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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 BIOCHEM 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.

MDMA Analgesia Serotonin Hot-plate test Tail-flick test

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 phenylethylamine 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 (0600–1800) 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 (IITC Life Science Instruments, Woodland Hills, CA). The temperature of the hot plate was maintained at 55 ± 0.5°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).

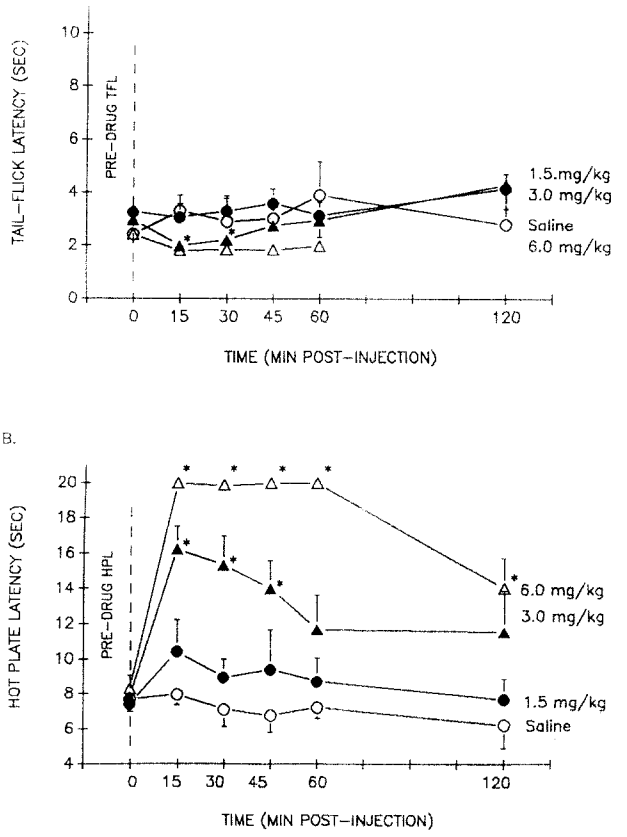

FIG. 1. Dose-response and duration of action of MDMA administered intraperitoneally on the tail-flick (A) and hot-plate (B) analgesiometric tests. Values are the means ± S.E.M. of 6 animals per treatment group. *Significantly different than predrug and saline TFL and HPL values, $p<0.05$, two-way ANOVA and a post hoc Dunnett's test.

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).

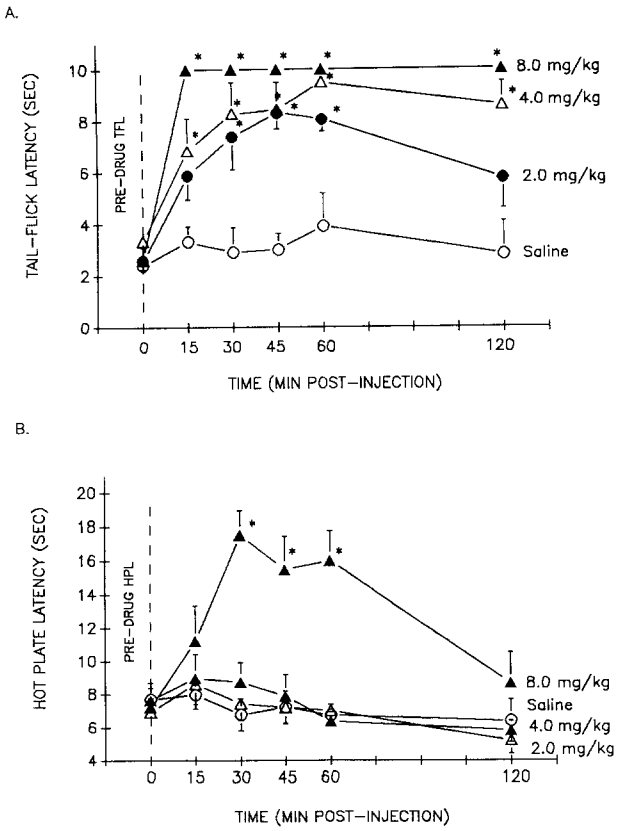

FIG. 2. Dose-response and duration of action of morphine sulfate administered intraperitoneally on the tail-flick and hot-plate tests. Values represent the means ± S.E.M. of 6 rats per treatment group. *Significantly greater than predrug and saline TFL and HPL values, $p<0.05$, ANOVA and a post hoc Dunnett's test.

