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Serotonergic Deficits and Impaired Passive-Avoidance Learning in Rats by MDEA: A Comparison With MDMA

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BARRIONUEVO, M., N. AGUIRRE, J. DEL RÍO AND B. LASHERAS. *Serotonergic deficits and impaired passive-avoidance learning in rats by MDEA: A comparison with MDMA.* PHARMACOL BIOCHEM BEHAV **65**(2) 233–240, 2000.—The serotonergic deficits induced by 3,4-methylenedioxyethamphetamine (MDEA, "eve"), were examined and compared with 3,4 methylenedioxymethamphetamine (MDMA, "ecstasy"). A single dose of MDEA (10, 20, or 40 mg/kg IP) induced a dose-related hyperthermia, but only the highest dose significantly reduced 5-HT content and 5-HT transporter density in the frontal cortex and in the hippocampus 7 days later. Long-term serotonergic deficits were much more marked when MDEA was given repeatedly (40 mg/kg IP., b.i.d., for 4 consecutive days). Single or repeated administration of MDEA induced no change on 5-HT$_{1A}$ receptor density in the frontal cortex, brain stem, or hippocampus, although 3 h after both treatments plasma corticosterone levels were significantly increased. MDEA (5–20 mg/kg, IP) produced significant retention deficits in a passive-avoidance learning task. Conversely, 7 days after the repeated administration of MDEA (40 mg/kg b.i.d., for 4 consecutive days) no effect on passive-avoidance performance was observed unless rats were treated again with another dose of MDEA (20 mg/kg IP) 30 min before the training trial. The 5-HT$_{1A}$ receptor antagonist, WAY 100635, prevented the impairment in retention performance induced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), but not by MDEA or MDMA, indicating that the effect of these amphetamine derivates was not mediated by 5-HT$_{1A}$ receptor activation. The results suggest the risk of serotonergic dysfunction associated with MDEA abuse in humans. © 2000 Elsevier Science Inc.

3,4-Methylenedioxyethamphetamine (MDEA) 5-Hydroxytryptamine (serotonin, 5-HT) 5-HT transporter
5-HT$_{1A}$ receptor Neurotoxicity Corticosterone Learning Memory

THE risk of adverse reactions to 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") is widely known [see reviews in (13,17)]. However, tablets or capsules sold as "ecstasy" are not always what they purport to be. Frequently, these "ecstasy" tablets also contain 3,4-methylenedioxyethamphetamine (MDEA "eve") (40), which has made the examination of its biological effects of considerable interest. Subjectively, the effects and pharmacological actions of MDEA are reported to be similar, but not identical, to those of MDMA (17). The massive and prolonged stimulation of 5-hydroxytryptamine (5-HT) release and the increased dopaminergic activity appears to be responsible for the acute psychosomimetic and psychostimulant effects of these drugs (6). The severity of this acute systemic response appears to be closely related to the severity of the long-term damage to 5-HT axon terminals caused by MDMA administration, and probably other substituted amphetamines [reviewed by (17,36)]. However, at variance with MDMA, studies about the pharmacological and toxic effects of MDEA are scarce.

Several studies have shown that repeated doses of MDEA (10–40 mg/kg SC) induce a selective and long-lasting 5-HTergic depletion in the rat brain (29,37). Ricaurte et al. (28,29) observed a dose-related reduction in the hippocampal 5-HT content, reaching a 50% decrease 2 weeks after MDEA (40 mg/kg SC) given twice daily for 4 consecutive days. Other authors (37) found a 30% reduction of hippocampal 5-HT content 2 weeks after 5 doses of MDEA (10 mg/kg SC). Immunocytochemical evidence for neurotoxicity comes from studies

in which the repeated administration of MDEA (40 mg/kg IP) caused morphologic changes highly suggestive of structural damage as well as the loss of 5-HT immunoreactive axons (35). On the contrary, MDEA or MDMA appear to induce no toxic effects on the dopaminergic system of the rat because no long-term reductions are observed in the hypothalamic and neostriatal concentrations of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) (2,37). It is also well known that MDMA-induced toxicity is markedly affected by ambient temperature, and a correlation appears to exist between hyperthermia and neurotoxicity engendered by MDMA and other amphetamines (18). Furthermore, hyperthermia is one of the most common and fatal side effects associated with MDEA or MDMA use in humans (13,15,38).

We had previously found that MDMA was able to increase $5-HT_{1A}$ receptor density in the frontal cortex, while $5-HT_{1A}$ receptors were downregulated in the hippocampus and brainstem of the rat (1,3). The latter effect appeared to be mediated, at least in the hippocampus, by an increased release of corticosterone induced by MDMA (3). It has been shown, on the other hand, that another serotonergic neurotoxin, p-chloramphetamine (PCA), which produces a massive acute release of 5-HT followed by long-term depletion, impairs passive-avoidance tests in rats (31). The authors indicated that these alterations in the mnemonic processes were due to the acute release of 5-HT and not to the long-term 5-HTergic neurotoxicity.

In the present work, we compared the toxic effects of single or repeated doses of MDEA (40 mg/kg IP) with those induced by MDMA (20 mg/kg IP), a dose of the latter drug widely used to cause long-lasting neurotoxicity in rats. In this sense, 5-HT content and [³H]paroxetine binding in the frontal cortex and hippocampus of the rat were studied as markers of toxicity. We also examined the acute effect of increasing doses of MDEA (10, 20, and 40 mg/kg IP) on body temperature, which were compared to the hyperthermic effect of MDMA (20 mg/kg IP). It also appeared of interest to determine whether the effects of single or repeated administrations of MDEA on plasma corticosterone levels and $5-HT_{1A}$ receptor density in the hippocampus, raphe nuclei, and frontal cortex of the rat were comparable to those previously described for MDMA. The acute and long-term effect of MDEA on learning in a passive-avoidance task after single or repeated administration of the drug was also studied. As MDEA and MDMA are potent 5-HT releasers (33), and it has been shown that postsynaptic $5-HT_{1A}$ receptor stimulation interferes with learning processes not only in passive-avoidance procedures (22) but also in other learning tasks (39), 5-HT released by these drugs could be stimulating postsynaptic $5-HT_{1A}$ receptors. Accordingly, we additionally studied the eventual role of $5-HT_{1A}$ receptors in the effects of MDEA or MDMA on passive-avoidance learning.

