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Enhancement of morphine-induced analgesia after repeated injections of methylenedioxymethamphetamine

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Repeated administration of methylenedioxymethamphetamine (MDMA) to rats results in long-term depletion of serotonin (5-hydroxytryptamine; 5-HT) in several brain regions. Because of the apparent role of 5-HT in morphine-induced antinociception, the present experiment was designed to determine the effects of repeated MDMA injections on morphine-induced analgesia. Rats (n = 48) received 8 s.c. injections (one every 12 h for 4 days) of MDMA (20 mg/kg) or saline (1.0 ml/kg). Two weeks after the last injection, the groups were divided into 4 subgroups that received either saline, or morphine 2.5, 3.55 or 5.0 mg/kg (s.c.). Nociception was assayed before and after saline or morphine administration by the method of tail immersion in warm water (55 °C). The day after analgesia testing, the animals were sacrificed, brains and spinal cords removed and 5-HT, norepinephrine (NE) and dopamine (DA) levels in various brain and spinal cord regions were assayed. The analgesic effect of morphine was enhanced in rats that had received repeated MDMA injections. MDMA selectively depleted 5-HT in the cortex, hippocampus, striatum, brainstem and in the cervical portion of spinal cord. However, 5-HT levels were not changed in the thoracic and lumbar segments of the spinal cord. Thus, a functional consequence of repeated MDMA administration in rats was to enhance morphine-induced antinociception in association with reductions in brain and cervical spinal cord 5-HT. The fact that MDMA did not deplete 5-HT at lower spinal levels suggests that its neurotoxic action has an anatomical selectivity and may explain why morphine-mediated analgesia was not reduced as has been found with other depletors of 5-HT.

INTRODUCTION

Methylenedioxymethamphetamine (MDMA) belongs to a group of phenylalkylamines, which has both psychomotor stimulant and hallucinogenic properties[10,11]. The interest in this compound derives from its popularity as a drug of abuse and from its reported ability to facilitate psychotherapy in some patients[13]. Since the close chemical congeners methamphetamine (MA) and methylenedioxyamphetamine (MDA) have been found to produce degenerative effects on monoaminergic neurons[21,25,28], recent studies evaluated the possibility that this neurotoxicity is shared by MDMA[6,24]. The results of these studies showed that MDMA was more toxic and selective than MA for serotonergic (5-HT) neurons. In addition, the effect of MDMA appeared to be unusually prolonged, a single administration producing a substantial depletion of 5-HT up to 8 weeks after the injection. It is interesting to observe that this selective and prolonged 5-HT neurotoxic effect of MDMA is shared by MDA[20] suggesting that the methylenedioxy substitution of the amphetamine moiety might be involved in the 5-HT neurotoxicity of these compounds.

Behavioral studies with MDMA strongly suggest that it has dependence potential, probably of the amphetamine type[4,18]. Given the possibility of MDMA-induced neurotoxicity in humans, it is important to determine the functional consequences of that toxici-

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ty. Because of the apparent role of 5-HT in nociception[3,7,9,12,14,19,23,27,29–31], the present study was designed to examine the possibility that MDMA-induced 5-HT depletion is associated with an alteration in baseline nociception or in the antinociceptive effects of morphine. The tail-immersion test, which measures the latency of tail withdrawal from a noxious thermal stimulus, was used in rats[17]. This reflex is considered to be spinal, although also under the control of supraspinal mechanisms.

MATERIALS AND METHODS

Animals

Forty-eight male Sprague–Dawley rats (Holtzman Co., Madison, WI) weighing 330 ± 1.9 (S.E.M.) g were used. They were singly housed in stainless-steel cages with free access to food (Teklad 4% Rat and Mouse Diet) and water. The colony room was maintained at 24 °C with a 12-h light/dark cycle (light on 08.00–20.00 h).

Procedure

Twenty-four rats were injected with MDMA 20 mg/kg (s.c.) twice a day (at 08.00 and 20.00 h) for 4 days. The remaining 24 rats received saline according to the same schedule. In the following days the animals were regularly handled and their body weights recorded. Two weeks after the last injection, both MDMA- and saline-treated rats were assigned to 4 groups receiving saline or morphine at the dose of 2.5, 3.55 and 5.0 mg/kg (s.c.), respectively. Immediately before and after saline and morphine administration, pain perception was measured by the tail immersion method as described by Janssen et al.[17]. Briefly, rats were loosely held in the hand while the tail was immersed in a water bath maintained at 55 °C. A rapid jerk of the tail was considered the nociceptive end-point and its latency was measured at the nearest 0.1 s. A cut-off of 15 s was adopted for the animals that failed to respond to the thermal stimulus.

