The Antinociceptive Effects of 3,4-Methylenedioxymethamphetamine (MDMA) in the Rat

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CRISP, T., J. L. STAFINSKY, J. W. BOJA AND M. D. SCHECHTER. The antinociceptive effects of 3,4-methylenedioxymethamphetamine (MDMA) in the rat. PHARMACOL BIOSCHEM BEHAV 34(3) 497-501, 1989. - The antinociceptive effects of MDMA and morphine were examined in rats using the tail-flick and hot-plate analgesiometric tests. MDMA, in the dose range of 1.5-6.0 mg/kg IP, produced a dose-dependent elevation in hot-plate latency, but did not elevate tail-flick latency. In contrast, morphine (2-8 mg/kg, IP) produced analgesia on both the tail-flick and hot-plate tests in a dose-dependent manner. Neither the opiate antagonist naltrexone nor the adrenoceptor antagonist phentolamine effectively attenuated MDMA-induced analgesia. Conversely, the serotonin antagonist methysergide significantly reversed the analgesic effects of MDMA on the hot-plate test. These findings suggest that the antinociceptive effects of MDMA are serotonergically mediated. Furthermore, the results verify earlier findings describing the test-specific effects of serotonin-induced pain modulation.

3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative with significant abuse potential. In a recent study conducted at Stanford University (19), 39% of the undergraduate students interviewed reported using MDMA at least once. Apparently, MDMA retains the stimulant properties of the amphetamines but lacks the hallucinogenic effects of the classic phenethylamine hallucinogens. Moreover, the reported mood-elevating and consciousness-altering properties of MDMA may support the use of the drug as an adjunct in psychotherapy (1). Although the mechanisms underlying the unique pharmacological properties of MDMA have not yet been determined, there is evidence to suggest that the effects of the drug may be mediated by serotonin (5-hydroxytryptamine; 5-HT) neurons in the brain. For instance, MDMA reportedly inhibits the active uptake of 5-HT and increases the release of the indoleamine in vitro (11, 16, 23).

It is well established that centrally acting compounds that directly mimic 5-HT at 5-HT receptor sites or those that indirectly enhance the release of endogenous 5-HT share similar pharmacological properties. As an example, the 5-HT uptake blocker fluoxetine elevates synaptic levels of the indoleamine and, as a result, elicits analgesia by itself and potentiates morphine-induced analgesia (14,15). Moreover, halogenated amphetamines, such as parachloroamphetamine and fenfluramine, possess antinociceptive properties via an ability to release serotonin from presynaptic nerve endings (for a review, see (7,25)). Data such as these suggest that MDMA may have analgesic properties (2,4).

The purpose of the present study was to determine the analgesic efficacy of MDMA in rats. The tail-flick and hot-plate analgesiometric tests were used as the nociceptive measures. The antinociceptive effects of MDMA were compared to the analgesic actions of morphine sulfate. In another group of experiments, rats were pretreated with either methysergide, phentolamine or naltrexone, and the ability of these receptor antagonists to alter MDMA-induced analgesia was assessed. In this manner, the role of central serotonergic, noradrenergic and/or opioid neuronal systems in the antinociceptive actions of MDMA could be evaluated.

METHOD

Subjects

Male Sprague-Dawley rats (300-350 g; Zivic-Miller Laboratories, Inc., Allison Park, PA) were used in all experiments. Animals were housed individually and maintained on a 12-hour light/dark cycle in temperature-controlled rooms (22-23°C). Rats received water and rat chow ad lib.

Nociceptive Tests

A Model 33 Tail-Flick Analgesia Meter (IITC Life Science Instruments, Woodland Hills, CA) was used to measure tail-flick latency following intraperitoneal (IP) drug administration. The rat's tail (blackened beforehand with India ink) was placed into a depression over a photocell on the tail-flick meter. The time (in sec) required for the rat to remove its tail from the light source was automatically determined and expressed as tail-flick latency (TFL). Four predrug TFL values were obtained at 10-min intervals and the last three of these were averaged to obtain predrug means. A 10-sec maximum exposure to the light source was employed as the
cut-off to avoid damage to the tail, and animals not responding to the light source within the allotted 10-sec interval were assigned a TFL of 10.