METHOD

Drugs

The source of the drugs was as follows: 3,4-methylenedioxyethamphetamine-HCl (MDEA-HCl) and 3,4-methylenedioxymethamphetamine-HCl (MDMA-HCl) were a generous gift from the "Servicio de Restricción de Estupefacientes" (Dr. L. Domínguez, Madrid, Spain); [³H] 8-hydroxy-2-(di-n-propylamino)tetralin ([³H] 8-OH-DPAT) (148.5 Ci/mmol) and [³H] paroxetine (22.5 Ci/mmol) were obtained from New England Nuclear (Boston, MA); buspirone-HCl was donated by Eli-Lilly and Co. (Indianapolis, IN); 8-OH-DPAT-HBr

was obtained from R.B.I. (Natick, MA); serotonin creatinine sulfate and p-chloroamphetamine-HCl (PCA) were from Sigma Chemical (St. Louis, MO); WAY 100635 was a gift from Wyeth Labs. (Princeton, NJ); all other chemicals were from Merck (Darmstadt, Germany).

Animals and Treatments

Male Wistar rats (200–250 g) were housed in plastic cages (three to four per cage) in a temperature-controlled room (22 ± 2°C) with free access to food and water, and maintained on a 12L:12D cycle (lights on at 0700 h).

Rats received a single or repeated injections of MDEA (40 mg/kg IP, b.i.d. for 4 consecutive days) or MDMA (20 mg/kg IP), and were killed 7 days later. In all cases, the doses of MDEA and MDMA refer to the hydrochloride. Rats were killed by decapitation, and their brains were removed rapidly and placed on ice. The appropriate brain regions were then frozen on dry ice and stored at −80°C until chromatographic and binding studies were performed.

All procedures for the treatment of these animals were in compliance with the European Community Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Ethical Committee of the University of Navarra.

Temperature Measurements

The rectal temperature of the rats was measured with a lubricated digital thermometer probe (pb 0331, Panlab, Barcelona) inserted 4 cm into the rectum and maintained until the temperature stabilized. Readings were taken 15 min before the administration of MDEA, MDMA or saline and 30, 60, and 90 min thereafter.

Determination of Brain 5-HT Concentration

The concentration of 5-HT in the different brain regions of the rat were determined by high-performance liquid chromatography with electrochemical detection as previously described.

[³H] Paroxetine Binding

[³H] Paroxetine binding studies were performed as described (19), with minor modifications. The tissue was homogenized in 15 ml of ice-cold buffer (Tris-HCl 50 mM, 120 mM NaCl, 5 mM KCl, pH 7.4) and centrifuged at $48,000 \times g$ for 10 min at 4°C. The pellet was resuspended in buffer and incubated at 37°C for 10 min. After a second centrifugation in the same conditions, the resultant pellet was resuspended in buffer (1.5 mg tissue/400 µl buffer). The incubation mixture contained 400 µl of tissue suspension, 200 µl of increasing concentrations of [³H] paroxetine (0.02–2 nM) and 1.4 ml of incubation buffer in the absence and presence of fluoxetine 10 µM. Tubes were incubated for 60 min at 22°C. After rapid filtering through GF/C Whatman filters, the filters were rinsed with 4×5 ml of ice-cold buffer and placed in vials containing 4 ml of liquid scintillation cocktail (Biogreen3, Scharlau). All determinations were carried out in duplicate. Data were subjected to Scatchard analysis to determine the number of binding sites B_{max}:fmol/mg of protein) and the dissociation constant (K_d:nM).

[³H]8-OH-DPAT Binding

[³H] 8-OH-DPAT binding studies were carried out according to a previously described procedure (12), with minor mod-

ifications. Briefly, the brain regions studied were homogenized in ice-cold buffer Tris-HCl 50 mM (pH 7.7) and centrifuged at 49,000 × g for 15 min at 4°C. The pellet was resuspended in the same buffer and incubated at 37°C for 15 min. After a second centrifugation under the same conditions, the resultant pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.7) containing $CaCl_2$ 4 mM at a final tissue concentration of approximately 20 mg/ml (wet tissue weight). The incubation mixture contained 100 μl of tissue suspension, 50 μl of six increasing concentrations of the labeled ligand (0.1–4 nM) and 50 μl of incubation buffer with or without buspirone 10 μM. Tubes were incubated for 15 min at 37°C. Filtration of incubation mixture and data analysis were performed as described for [³H] paroxetine binding studies.

Plasma Corticosterone Levels

Trunk blood was collected into heparinized tubes. After centrifugation at 2000 × g for 10 min, aliquots (100 μl) of plasma were stored at −40°C until assay. Plasma corticosterone levels were determined with a radioimmunoassay kit (rat corticosterone [¹²⁵I] assay system, Amersham). Duplicate determinations were made for each animal.

Passive Avoidance Learning

A two-compartment passive avoidance apparatus was used (4). The apparatus consisted of an illuminated white compartment (42 × 44 × 46 cm) and a dark compartment (15 × 15 × 30 cm), both the white and black areas being equipped with a grid floor (Letica, model 1516). The two compartments were separated by a guillotine door. The rat was placed in the illuminated area and 3 s later, the door was raised. For 90 s the animal explored the apparatus freely (habituation trial). Twelve minutes later, the rat was placed again in the illuminated chamber. When the rat entered the dark chamber, the guillotine door was closed, and after 10 s the animal was returned to its home cage. Sixty minutes later, the animal was placed again in the white compartment (acquisition trial). When the rat entered the dark chamber the guillotine door was closed again and after 10 s an inescapable 2-mA scrambled electrical foot shock was delivered for 3 s through the grid floor using a shock generator (Letica). A retention trial was given 24 h after the acquisition trial by placing the rat in the illuminated compartment and measuring the response latency to reenter the dark compartment using a cutoff time of 300 s.