The day after nociception testing, all the animals were sacrificed for biochemical assay. Immediately after decapitation, the brain and spinal cord were quickly removed and placed on ice. Cortex, striatum, hippocampus and brainstem were then dissected according to the method described by Heffner et al.[15]. Spinal cord was divided into cervical, thoracic and lumbar segments. The tissue samples were stored in liquid nitrogen until assayed for 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and norepinephrine (NE) by high-performance liquid chromatography with electrochemical detection, as described by Ricaurte et al.[20]. Since differences in reaction times between MDMA- and saline-pretreated animals were statistically significant when morphine 5.0 mg/kg was administered, tissue samples from rats treated with 2.5 and 3.55 mg/kg were not considered for neurochemical assays.

Data analysis

Latencies obtained in the tail-immersion test were analyzed in two different ways: (1) data were transformed to the percent of the maximal possible effect (%MPE):

$$\%\text{MPE} = ((\text{postdrug latency} - \text{predrug latency})/(\text{cutoff time} - \text{predrug latency})) \times 100).$$

In order to further compare the analgesic effect of morphine in the two groups, data were converted to the area under the curve that represented the 120-min time effects of each dose. Area for each animal was calculated using the trapezoidal rule method of Tallarida and Murray[26]. In both the cases a two way analysis of variance (ANOVA) was then used to evaluate statistical differences between groups.

Neurochemical data are expressed as ng/mg of wet tissue. Differences between treatments were assessed using a two-way ANOVA.

Drugs

(±)-Methylenedioxymethamphetamine (MDMA) and morphine sulfate were provided by the National Institute on Drug Abuse (Rockville, MD, U.S.A.). Both compounds were dissolved in 0.9% saline for injection in a volume of 1.0 ml/kg.

RESULTS

Acute injections of MDMA had observable behavioral effects that included periodic bursts of crawling with the abdomen on the cage floor. After 2 h the rats recovered their normal posture and engaged in stereotyped behavior that lasted at least another 2 h. Piloerection and salivation were also present. Repeat-

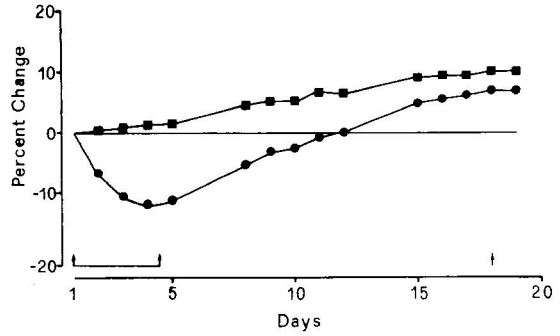

Fig. 1. Body weight changes in rats treated with MDMA (20 mg/kg s.c. twice a day for 4 days; circles) or saline (1.0 ml/kg, same schedule; squares). Data are expressed as mean percent (n = 24/group) of the body weight measured on the first day of treatment. The two arrows joined by a line enclose the days with MDMA treatment; the single arrow indicates the day of morphine treatment.

ed injections were associated with a decrease in body weight of about 12% (Fig. 1). The maximal decrease was reached after 7 injections (day 4) and was followed by a recovery in body weight. At the time of sacrifice the groups still differed slightly in body weight. The behavioral effects of MDMA did not appear to change during the 4-day treatment.

Fourteen days after the last MDMA injection there was no apparent difference in gross behavior between MDMA- and saline-treated rats. When nociception was measured, the two groups did not differ in baseline latencies in the tail-immersion test (2.0 ± 0.1 and 2.2 ± 0.1, for MDMA and saline groups, respectively). The analgesic effect of morphine was enhanced and prolonged in MDMA-treated rats (Fig. 2). A two-way ANOVA showed a significant interaction between pretreatment and both morphine treatment and time course of the analgesic response (Table I). When latencies were transformed to %MPE, the ANOVA showed that MDMA pretreatment significantly increased morphine analgesic potency and efficacy at 45, 60 and 90 min (Fig. 3). When the overall analgesic effect was evaluated by calculating the area under the curve, dose–response functions were not parallel in the two groups (Fig. 4). The response to 5.0 mg/kg was greater in the MDMA-group than in the saline-treated group.

