-801 (one or two injections) with PCA increased 5-HT 25–66% over PCA-induced depletions. Sabol et al. have recently reported that MK-801 does not protect against 5-HT depletions engendered by another halogen-substituted amphetamine, DL-fenfluramine, and in fact appears to enhance the depletions[25]. We cannot draw firm conclusions from this study about the relative efficacy of MK-801 in protecting against 5-HT depletions from different substituted amphetamines, but our results suggest that this efficacy varies with the ring- and side-chain substitutions. Systematic dose–response and time-course studies are warranted to further characterize these effects.

The mechanism by which MK-801 protects against neurotoxicity engendered by substituted amphetamines is still in question, although it appears that MK-801 must be administered early in the injection regimen to have an effect. Sonsalla et al.[38] showed that a single injection of MK-801 given to mice at time 0 or 1 h concomitant with four MA injections spaced 2 h apart partially protects against dopaminergic toxicity, while MK-801 given at hour 3, 5, or 7 does not protect against neurotoxicity. Since the elimination half-life of MK-801 from brain and plasma is approximately 2 h[41], MK-801 given at time 0 or 1 h was virtually eliminated by the time of the last injection. MK-801 given at hour 3, 5, or 7 is in higher concentration at the time of the last injection but is not present for the first two injections, indicating that it is necessary to administer MK-801 within 1 h of the first MA injection for protection against dopaminergic toxicity due to MA, and presumably, serotonergic toxicity as well.

Evidence indicates that release of synaptic DA is necessary to engender serotonergic toxicity[1,10,13,14,32,39], and both DA and 5-HT are released after administration of MA, MDMA and PCA[29,31,34]. Since MK-801 must be administered within 1 h of MA injection for neuroprotection, it has been suggested[5,15,43] that MK-801 might attenuate or block release of DA, thereby protecting against dopaminergic (and serotonergic) toxicity. Unfortunately, the data which support this hypothesis are conflicting. Marshall and coworkers[43] have shown that while MK-801 alone has no effect on striatal DA release, MK-801 significantly attenuates MA-induced DA release. Kashihara et al., however, have demonstrated that MK-801 alone significantly attenuates striatal DA release in a dose-dependent manner[15], while having no effect on MA-induced DA release[16]. Bowyer et al. have also shown that MK-801 does not attenuate DA release due to MA, but instead show that MK-801 attenuates DA release due to glutamate stimulation[5] and suggest this effect may underlie neuroprotection by MK-801. Clearly, further work needs to be done to clarify the effect of MK-801 on DA release, and its relationship to the mediation of dopaminergic and serotonergic toxicity.

In summary, administration of MK-801 with MA, MDMA or PCA protects against depletion of 5-HT in hippocampus and striatum. The protection against serotonergic toxicity may be a phenomenon which is linked to the effect of MK-801 on DA release, and more data is needed to elucidate the mechanism of this effect of MK-801.

Acknowledgements. This research was supported by Grant DA-00085 from the National Institute on Drug Abuse. L.S.S. is a recipient of Research Scientist Award MH-10562.

REFERENCES

1 Axt, K.J., Commins, D.L., Vosmer, G. and Seiden, L.S., Alpha-methyl-*p*-tyrosine pretreatment partially prevents methamphetamine-induced endogenous neurotoxin formation, *Brain Res.*, 515 (1990) 269–276.

2 Bakhit, C. and Gibb, J.W., Methamphetamine-induced depression of tryptophan hydroxylase: Recovery following acute treatment, *Eur. J. Pharmacol.*, 76 (1981) 229–233.

3 Battaglia, G., Sharkey, J., Kuhar, M.J. and DeSouza, E.B., Neuroanatomic specificity and time course of alterations in rat brain serotonergic pathways induced by MDMA (3,4-methylenedioxymethamphetamine): assessment using quantitative autoradiography, *Synapse*, 8 (1991) 249–260.

4 Battaglia, G., Yeh, S.Y. and De Souza, E.B., MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons, *Pharm. Biochem. Behav.*, 29 (1988) 269–274.

5 Bowyer, J.F., Scallet, A.C., Holson, R.R., Lipe, G.W., Slikker, W. and Ali, S.F., Interactions of MK-801 with glutamate-, glutamine- and methamphetamine-evoked release of [^{3}H]dopamine from striatal slices, *J. Pharm. Exp. Ther.*, 257 (1991) 262–270.