MDEA, MDMA, and 8-OH-DPAT were administered 30 min before the acquisition trial. The 5-HT$_{1A}$ receptor antagonist N-[2-[4-(2-methoxyphenil)-1-piperazinyl]ethyl]-N-(2-pyridinyl) ciclohexanecarboxamide trihydrochloride (WAY 100635) was given 15 min before MDEA, MDMA, or 8-OH-DPAT. In another series of experiments rats were repeatedly treated with MDEA (40 mg/kg IP, b.i.d. for 4 consecutive days), and 7 days after the last injection rats were treated again with saline or MDEA (20 mg/kg IP) to assess whether the acute or long-term effects of MDEA were related or not to 5-HT depletion.

Statistics

Analyses of the differences between multiple treatment groups consisted of ANOVA followed by Tukey post hoc test. For the rectal temperature analysis, two-way ANOVA for repeated measures was used to compare treatment groups. Single time-point comparisons between groups were made using Tukey's test. Significant differences were defined at $p < 0.05$.

RESULTS

MDEA and MDMA-Induced Hyperthermia

Two-way ANOVA revealed a significant treatment effect, $F(4, 3) = 4.311$, $p < 0.01$. Single time-point comparisons indicated that all doses of MDEA (10, 20, and 40 mg/kg) as well as MDMA (20 mg/kg) produced a marked hyperthermia 30 and 60 min after injection. MDMA and the two higher doses of MDEA induced significant increases in body temperature of the rats that lasted beyond 90 min postinjection, $F(4, 23) = 13.481$, $p < 0.05$ (Fig. 1). The highest increase in body temperature induced by MDMA and MDEA (40 mg/kg) was 2.37 and 1.85°C, 60 and 30 min postinjection, respectively.

Effect of MDEA and MDMA on 5-HT Levels and 5-HT Transporter Density in the Frontal Cortex and in the Hippocampus of the Rat

No change in 5-HT content was found 7 days after a single dose of MDEA (10 or 20 mg/kg IP) (not shown). A slight but significant decrease of 5-HT content in the frontal cortex, $F(2, 15) = 15.531$, $p < 0.05$, and in the hippocampus, $F(2, 15) = 23.293$, $p < 0.05$, was found 7 days after a single injection of MDEA (40 mg/kg IP) or MDMA (20 mg/kg IP). The effect after repeated injections of MDEA or MDMA (40 or 20 mg/kg IP, b.i.d. for 4 consecutive days) was much more marked. In both brain regions, 5-HT content was reduced by 35–40% or by 50–55% 7 days after the last injection of MDEA or MDMA, respectively. The results of both acute and repeated treatments are shown in Fig. 2.
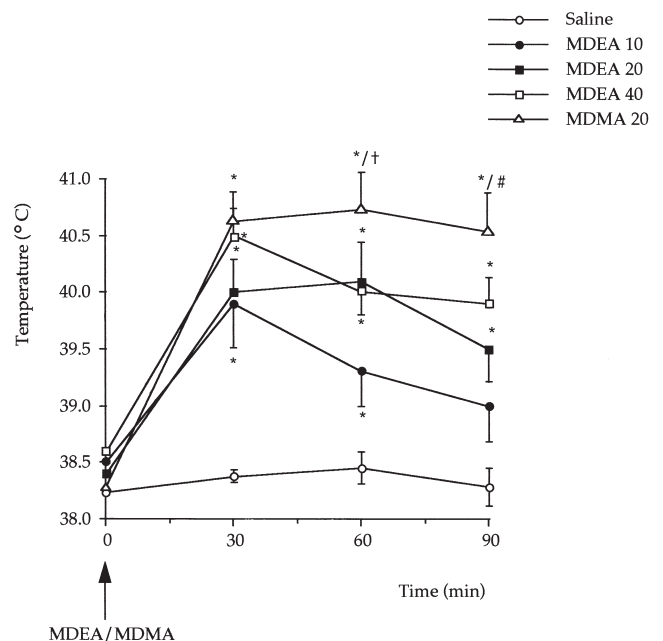