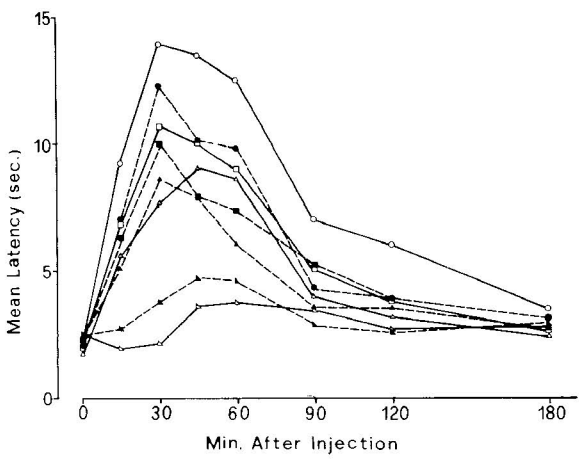

Fig. 2. Time course of the analgesic effect of morphine in MDMA (open symbols joined by solid lines) and saline (closed symbols joined by dashed lines) pretreated rats. ▷, ▶, saline; △, ▲, morphine 2.5 mg/kg sc; □, ■, morphine 3.55 mg/kg sc; ○, ●, morphine 5 mg/kg s.c. Each point is the mean of 6 rats. S.E.M.s are omitted for the sake of clarity.

Table II shows 5-HT, NE and DA content in different areas of brain 2 weeks after MDMA administration. 5-HT levels were markedly reduced in the cortex, hippocampus, striatum and brainstem of MDMA groups while DA and NE levels were unaffected. A two-way ANOVA showed that this MDMA effect was not influenced by morphine treatment. MDMA also reduced 5-HIAA content in the brainstem and striatum. MDMA significantly reduced 5-HT content also in the cervical portion of spinal cord and to a smaller extent in the thoracic segment (Table III), an effect that was not seen in the lumbar segment. As was seen in the brain, neither MDMA pretreatment nor morphine treatment affected NE and DA concentrations in the spinal cord areas examined.

DISCUSSION

MDMA has been found to cause long-lasting (at least 2 weeks) depletions of 5-HT in several brain

TABLE I

Values of the two-way ANOVA for the data shown in Fig. 2.

Factor	*F*	*df*	*P*
Pretreatment	4.59	1, 40	<0.05
Treatment	45.78	3, 40	<0.001
Time	164.79	7, 280	<0.001
Pretreatment × treatment	3.44	3, 40	<0.05
Pretreatment × time	2.22	7, 280	<0.001
Treatment × time	13.52	21, 28	<0.001