6 Commins, D.L., Vosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R. and Seiden, L.S., Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain, *J. Pharmacol. Exp. Ther.*, 241 (1987) 338.

7 Fuller, R.W. and Snoddy, H.D., Long-term effects of 4-chloroamphetamine on brain 5-hydroxyindole metabolism in rats, *Neuropharmacology*, 13 (1974) 85.

8 Harvey, J.A. and McMaster, S.E., Neurotoxic action of *p*-chloroamphetamine in the rat as revealed by Nissl and silver stains, *Psychopharmacol. Bull.*, 12 (1976) 62–64.

9 Hiramatsu, M. and Cho, A.K., Enantiomeric differences in the effects of 3,4-methylenedioxymethamphetamine on extracellular monoamines and metabolites in the striatum of freely-moving rats: an in vivo microdialysis study, *Neuropharmacology*, 29 (1990) 269–275.

10 Hotchkiss, A.J. and Gibb, J.W., Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain, *J. Pharmacol. Exp. Ther.*, 214 (1980) 257.

11 Hotchkiss, A.J., Morgan, M.E. and Gibb, J.W., The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities, *Life Sci.*, 25 (1979) 1373.

12 Johnson, M., Hanson, G.R. and Gibb, J.W., Effect of MK-801 on the decrease in tryptophan hydroxylase induced by methamphetamine and its methylenedioxy analog, *Eur. J. Pharmacol.*, 165 (1989) 315–318.

13 Johnson, M., Stone, D., Hanson, G.R. and Gibb, J.W., Role of the dopaminergic nigrostriatal pathway in methamphetamine-induced depression of the neostriatal serotonergic system, *Eur. J. Pharmacol.*, 135 (1987) 231–234.

14 Johnson, M.P. and Nichols, D.E., Combined administration of a non-neurotoxic 3,4-methylenedioxymethamphetamine analogue with amphetamine produces serotonin neurotoxicity in rats, *Neuropharmacology*, 30 (1991) 819–822.

15 Kashihara, K., Hamamura, T., Okumura, K. and Otsuki, S., Effect of MK-801 on endogenous dopamine release in vivo, *Brain Res.*, 528 (1990) 80–82.

16 Kashihara, K., Okumura, K., Onishi, M. and Otsuki, S., MK-801 fails to modify the effect of methamphetamine on dopamine release in the rat striatum, *NeuroRep.*, 2 (1991) 236–238.

17 Kirk, R.E., *Experimental Design. Procedures for the Behavioral Sciences*, Brooks/Cole Publishing Co., 1982.

18 Kotake, C., Heffner, T., Vosmer, G. and Seiden, L., Determination of dopamine, norepinephrine, serotonin and their major metabolic products in rat brain by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection, *Pharm. Biochem. Behav.*, 22 (1985) 85–89.

19 Kovavich, G.B., Aronson, C.E. and Brunswick, D.J., Effects of high-dose methamphetamine administration on serotonin uptake sites in rat brain measured using [^{3}H]cyanoimipramine autoradiography, *Brain Res.*, 505 (1989) 123–129.

20 Mamounas, L.A. and Molliver, M.E., Evidence for dual serotonergic projections to neocortex: axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin *p*-chloroamphetamine, *Exp. Neurol.*, 102 (1988) 23–36.

21 Massari, V.J., Tizabi, Y. and Sanders-Bush, E., Evaluation of the neurotoxic effects of *p*-chloroamphetamine: a histological and biochemcial study, *Neuropharmacology*, 17 (1978) 541–548.

22 O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J. and Molliver, M.E., Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonin axon terminals in forebrain: immunocytochemical evidence for neurotoxicity, *J. Neurosci.*, 8 (1988) 2788–2803.

23 Preston, K.L., Wagner, G.C., Schuster, C.R. and Seiden, L.S., Long-term effects of repeated methylamphetamine administration on monoamine neurons in the rhesus monkey brain, *Brain Res.*, 338 (1985) 243–248.

24 Ricaurte, G.A., Schuster, C.R. and Seiden, L.S., Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study, *Brain Res.*, 193 (1980) 153–163.