FIG. 1. Rectal temperature of rats 30, 60, and 90 min after the administration of different doses of MDEA (10, 20, and 40 mg/kg IP) or MDMA (20 mg/kg IP). Data are means ± SEM from five to six rats. *$p < 0.05$ vs. control group. †$p < 0.05$ vs. MDEA (10 mg/kg IP). #$p < 0.05$ vs. MDEA (10–20 mg/kg IP).
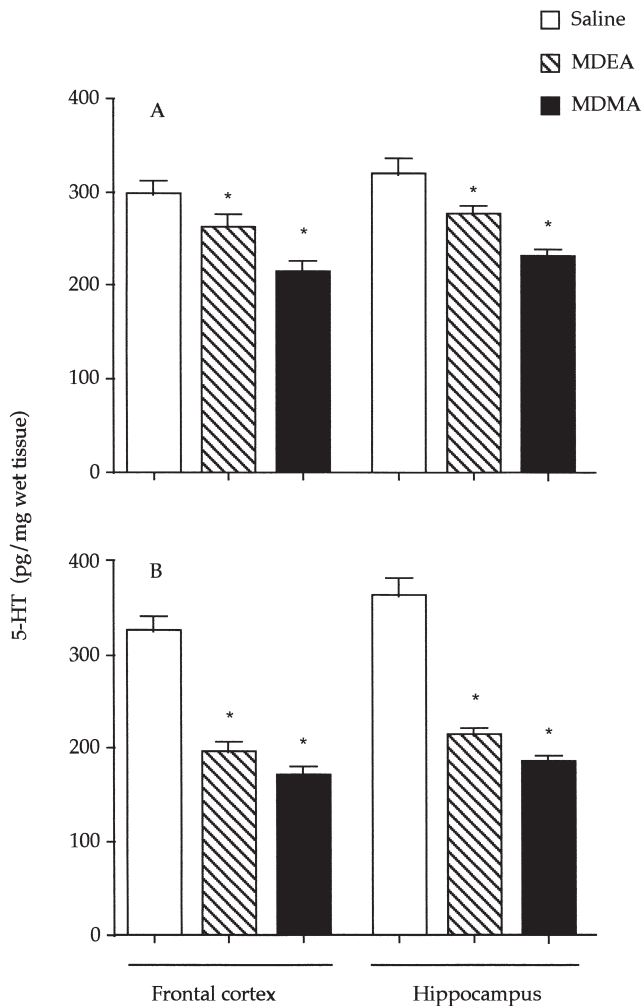
FIG. 2. Effect of (A) single MDEA (40 mg/kg, IP) or MDMA (20 mg/kg, IP) and (B) repeated injections of MDEA or MDMA (40 or 20 mg/kg IP, b.i.d., for 4 consecutive days) on 5-HT levels in the frontal cortex and hippocampus of the rat. Animals were killed 7 days after the last MDEA or MDMA injection. Values are means ± SEM from five to seven rats. *$p < 0.05$ vs. control group.
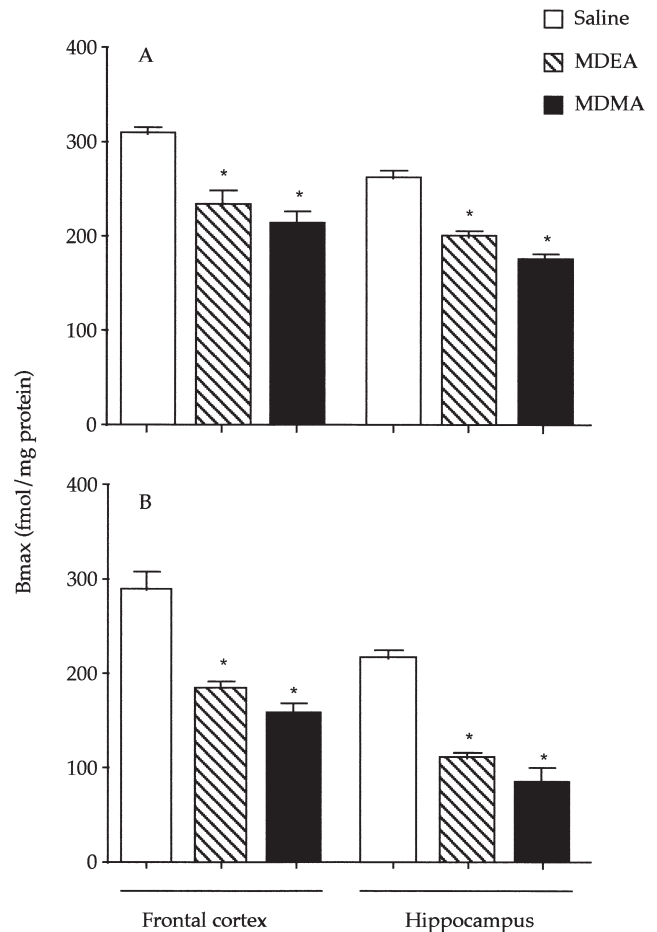

FIG. 3. Effect of (A) single MDEA (40 mg/kg, IP) or MDMA (20 mg/kg, IP) and (B) repeated injections of MDEA or MDMA (40 or 20 mg/kg IP, b.i.d., for 4 consecutive days) on 5-HT transporter density (B_{max}) in the frontal cortex and hippocampus of the rat. Animals were killed 7 days after the last MDEA or MDMA injection. Values are means ± SEM from five to seven rats. *$p < 0.05$ vs. control.

In an analogous fashion, a single administration of MDEA or MDMA (same doses as above) significantly decreased 7 days later 5-HT transporter density in the frontal cortex, $F_{(2, 15)} = 23.857$, $p < 0.05$, and in the hippocampus, $F_{(2, 15)} = 31.53$, $p < 0.05$, of the rat. Again, repeated drug exposure caused a more marked effect in both brain regions. Thus, MDEA and MDMA reduced 5-HT transporter density by 38 and 55% in the frontal cortex, $F_{(2, 15)} = 65.097$, $p < 0.05$, and by 49 and 60% in the hippocampus, $F_{(2, 15)} = 144.15$, $p < 0.05$, respectively (Fig. 3).

5-HT$_{1A}$ Receptor Density in the Frontal Cortex and in the Hippocampus

[^{3}H] 8-OH-DPAT binding to 5-HT$_{1A}$ receptors 1 week after either acute or repeated MDEA treatment was not significantly changed in the frontal cortex, hippocampus, or brainstem (Fig. 4). However, a tendency to an increase in the frontal cortex, as well as a slight nonsignficant decrease in the hippocampus was observed. This tendency was more marked after the repeated MDEA administration.

Plasma Corticosterone Levels

Three hours after single or repeated administration of MDEA there was a three- to fourfold increase in plasma corticosterone levels, $F_{(2, 14)} = 10.2499$, $p < 0.05$. However, 7 days after the last dose of MDEA, plasma corticosterone levels had returned to control values, $F_{(2, 14)} = 1.0564$, $p = 0.3779$ (Table 1).