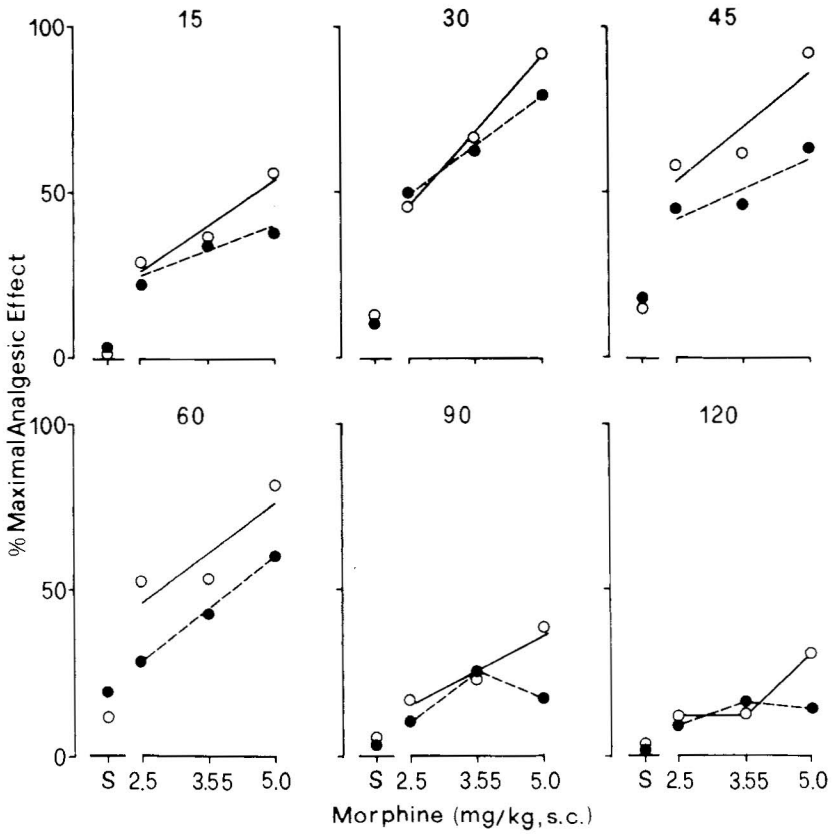

Fig. 3. Analgesic effect of morphine in MDMA- (open symbols) and saline- (closed symbols) pretreated rats. Data shown in Fig. 2 were transformed as percent of maximal analgesic effect. S, saline. The number on the top of each panel indicates minutes from the morphine injection.

TABLE II

Monoamine concentrations in brains of rats pretreated with MDMA or saline and administered saline or morphine (5.0 mg/kg) 24 h before sacrifice.

Data are expressed in mean ± S.E.M. ng/mg of wet tissue.

Treatment	*Whole cortex*				*Hippocampus*		
	5-HT	*5-HIAA*	*NE*	*DA*	*5-HT*	*5-HIAA*	*NE*
Saline + saline	0.18	0.09	0.25	0.24	0.25	0.25	0.50
	0.03	0.02	0.05	0.03	0.02	0.01	0.02
Saline + morphine	0.16	0.15	0.22	0.22	0.25	0.20	0.42
	0.02	0.06	0.01	0.04	0.03	0.02	0.03
MDMA + saline	0.08*	0.08	0.24	0.23	0.12*	0.43	0.43
	0.03	0.01	0.01	0.03	0.01	0.29	0.03
MDMA + morphine	0.06*	0.03	0.25	0.24	0.09*	0.08	0.47
	0.01	0.003	0.01	0.03	0.01	0.01	0.02
	Striatum			*Brainstem*			
	5-HT	*5-HIAA*	*DA*	*5-HT*	*5-HIAA*	*NE*	*DA*
Saline + saline	0.27	0.32	10.15	0.30	0.20	0.48	0.08
	0.02	0.02	0.02	0.02	0.01	0.03	0.01
Saline + morphine	0.23	0.30	9.67	0.31	0.20	0.42	0.09
	0.02	0.01	0.48	0.01	0.01	0.02	0.01
MDMA + saline	0.12*	0.15*	10.60	0.23*	0.16*	0.42	0.09
	0.01	0.01	0.39	0.01	0.01	0.02	0.01
MDMA + morphine	0.13*	0.16*	9.27	0.22*	0.15*	0.43	0.09
	0.02	0.02	0.15	0.01	0.01	0.01	0.01

* Significant influence of pretreatment, $P < 0.05$ (two-way ANOVA).

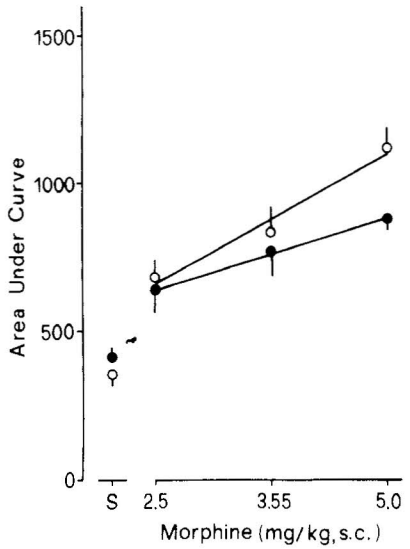

Fig. 4. Analgesic effect of morphine in MDMA- (open symbols) and saline- (closed symbols) pretreated rats. Each point was derived from the area under the 120-min time course curve shown in Fig. 2.

areas and a non-competitive reduction of synaptosomal 5-HT uptake[6,24]. Morphological observations give evidence that this depletion is due to a selective destruction of serotonergic neurons[6]. The present study confirms that MDMA administration results in a long-lasting depletion of 5-HT and metabolites in several brain areas and extends those findings to include the cervical spinal cord. However, 5-HT depletions were not found in more caudal regions of the spinal cord suggesting a progressive resistance (in the caudal direction) of spinal cord to the neurotoxic action of MDMA. Since the neurotoxicity of both serotonin and amphetamine analogs depends on their synoptic uptake[5], it is possible that the distribution to or the amount of MDMA taken up by the serotonergic terminals in more caudal regions of the spinal cord is insufficient to induce degeneration of the fibers.