Morphine sulfate dose-dependently elevated both TFL and HPL (Fig. 2). On the tail-flick test, the highest dose of morphine tested (8 mg/kg) brought 100% of the animals to cut-off. The onset of morphine-induced elevations in TFL occurred within 15 min following IP injections, and the antinociceptive effects of the 4 and 8 mg/kg doses of the opiate were significantly greater than predrug and saline values for at least 120 min (Fig. 2A). Morphine-induced elevations in HPL were significantly greater than baseline values by 30 min postinjection (Fig. 2B), and the hot-plate response remained significantly elevated above predrug and saline HPL values for 60 min.

Effect of Methysergide, Phentolamine 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, phentolamine 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 phentolamine (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 antinociceptive efficacy of MDMA and to determine how endogenous monoaminergic and/or opioid systems might contribute to the analgesic 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 serotonergically-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 analgesic efficacy of MDMA against reflexive types of nociception using the spinally mediated tail-flick test. The present finding that systemically 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-HT_1, 5-HT_2 and 5-HT_3 sites) subserve different pharmacological functions, depending upon the specific type of noxious input. For instance, local spinal reflexes are facilitated by serotonin agonists that preferentially bind to 5-HT_1 sites, whereas ascending nociceptive input is blocked by 5-HT_1 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-HT 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-