Passive-Avoidance Learning

Experiments carried out with increasing doses of MDEA (2.5–20 mg/kg) given 30 min before the acquisition trial showed a dose-dependent impairment in passive-avoidance learning. A significant treatment effect was found, $F_{(4, 38)} = 23.1$, $p < 0.05$ (Fig. 5).
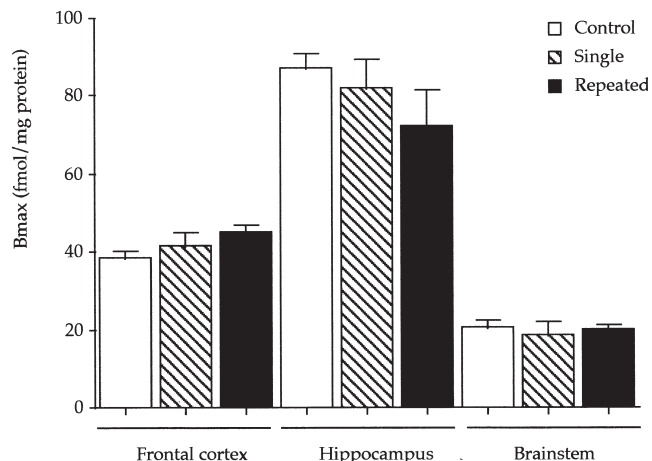
FIG. 4. Effect of MDEA administration on 5-HT$_{1A}$ receptor density in several brain regions of the rat. Treatments are detailed in the legend to Fig. 2. Animals were killed 7 days after the last MDEA injection. Data are means ± SEM from five to seven rats.
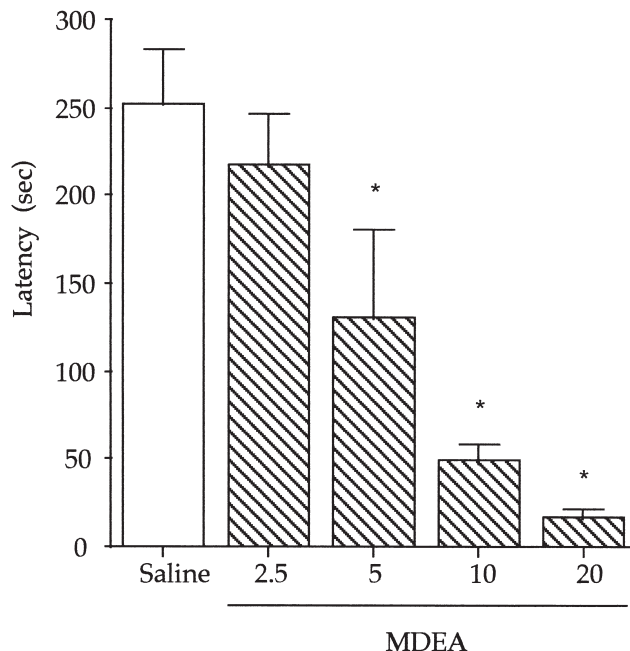

FIG. 5. Dose-related effect of MDEA on passive-avoidance performance in rats. MDEA (doses in mg/kg IP) was given 30 min before the training test, and rats were tested for retention 24 h later. Values are means ± SEM of 8–10 animals. *$p < 0.05$ vs. saline-treated group using one-way ANOVA followed by Tukey's test.

When rats received repeated injections of saline or MDEA (40 mg/kg, b.i.d. for 4 consecutive days) and were treated again with saline or MDEA (20 mg/kg IP) 30 min before the acquisition session, no effect on passive-avoidance performance was found in rats that had been repeatedly treated with MDEA and received 1 week later a pretraining injection of saline. In the rats pretreated with MDEA, a new pretraining injection of MDEA produced, however, a significant reduction in retention latency 24 h later (Fig. 6).

The compared effects of MDEA (20 mg/kg IP), MDMA (10 mg/kg IP) and the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (0.5 mg/kg SC) on passive-avoidance learning are shown in Fig. 6. All of these drugs significantly reduced, $F(7, 72) = 24.333$, $p < 0.05$, retention latency of rats 24 h after the training test, the effect being more evident for both amphetamines. WAY 100635 (1 mg/kg SC), given 45 min before the acquisition trial, had no effect on passive-avoidance learn-

ing by itself, but completely prevented 8-OH-DPAT–induced impairment. The 5-HT$_{1A}$ receptor antagonist, however, was unable to reverse the impairing effect of MDEA or MDMA on the acquisition process (Fig. 7).

DISCUSSION

In the present experiments, single or repeated administrations of MDEA or MDMA caused significant reductions in 5-HT levels and 5-HT transporter density in the frontal cortex and in the hippocampus of the rat 1 week later. Both the single and repeated MDEA treatments used in the present study have been reported to produce a long-term reduction in brain 5-HT content, a decrease in the activity of tryptophan hydroxylase, and a degeneration of fine 5-HT terminals projecting from the dorsal raphe nucleus to different brain areas of the rat (29,35,37); however, to our knowledge, long-term reductions in [^{3}H]paroxetine binding after MDEA administration to rats had not been reported before. According to the studies above and to our own results, it appears that MDEA causes a neurodegenerative pattern similar to MDMA in rats. However, when compared to MDMA, MDEA was approximately half as potent in producing long-term 5-HTergic deficits. These data are in line with those of Ricaurte et al. (29), who found that two- to threefold higher doses of MDEA were required to produce a long-term 5-HT depletion comparable to that of MDMA. The precise mechanism by which MDMA and related drugs selectively damages 5-HT axon terminals remains unknown; however, increasing evidence suggests that MDMA generates reactive oxygen species as a result of its metabolism into catechols and reactive quinones (16), responsible for the formation of free radicals and final 5-HT terminal degeneration (11). The lower toxic potency of MDEA rel-

TABLE 1
ACUTE AND LONG-TERM EFFECTS OF MDEA ON PLASMA CORTICOSTERONE LEVELS OF THE RAT

Treatment	Corticosterone (μg/100 ml)
Saline (3 h)	7.72 ± 1.41
Single MDEA (3 h)	23.27 ± 1.57*
Repeated MDEA (3 h)	17.45 ± 3.68*
Saline (7 days)	3.69 ± 0.55
Single MDEA (7 days)	5.29 ± 1.02
Repeated MDEA (7 days)	4.64 ± 1.14

Rats were killed 3 h or 7 days after single (40 mg/kg, IP) or repeated (40 mg/kg, IP, b.i.d., for 4 consecutive days) MDEA administration. Data are means ± SEM of five rats.