Although MDMA is toxic to 5-HT neurons, the functional consequences of that toxicity have not been established. Pharmacological, neurochemical and anatomical evidence suggest the existence of a centripetal serotonergic pathway that at the spinal level is involved in the inhibition of pain perception produced by drugs or environmental stimuli[14]. Several studies have provided direct evidence that 5-HT plays an inhibitory role in pain perception (for reviews see ref. 3 and 14). For instance, iontophoretic application of 5-HT to dorsal horn neurons of spinal cord inhibits their response to noxious stimuli. The same inhibition is obtained when 5-HT is administered intrathecally or when mesencephalic nuclei containing 5-HT are stimulated. The antinociception produced by morphine is prevented by other neurotoxins selective for 5-HT neurons, such as 5,6-DHT[9] and 5,7-DHT[22]. Moreover, the 5-HT antagonist methysergide has been found to antagonize the analgesic effects of morphine[30]. Consequently, we hypothesized that 5-HT reductions produced by MDMA would reduce sensitivity to a noxious stimulus or reduce the analgesic efficacy of morphine. Surprisingly, MDMA-induced 5-HT depletions did not alter baseline sensitivity to a noxious stimulus and were associated with enhanced rather than reduced analgesic efficacy of morphine. The reason(s) for this apparently paradoxical effect is unclear. Nociceptive tests in which a thermal stimulus is applied to the tail

TABLE III

Monoamine concentrations (ng/mg of wet tissue) in segments of the spinal cord in the same groups considered in Table II

Treatment	*Cervical*				*Thoracic*				*Lumbar*			
	5-HT	*5-HIAA*	*NE*	*DA*	*5-HT*	*5-HIAA*	*NE*	*DA*	*5-HT*	*5-HIAA*	*NE*	*DA*
Saline + saline	0.31	0.20	0.28	0.18	0.18	0.11	0.25	0.05	0.34	0.10	0.30	0.13
	0.03	0.01	0.01	0.07	0.01	0.01	0.01	0.00	0.03	0.01	0.02	0.05
Saline + morphine	0.28	0.21	0.31	0.28	0.19	0.10	0.26	0.07	0.41	0.11	0.31	0.08
	0.01	0.06	0.01	0.10	0.01	0.00	0.01	0.02	0.03	0.00	0.01	0.02
MDMA + saline	0.20*	0.12	0.29	0.19	0.17	0.08*	0.25	0.09	0.36	0.11	0.28	0.11
	0.01	0.01	0.02	0.05	0.01	0.01	0.01	0.04	0.01	0.00	0.02	0.04
MDMA + morphine	0.20*	0.11	0.30	0.13	0.14*	0.07*	0.27	0.04	0.37	0.11	0.31	0.11
	0.01	0.02	0.01	0.06	0.01	0.05	0.02	0.03	0.03	0.00	0.03	0.06

* Significant influence of pretreatment, $P < 0.05$ (two-way ANOVA).

are considered to involve mainly spinal reflexes[16]. It is possible, therefore, that nociception was either unaffected or decreased because of the rather limited spinal effect of MDMA. The enhancement of morphine analgesia produced by MDMA may reflect the changes in 5-HT content caused by this agent at a supraspinal, more than at a spinal, level. Whatever the mechanism, 5-HT neurotoxicity produced by MDMA shows a topographic selectivity, sparing CNS segments such as thoracic and lumbar portions of spinal cord that are functionally crucial for morphine-induced analgesia.

The observation that the baseline response to a thermal stimulus was not altered by MDMA-induced 5-HT depletions is consistent with previous findings concerning the functional consequences of monoamine depletions induced by phenethylamines. For instance, changes in behavior were not readily apparent following a regimen of MA administration that destroys DA-containing neurons in the brain[1,2,8]. As in the present study, this functionally occult neurotoxicity became apparent as a change in sensitivity to the behavioral effects of another pharmacological agent, i.e. via a pharmacological challenge. It is possible that the functional deficiencies might occur with the additional neuronal degeneration of aging or under more complex behavioral conditions. It remains for future research to establish the behavioral consequences of drug-induced brain damage.

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