The effects of MDMA and morphine sulfate on supraspinally mediated nociception were tested using a Model 39-D Hot Plate Analgesia Meter (ITC Life Science Instruments, Woodland Hills, CA). The temperature of the hot plate was maintained at $55 \pm 0.5^\circ C$. Hot-plate latency (HPL) was defined in these studies as the interval of time (in sec) between placement of the rat onto the hot plate and the instant a nociceptive response was elicited (e.g., licking of a forepaw or hindpaw, or hopping off the plate). Four predrug HPL values were obtained at 10-min intervals, and the last three were averaged for predrug means. Animals not responding to the hot plate within 20 sec postdrug administration were removed from the plate and assigned a HPL of 20.

**Antinociceptive Effects of MDMA and Morphine**

The first set of experiments was conducted to determine the analgesic efficacy of different doses of MDMA (1.5, 3.0 or 6.0 mg/kg) or morphine sulfate (2.0, 4.0 or 8.0 mg/kg) administered intraperitoneally (IP). All drugs were dissolved in 0.9% saline for injection in a volume of 1.0 ml/kg. TFL and HPL values were obtained 15, 30, 45, 60 and 120 min postdrug injection. Dose-response and duration curves were generated for both MDMA and morphine.

**Effect of Methysergide, Naltrexone or Phentolamine Upon MDMA-Induced Analgesia**

In an effort to determine the extent of serotonergic and/or opioid involvement in the antinociceptive effects of MDMA, either the serotonin receptor antagonist methysergide (2 mg/kg) or the opiate antagonist naltrexone (2 mg/kg) was administered IP 10 min prior to MDMA (3 mg/kg), and the ability of these drugs to reverse MDMA-induced alterations in TFL and HPL was assessed. Preliminary studies (5,6) have shown that the 2 mg/kg dose of methysergide and naltrexone used in the present study clearly reversed the analgesic effects of 5-HT or morphine, respectively.

Additional studies were designed to test the possibility that norepinephrine might contribute to the antinociceptive effects of MDMA. In this group of experiments, the adrenoceptor antagonist phentolamine was administered intrathecally (15 μg/10 μl) and tested for its ability to alter MDMA-induced analgesia. The intrathecal route of administration was required since previous reports have shown that norepinephrine elicits analgesia when injected spinally (20) and decreases nociceptive thresholds when administered supraspinally (10). The intrathecal catheter was inserted into the spinal subarachnoid space, and phentolamine was injected spinally at the level of the lumbar enlargement (12). In addition to the pretreatment experiments, methysergide, phentolamine and naltrexone were administered alone and tested for an ability to alter TFL and HPL.

**Data Analyses**

Experimental results are expressed as means ± S.E.M., and all data were derived from experiments having an n of 6 animals per treatment group. The effects of drug treatments over time on TFL and HPL were statistically analyzed using a two-way analysis of variance (ANOVA) and a Dunnett's test, as applicable, for multiple post hoc comparisons (p<0.05).

**Drugs**

3,4-Methylenedioxymethamphetamine (MDMA) and morphine sulfate were supplied by the National Institute on Drug Abuse (Rockville, MD). Methysergide maleate was generously donated by Sandoz Research Institute (East Hanover, NJ) and phentolamine mesylate (Regitine) by CIBA-GEIGY ( Suffern, NY). Naltrexone was obtained from a commercial source (Resource Biochemicals, Inc., Natick, MA).

**RESULTS**

**Antinociceptive Effects of MDMA and Morphine**

MDMA administered IP was ineffective at producing analgesia on the spinally mediated tail-flick test (Fig. 1A). As a matter of fact, the mean tail-flick response from rats treated with 3 mg/kg MDMA was significantly less than predrug latencies for this group of rats and from saline-treated animals (p<0.05). The MDMA-induced hyperalgesic response on the tail-flick test persisted for 30 min in rats treated with the 3 mg/kg dose of the drug. On the other hand, MDMA dose-dependently elevated HPL in rats (Fig. 1B). The onset of MDMA-induced analgesia on the hot-plate test occurred within 15 min postinjection, and its duration of action was dose-dependent. For example, the 3 mg/kg dose of MDMA significantly elevated HPL above predrug and saline values for 45 min, whereas the antinociceptive effects of the 6 mg/kg dose persisted for 120 min (Fig. 1B).
MDMA AND ANALGESIA

**Effect of Methysergide. Phenolamine or Naltrexone Upon MDMA-Induced Analgesia**

To determine if monoaminergic and/or opioid systems play a role in mediating the antinociceptive effects of MDMA on the hot-plate test, rats were pretreated with either methysergide, phenolamine or naltrexone, and the ability of the various receptor antagonists to ALTER MDMA-induced analgesia was assessed. The effects of the various antagonists were tested against the 3 mg/kg dose of MDMA because this dose of the amphetamine derivative produced significant elevations in HPL without taking animals to the 20-sec hot-plate cut-off point (Fig. 1B). In this manner, detections could be made of antagonist-induced increases or decreases in the antinociceptive effects of MDMA.