*$p < 0.05$ vs. control group using one-way ANOVA followed by Tukey's test.
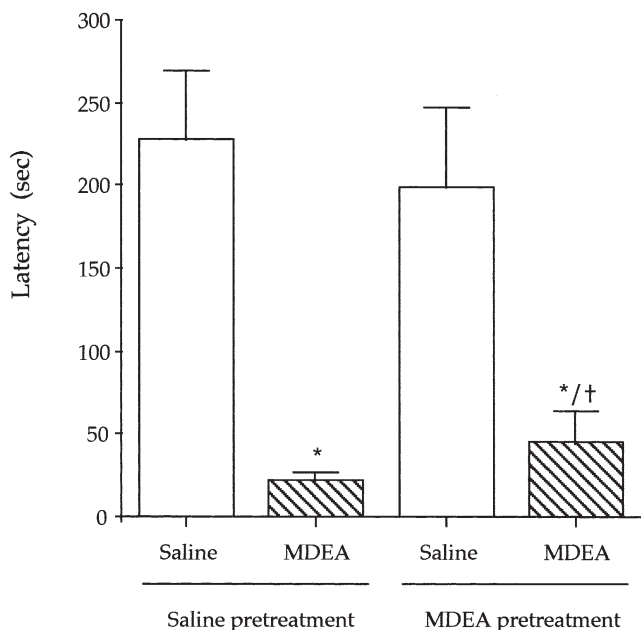
FIG. 6. Effect of repeated MDEA pretreatment on passive-avoidance performance in rats. Seven days after the last administration of saline or MDEA (40 mg/kg, IP, b.i.d., for 4 consecutive days) rats were given a new injection of either saline or MDEA (20 mg.kg, IP) 30 min before the training test. Rats were tested for retention 24 h later. Values are means ± SEM of 8 to 10 animals. $*p < 0.05$ vs. saline/saline; †p 0.05 vs. MDEA/saline.
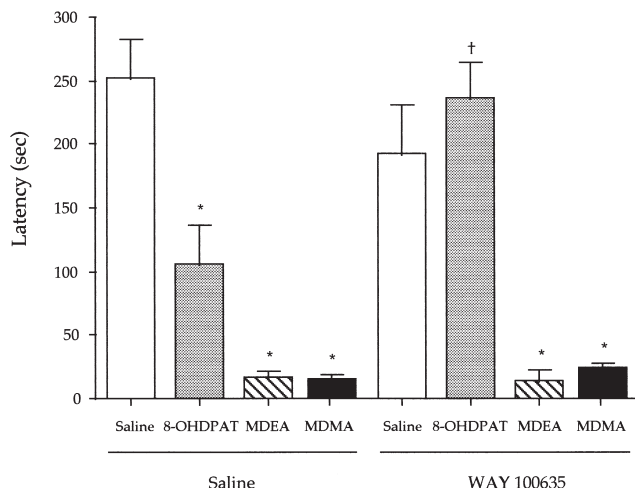

FIG. 7. Effect of pretraining administration of 8-OH-DPAT (0.5 mg/kg SC), MDEA (20 mg/kg, IP) or MDMA (10 mg/kg, IP) alone or in combination with WAY 100635 (1 mg/kg, SC) on passive-avoidance performance in rats. Retention was tested 24 h after the training session. Values are means ± SEM of 8–10 animals. Data were analyzed by one-way ANOVA followed by Tukey's test. $*p < 0.05$ vs. saline/saline. †$p < 0.05$ vs. 8-OH-DPAT/saline.

ative to MDMA might be related to the lower levels of the parent compound seen in brain tissues, as it has been reported that 1 and 4 h after the same dose of both drugs, brain MDEA levels are approximately 50% of the levels of MDMA (14). It has been also suggested that the large concentrations of extracellular dopamine that follows MDMA could be the source of reactive oxygen species responsible for 5-HT neurotoxicity due to its autoxidation or enzymatic breakdown within the serotonergic terminal [see (36) for review]. MDEA not only reaches the brain in a lower proportion than MDMA, but it is also a drug less potent than MDMA in releasing dopamine both "in vitro" (33) and "in vivo" (25). These differences between both drugs could account for their different toxic potency. On the other hand, it has been recently shown that MDMA-induced neurodegeneration occurs when endogenous free radical scavenging mechanisms become overwhelmed or exhausted, which is related to the dose and frequency of drug administration (26); this could explain why the neurotoxic effects caused by both drugs are more marked after the repeated treatments.

Hyperthermia induced by amphetamines is of clinical interest because it often accompanies deaths or complications associated not only to MDMA but also to MDEA use in humans (15,21,38). There is a large body of evidence indicating that the hyperthermia induced by MDMA and methamphetamine exacerbates neurotoxicity [e.g. (18)]. One common feature for different amphetamines, including MDEA, is their pronounced stimulant effect on 5-HT release from presynaptic nerve terminals (5,33). It has been proposed that the hyperthermic effect of MDMA, and probably other amphetamines, is due to the stimulation of $5-HT_{2A/C}$ receptors

mediated either by a direct action of MDMA or by the released 5-HT (24,34). As it could be expected, MDEA administration to rats resulted in a dose-related hyperthermia. Although this rise in body temperature was not as high as that induced by MDMA, the hyperthermic effect of MDEA lasted beyond the measured time period (90 min) for the two higher doses used (20 and 40 mg/kg). As in the case of MDMA (18), hyperthermia induced by MDEA may exacerbate its neurotoxicity. It is important to note, however, that hyperthermia does not by itself alter the neurochemical indices of serotonergic function (1).

We previously reported that repeated MDMA administration to rats induced opposite changes in central pre- and postsynaptic $5-HT_{1A}$ receptors (3). In the present study, MDEA only tended to slightly increase $5-HT_{1A}$ receptor density in the frontal cortex and to decrease receptor density in the hippocampus and in the brain stem; however, none of these differences reached statistical significance. Abundant evidence indicates, on the other hand, that 5-HT has stimulant effects on the rodent hypothalamic neurons that regulate pituitary–adrenocortical function and, reciprocally, corticosteroids exert multiple actions on the serotonergic system and on 5-HT receptors [e.g. (10)]. Plasma corticosterone levels were measured 3 h and 7 days after single and repeated MDEA treatments. In line with previous studies with MDMA (3,24), we found a significant elevation in plasma corticosterone levels 3 h but not 7 days after single or repeated doses of MDEA. It has been shown that MDMA-induced corticosterone secretion in rats is mediated by the stimulation of $5-HT_2$ receptors, probably through the released 5-HT (24). As in the case of hyperthermia, the increase in plasma corticosterone levels induced by MDEA (40 mg/kg IP) was lower than that found for MDMA (20 mg/kg IP) (3). Whether these differences between MDMA and MDEA are due to a lower potency of this drug in releasing 5-HT (33) is an issue that needs to be addressed.