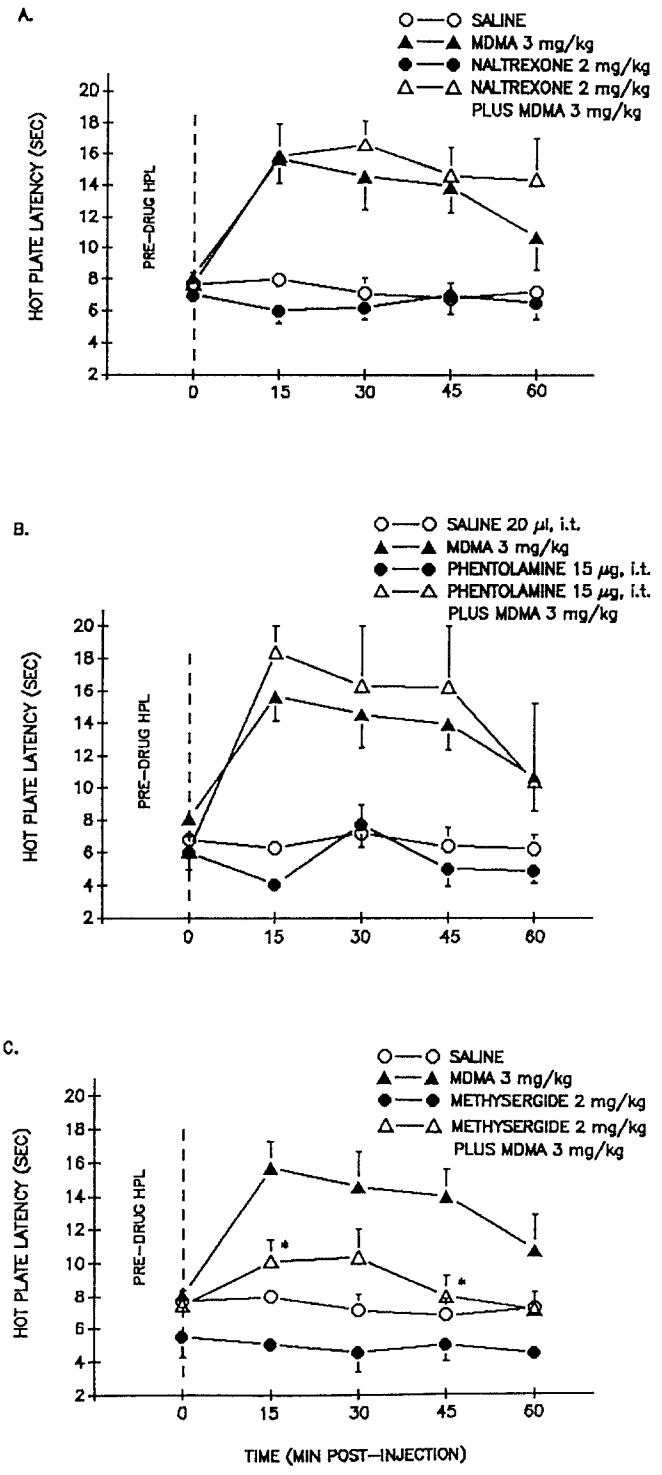

FIG. 3. The effects of (A) naltrexone, (B) phentolamine or (C) methysergide upon MDMA-induced analgesia. Values are the means ± S.E.M. of 6 animals per treatment group. *Significantly less than HPL values for MDMA (3 mg/kg) alone, $p<0.05$ ANOVA and a post hoc Dunnett's test.

gide-reversible, it was observed that methysergide produces hyperalgesia on the tail-flick test when administered alone. Hyperalgesia resulting from the administration of serotonergic antagonists is supposedly due to a blockade of 5-HT released from tonically active descending pain-inhibitory nerves (3). That methysergide was ineffective against MDMA-induced decreases in TFL in the present study does not necessarily suggest that the hyperalgesic effects of MDMA are not serotonergically mediated. One group of investigators recently suggested that 5-HT_3 receptors play an important role in mediating spinal nociceptive responses to 5-HT (9). Moreover, receptors other than the 5-HT_3 sites (e.g., 5-HT_{1A} or 5-HT_{1B} subtypes) may facilitate nociceptive reflexes (9,24). Methysergide does not preferentially bind to specific 5-HT receptor subtypes. Therefore, it will be necessary to test more selective 5-HT receptor antagonists against MDMA to accurately identify the 5-HT receptor subtype(s) that may be involved in mediating the analgesic and hyperalgesic effects of MDMA. These studies are currently in progress.

It should also be emphasized that the nociceptive test-specific effects of MDMA in this study may have been a function of the systemic route of drug administration. For instance, other investigators have shown that spinally administered 5-HT produces elevations in tail-flick latency (9,26). Thus, before more conclusive statements can be drawn about the role of 5-HT in the mediation of MDMA-induced analgesia, further research will need to be done to test the antinociceptive efficacy of intrathecally and supraspinally administered MDMA.

Recent radioligand binding studies have shown that MDMA binds directly to 5-HT_1 and 5-HT_2 binding sites (13). These results might imply that the methysergide-induced attenuation of MDMA analgesia in the present study is due to a direct competitive interaction between the two drugs at 5-HT receptors. Alternatively, the 5-HT releasing action of MDMA may account for the analgesic effects of the drug. Research is currently underway in this laboratory to determine whether the antinociceptive effects of MDMA and other phenylethylamine hallucinogens are mediated by their interactions with 5-HT receptors.

It is also apparent from these data that MDMA does not interact with opioid or noradrenergic systems to produce antinociception on the hot-plate test. This is evidenced by the lack of an inhibitory effect of naltrexone and phentolamine upon MDMA. Preliminary data demonstrated that the doses of naltrexone and phentolamine used in the present studies were sufficient to block the respective antinociceptive effects of morphine and norepinephrine (5,6). Therefore, it was unlikely that the inability of the two receptor antagonists to inhibit MDMA-induced analgesia was a dose-related problem.

Systemic morphine-induced analgesia is naloxone-reversible (18), suggesting that morphine interacts with opioid receptors. On the other hand, data from this study suggest that MDMA-induced analgesia is mediated by endogenous 5-HT. Noradrenergic systems apparently do not contribute to the antinociceptive effects of either MDMA (present study) or morphine (18). This type of research may shed some light on the similarities and differences in the mechanisms of action of drugs that produce psychological and behavioral effects similar to MDMA. Research is currently underway in this laboratory to investigate the mechanisms underlying the analgesic actions of an n-ethylated congener of MDMA known as n-ethyl-methylenedioxymethamphetamine (MDE). Additional research will be necessary before conclusions can be drawn regarding how particular drug-induced behavioral changes (e.g., analgesia) may contribute to the abuse potential of MDMA-like drugs.

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