As graphically represented in Fig. 3A, naltrexone (2 mg/kg) did not reverse MDMA-induced analgesia on the hot-plate test. The adrenoceptor antagonist phenolamine (15 μg) administered IT similarly did not block the hot-plate effects of MDMA (Fig. 3B). In contrast, the serotonin receptor blocker methysergide (2 mg/kg) diminished MDMA-induced elevations in HPL for at least 45 min (Fig. 3C). Since the 5-HT receptor blocker attenuated the analgesic effects of MDMA on the hot-plate test, another group of experiments were performed to determine if methysergide might also reverse MDMA-induced hyperalgesia on the tail-flick test. Methysergide was ineffective at reversing MDMA-induced decreases in TFL, and the 5-HT receptor antagonist produced hyperalgesia on the tail-flick test when administered alone. Furthermore, when the various receptor antagonists were tested by themselves on the hot-plate test, none of them produced significant decreases in HPL from predrug values 15, 30, 45 or 60 min postinjection (Fig. 3A, B and C).

**DISCUSSION**

The purpose of the present study was to investigate the antinoceptive efficacy of MDMA and to determine how endogenous monoaminergic and/or opioid systems might contribute to the antinociceptive actions of the drug. A recent study (2) suggested that the antinociceptive properties of MDMA may contribute to the popularity of the agent as a recreational drug of abuse. Thus, further experimentation to establish a more thorough understanding of the mechanisms underlying the behavioral and psychological effects of MDMA seemed warranted.

Previous studies have shown that some of the pharmacological effects of MDMA result from the release of endogenous 5-HT (11, 16, 23). The present finding that MDMA-induced elevations in HPL were inhibited by methysergide further suggests that endogenous serotonin plays a significant role in mediating the antinociceptive effects of MDMA. Further evidence to support the hypothesis that MDMA-induced analgesia might be serotoninically-mediated is provided by the finding that fenfluramine produced analgesia on the hot-plate test, and these effects were reversed by the serotonin receptor antagonist metergoline (21). Reportedly, MDMA and fenfluramine produce similar effects upon serotonergic neurons, which explains their pharmacological and toxicological actions (8, 11, 21, 22). The present results strongly suggest that MDMA and serotonin interact to produce behavioral analgesia.

Experiments were also conducted to evaluate the antinociceptive efficacy of MDMA against reflexive types of nociception using the spinally mediated tail-flick test. The present finding that systematically administered MDMA did not inhibit the tail-flick reflex is congruent with earlier work showing that serotonin agonists (e.g., 5-methoxydimethyltryptamine and quipazine) and serotonin releasing agents (e.g., fenfluramine) are ineffective against reflexive types of nociception (21, 27). Other investigators (17) reported that drugs which enhance central serotonergic neurotransmission (e.g., p-chloroamphetamine and zimelidine) produce analgesia on the hot-plate test, but not on the tail-flick test.

Apparently, serotonin receptors in the spinal cord (e.g., 5-HT1, 5-HT2 and 5-HT3 sites) subserve different pharmacological functions, depending upon the specific type of nociceptive input. For instance, local spinal reflexes are facilitated by serotonin agonists that preferentially bind to 5-HT1 sites, whereas ascending nociceptive input is blocked by 5-HT1 agonists (6, 27). The present finding that MDMA elicits hyperalgesia on the tail-flick test and analgesia on the hot-plate test provides further evidence for a nociception-specific function of 5-HT1 receptor sites in the spinal cord (6, 17, 24, 27). However, when attempts were made to determine if MDMA-induced decreases in TFL were methyser-
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nists is supposedly due to a blockade of 5-HT released from CIBA-GEIGY and Sandoz Research Institute for their generous gifts
algesia resulting from the administration of serotonergic antago- Care System in Youngstown, OH. The authors would also like to thank
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