Both clinical and preclinical findings suggest that alterations in serotonergic neurotransmission impair learning and memory (30). In line with these observations, memory deficits have been reported for "ecstasy" users, and have been correlated with the extent of "ecstasy" exposure (7,23,27). In the present study, the administration of increasing doses of MDEA (2.5–20 mg/kg) 30 min before the training test produced a dose-related impairment in passive-avoidance retention 24 h later. MDEA (10–20 mg/kg IP) caused comparable effects to those produced by MDMA or PCA. Conversely, repeated administration of MDEA (40 mg/kg IP, b.i.d., for 4 consecutive days) failed to alter retention 7 days later, although it caused a significant loss of cortical and hippocampal 5-HT content. However, if a new injection of MDEA was given to rats 7 days after the repeated drug treatment, passive-avoidance retention was again impaired. The present findings are consistent with those indicating that the long-term 5-HT depletion induced by amphetamines, such as MDMA or PCA, is not determinant of impaired performance in different learning and memory tasks (30,31). Memory impairment acutely induced by MDEA appears to be due to an excessive release of 5-HT (33), and not to depletion, as it happens with PCA (31).

It is well known that the administration of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, impairs passive avoidance retention in rats and induces different memory deficits in other cognitive tasks, which are apparently mediated by stimulation of postsynaptic 5-HT$_{1A}$ receptors (9,22,39). As mentioned above, the impairing effects of the amphetamines used in this study have been attributed to their ability for releasing 5-HT from the presynaptic terminal (31). It was then conceivable that the deficits in passive-avoidance retention induced by MDEA and MDMA could be mediated by postsynaptic 5-HT$_{1A}$ receptor activation. Pretraining administration of 8-OH-DPAT significantly impaired passive-avoidance retention in rats, an effect blocked by the 5-HT$_{1A}$ receptor antagonist WAY 100635, at a dose with no intrinsic effect on the learning task. WAY 100635, however, failed to modify the memory deficits induced by both amphetamines.

These data appear to indicate that 5-HT$_{1A}$ receptors do not mediate the deficits in passive-avoidance learning induced by MDEA or MDMA. From the results of the present study, it cannot be obviously excluded that other 5-HT receptor subtypes may be responsible for the results obtained. For example, some behavioral effects induced by MDMA have been attributed to 5-HT$_{1B}$ receptors (32), and it is known that stimulation of these receptors causes memory deficits in rats [see (8) for review]. Whether this or any other 5-HT receptor subtype is involved in the impairing effect of MDEA or MDMA requires further investigation.

In summary, the ring-substituted amphetamine MDEA induces effects probably indicative of neurotoxicity when given to rats at doses higher than MDMA. It is not know whether MDEA ("eve") will produce the same serotonergic deficits recently shown for MDMA ("ecstasy") in human users (20), although the high percentage of "eve" occasionally found in "ecstasy" tablets (40) is obviously a matter of concern. It should be finally noted that similar impairing effects in passive-avoidance learning were herein shown for these two amphetamines. Whether MDEA alters cognition in humans is also unknown; however, memory deficits have been reported for "ecstasy" users, and have been correlated with the extent of "ecstasy" exposure (7,23,27).

REFERENCES

1. Aguirre, N.; Ballaz, S.; Lasheras, B.; Del Río, J.: MDMA ("Ecstasy") enhances 5-HT$_{1A}$ receptor density and 8-OH-DPAT-induced hypothermia: Blockade by drugs preventing 5-hydroxytryptamine depletion. Eur. J. Pharmcol. 346:181–188; 1998.

2. Aguirre, N.; Barrionuevo, M.; Lasheras, B.; Del Río, J.: The role of dopaminergic systems in the perinatal sensitivity to 3,4-methylenedioxymethamphetamine-induced neurotoxicity in rats. J. Pharmacol. Exp. Ther. 286:1159–1165; 1998.

3. Aguirre, N.; Frechilla, D.; García-Osta, A.; Lasheras, B.; Del Río, J.: Differential regulation by methylenedioxymethamphetamine of 5-hydroxytryptamine$_{1A}$ receptor density and mRNA expression in rat hippocampus, frontal cortex, and brainstem: The role of corticosteroids. J. Neurochem. 68:1099–1105; 1997.

4. Artaiz, I.; Romero, G.; Zazpe, A.; Monge, A.; Calderó, J. M.; Roca, J.; Lasheras, B.; Del Río, J.: The pharmacology of VA21B7: An atypical 5-HT$_3$ receptor antagonist with anxiolytic-like properties in animal models. Psychopharmacology (Berlin) 117:137–148; 1995.

5. Berger, U. V.; Gu, X. F.; Azmitia, E. C.: The substituted amphetamines 3,4-methylenedioxymethamphetamine, methamphetamine, p-chloroamphetamine and fenfluramine induce 5-hydroxytryptamine release via a common mechanism blocked by fluoxetine and cocaine. Eur. J. Pharmaol. 215:153–160; 1992.

6. Boja, J. W.; Schechter, M. D.: Possible serotonergic and dopaminergic mediation of the N-ethyl-3,4-methylenedioxyamphetamine discriminative stimulus. Eur. J. Pharamcol. 202:347–353; 1991.

7. Bolla, K. I.; McCann, U. D.; Ricaurte, G. A.: Memory impairment in abstinent MDMA ("Ecstasy") users. Neurology 51:1532–1537; 1998.

8. Buhot, M. C.: Serotonin receptors in cognitive behaviors. Curr. Opin. Neurobiol. 7:243–254; 1997.

9. Carli, M.; Tranchina, S.; Samanin, R.: 8-Hydroxy-2-(di-n-propylamino)tetralin, a 5-HT$_{1A}$ receptor agonist, impairs performance in a passive avoidance task. Eur. J. Pharmacol. 211:227–234; 1992.

10. Chaouloff, F.: Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Res. Rev. 18:1–32; 1993.

11. Colado, M. I.; Green, A. R.: The spin trap reagent α-phenyl-N-tert-butyl nitrone prevents "ecstasy"-induced neurodegeneration of 5-hydroxytrptamine neurones. Eur. J. Pharmacol. 280:343–346; 1995.

12. Gozlan, H.; El Mestikawy, S.; Glowinski, J.; Hamon, M.: Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature 305:140–142; 1983.