25 Sabol, K.E., Richards, J.B. and Seiden, L.S., The NMDA receptor antagonist MK-801 does not protect against serotonin depletions caused by high doses of DL-fenfluramine, *Brain Res.*, 582 (1992) 129–133.

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

27 Schmidt, C.J., Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 240 (1987) 1.

28 Schmidt, C.J., Black, C.K. and Taylor, V.L., Antagonism of the neurotoxicity due to a single administration of methylenedioxymethamphetamine, *Eur. J. Pharmacol.*, 181 (1990) 59–70.

29 Schmidt, C.J., Levin, J.A. and Lovenberg, W., In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, *Biochem. Pharmacol.*, 36 (1987) 747–755.

30 Schmidt, C.J., Ritter, J.K., Sonsalla, P.K., Hanson, G.R. and Gibb, J.W., Role of dopamine in the neurotoxic effects of methamphetamine, *J. Pharm. Exp. Ther.*, 233 (1985) 539–544.

31 Schmidt, C.J. and Taylor, V., Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons, *Eur. J. Pharmacol.*, 156 (1988) 121–131.

32 Schmidt, C.J., Taylor, V.L., Abbate, G.M. and Nieduzak, T.R., 5-HT2 Antagonists stereoselectively prevent the neurotoxicity of 3,4-methylenedioxymethamphetamine by blocking the acute stimulation of dopamine synthesis: Reversal by L-dopa, *J. Pharm. Exp. Ther.*, 256 (1991) 230–235.

33 Schmidt, C.J., Wu, L. and Lovenberg, W., Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue, *Eur. J. Pharmacol.*, 124 (1986) 175.

34 Seiden, L.S. and Ricaurte, G.A., Neurotoxicity of methamphetamine and related drugs, In H.Y. Meltzer (Eds.), *Psychopharmacology: The Third Generation of Progress*, Raven, New York, 1987.

35 Sekerke, H.J., Smith, H.E., Bushing, J.A. and Sanders-Bush, E., Correlation between brain levels and biochemical effects of the optical isomers of *p*-chloroamphetamine, *J. Pharm. Exp. Ther.*, 193 (1975) 835–844.

36 Sonsalla, P.K., Gibb, J.W. and Hanson, G.R., Roles of D1 and D2 dopamine receptor subtypes in mediating the methamphetamine-induced changes in monoamine systems, *J. Pharm. Exp. Ther.*, 238 (1986) 932–937.

37 Sonsalla, P.K., Nicklas, W.J. and Heikkila, R.E., Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity, *Science*, 243 (1989) 398–400.

38 Sonsalla, P.K., Riordan, D.E. and Heikkila, R.E., Competitive and noncompetitive antagonists at *N*-methyl-D-aspartate receptors protect against methamphetamine-induced dopaminergic damage in mice, *J. Pharm. Exp. Ther.*, 256 (1991) 506–511.

39 Stone, D.M., Johnson, M., Hanson, G.R. and Gibb, J.W., Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine, *J. Pharm. Exp. Ther.*, 247 (1988) 79–87.

40 Stone, D.M., Stahl, D.C., Hanson, G.R. and Gibb, J.W., The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain, *Eur. J. Pharmacol.*, 128 (1986) 41.

41 Vezzani, A., Serafini, R., Stasi, M.A., Caccia, S., Conti, I., Tridico, R.V. and Samanin, R., Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity in rats, *J. Pharm. Exp. Ther.*, 249 (1989) 278–283.

42 Wagner, J. and Peroutka, S.J., Neurochemistry and neurotoxicity of substituted amphetamines, *Neuropsychopharmacology*, 3 (1990) 219–220.

43 Weihmuller, F.B., O'Dell, S.J., Cole, B.N. and Marshall, J.F., MK-801 attenuates the dopamine-releasing but not the behavioral effects of methamphetamine: an in vivo microdialysis study, *Brain Res.*, 549 (1991) 230–235.

44 Wilson, M.A., Ricaurte, G.A. and Molliver, M.E., Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine, *Neurosci.*, 28 (1989) 121–137.

45 Woolverton, W.L., Ricaurte, G.A., Forno, L.S. and Seiden, L.S., Long-term effects of chronic methamphetamine administration in rhesus monkeys, *Brain Res.*, 486 (1989) 73–78.