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

14. Hegadoen, K. M.; Baker, G. B; Coutts, R. T: Analysis of the enantiomers of 3,4-methylenedioxy-N-ethylamphetamine (MDE, "Eve") and its metabolite 3,4-methylenedioxyamphetamine (MDA) in rat brain. J. Pharmacol. Toxicol. Methods 34:117–123; 1995.

15. Henry, J. A; Jefferys, K. T.; Dawling, S.: Toxicity and deaths from 3,4-methylenedioxymethamphetamine. Lancet 340:384–387; 1992.

16. Hiramatsu, M.; Kumagai, Y.; Unger, S. E.; Cho, A. K.: Metabolism of methylenedioxymethamphetamine: Formation of dihydroxymethamphetamine and quinone identified as its glutathione adduct. J. Pharmacol. Exp. Ther. 254:521–528; 1990.

17. Huether, G.; Zhou, D.; Rüther, E.: Causes and consequences of the loss of serotonergic presynapses elicited by the consumption of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its cogeners. J. Neural. Transm. 104:771–794; 1997.
18. Malberg, J. E.; Seiden, L. S.: Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. J. Neurosci. 18:5086–5094; 1998.
19. Marcusson, J. O.; Bergström, M.; Eriksson, K.; Ross, S. B.: Characterization of [^{3}H]-paroxetine binding in rat brain. J. Neurochem. 50:1783–1790; 1988.
20. McCann, U. D.; Szabo, Z.; Scheffel, U.; Dannals, R. F.; Ricaurte, G. A.: Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet 352:1433–1437; 1998.
21. Milroy, C. M.; Clarck, J. C.; Forrest, A. R.: Pathology of deaths associated with "ecstasy" and "eve" misuse. J. Clin. Pathol. 49:149–153; 1996.
22. Misane, I.; Johansson, C.; Ögren, S. O.: Analysis of the 5-HT$_{1A}$ receptor involvement in passive avoidance in the rat. Br. J. Pharmacol. 125:499–509; 1998.
23. Morgan, M. J.: Memory deficits associated with recreational use of "ecstasy." Psychopharmacology (Berlin) 141:30–36; 1999.
24. Nash, J. F.; Meltzer, H. Y.; Gudelsky, G. A.: Elevation of serum prolactine and corticosterone concentrations in the rat after the administration of 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 245:873–879; 1988.
25. Nash, J. F.; Nichols, D. E.: Microdialysis studies on 3,4-methylenedioxymethamphetamine and structurally related analogues. Eur. J. Pharmacol. 200:53–58; 1991.
26. O'Shea, E.; Granados, R.; Esteban, B.; Colado, M. I.; Green, A. R.: The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA ("ecstasy"). Neuropharmacology 37:919–926; 1998.
27. Parrott, A. C.; Lees, A.; Garnham, N. J.; Jones, M.; Wesnes, K.: Cognitive performance in recreational users of MDMA or "ecstasy": Evidence for memory deficits. J. Psychopharmacol. 12:79–83; 1998.
28. Pérez-Otaño, I.; Herrero, M. T.: Oñset, C.; De Ceballos, M. L.; Luquin, M. R.; Obeso, J. A.; Del Rió, J.: Extensive loss of brain dopamine and serotonin induced by chronic administration of MPTP in the marmoset. Brain Res. 567:127–132; 1991.
29. Ricaurte, G. A.; Finnegan, K. F.; Nichols, D. E.; DeLanney, L. E.; Irwin, I.; Langston, J. W.: 3,4-Methylenedioxyethylamphetamine (MDE), a novel analogue of MDMA, produces long-lasting depletion of serotonin in the rat brain. Eur. J. Pharmacol. 137:265–268; 1987.
30. Ricaurte, G. A.; Markowska, A. L.; Wenk, G. L; Hatzidimitriou, G.; Wlos, J.; Olton, D. S.: 3,4-Methylenedioxymethamphetamine, serotonin and memory. J. Pharmacol. Exp. Ther. 266:1097–1105; 1993.
31. Santucci, A. C.; Knott, P. J.; Haroutunian, V.: Excessive serotonin release, not depletion, leads to memory impairments in rats. Eur. J. Pharmacol. 295:7–17; 1996.
32. Scearce-Levie, K.; Viswanathan, S. S.; Hen, R.: Locomotor response to MDMA is attenuated in knockout mice lacking the 5-HT$_{1B}$ receptor. Psychopharmacology (Berlin) 141:154–162; 1999.
33. Schmidt, C. J.: Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs. Eur. J. Pharamcol. 136:81–88; 1987.
34. Schmidt, C. J.; Black, C. K.; Abbate, G. M.; Taylor, V. L.: Methylenedioxymethamphetamine-nduced hyperthermia and neurotoxicity are independently mediated by 5-HT$_2$ receptors. Brain. Res. 529:85–90; 1990.
35. Series, H. G.; Molliver, M. E.: Immunocytochemical evidence for serotonergic neurotoxicity of N-ethyl-methylenedioxyamphetamine (MDE). Exp. Neurol. 128:50–58; 1994.
36. Sprague, J. E.; Everman, S. L.; Nichols, D. E.: An integrated hypothesis for the axonal loss induced by 3,4-methylenedioxymethamphetamine. Neurotoxicology 19:427–442; 1998.
37. Stone, D. M. Johnson, M.; Hanson, G. R.; Gibb, J. W.: A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its N-methylated and N-ethylated derivatives. Eur. J. Pharmacol. 134:245–248; 1987.
38. Tehan, B.; Hardern, R.; Bodenham, S.: Hyperthermia associated with 3,4-methylenedioxyethamphetamine ("Eve"). Anaesthesia 48:507–510; 1993.
39. Warburton, E. C.; Harrison, A. A.; Robbins, T. W.; Everitt, B. J.: Contrasting effects of systemic and intracerebral infusions of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on spatial short-term working memory in rats. Behav. Brain Res. 84:247–258; 1997.
40. Wolff, K.; Hay, A. W.; Sherlock, K.; Conner, M.: Contents of "ecstasy." Lancet 346:1100–1101; 1995.