Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 2, pp. 357-361, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0043-4

Effect on Morphine-Induced Catalepsy, Lethality, and Analgesia by a Benzodiazepine Receptor Agonist Midazolam in the Rat

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Received 21 July 1993

RATTAN, A. K. AND P. SRIBANDITMONGKOL. *Effect on morphine-induced catalepsy, lethality, and analgesia by a benzodiazepine receptor agonist midazolam in the rat.* PHARMACOL BIOCHEM BEHAV **48**(2) 357-361, 1994.—Previously we have shown that intrathecal administration of midazolam can increase or decrease morphine-induced antinociception, depending upon relative concentration of these drugs by modulating spinal opioid receptors, and it also can inhibit morphine-induced tolerance and dependence in the rat. Now we report that midazolam also influences catalepsy, lethality, and analgesia induced by morphine in the rat. In the acute treatment, animals were first treated with saline or midazolam (0.03 to 30.0 mg/kg, b.wt., IP), and 30 min later with a second injection of saline or morphine (1.0 to 100.0 mg/kg, b.wt., SC). The catalepsy was measured 60 min after the second injection and lethality was checked after 24 h. Midazolam injection increased the morphine-induced catalepsy and lethality. In the chronic treatment, animals were injected with two injections daily for 11 days. The first injection consisted of saline or midazolam (0.03 to 3.0 mg/kg, b.wt., IP), and 30 min later with a second injection of saline or morphine (10.0 mg/kg, b.wt., IP) was given. Lethality, antinociception, and body weight were measured. Chronic morphine treatment also increased lethality in a dose-dependent manner. Chronic treatment with midazolam and morphine increased the antinociception on day 11, as measured in the tail-flick and hot-plate tests. Midazolam administration also prevented the morphine-induced weight loss. These results suggest a strong interaction between midazolam and morphine in altering catalepsy, lethality, and analgesia in rat.

Midazolam Morphine Catalepsy Lethality Opioid Benzodiazepine Body weight Tail flick Hot plate Analgesia

MIDAZOLAM is an ultra short-acting benzodiazepine and is used clinically for preoperative medication, an induction agent for general anesthesia, and intravenous sedation. It possesses anxiolytic, hypnotic, anticonvulsant, muscle relaxant, and anterograde amnestic properties, characterstic of the benzodiazepine class of drug (24). Midazolam is also administered to supplement opioids or inhaled anesthetics during maintenance of anesthesia (17,25). Recently, we have observed that a benzodiazepine receptor agonist, midazolam, can either increase or decrease spinal analgesia, depending on the relative concentrations of morphine and midazolam in the subarachnoid space in rats (12). The morphine- and midazolam-induced analgesia in rats is antagonized by the opioid antagonist naloxone (12). We have also observed that midazolam inhibits the binding of opioid ligands to spinal receptors in the following order: $\kappa > \delta > \mu$ (12,13). In addition, midazolam may prolong the effects of morphine treatment by delaying development of tolerance to the antinociceptive effect of morphine (14,18). Interactions between benzodiazepines and morphine in producing analgesia are controversial. For example, diazepam has been found to decrease (15), increase (2), or have no effect on morphine-induced antinociception. In view of these reports on the interaction between benzodiazepines and opioids, we have examined whether midazolam has any effect on morphine-induced catalepsy, lethality, and analgesia in rats.

METHOD

This study was approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee.

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Male Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA), weighing 225–275 g, were used. Before the start of the experiment, they were housed in groups of four in a room maintained at 22 ± 1°C with a 12 h on-and-off lighting schedule. Laboratory rat food and water were given ad lib.

Morphine sulfate (Steris Laboratories, Phoenix, AZ) and midazolam hydrochloride (Hoffmann-La Roche, Nutley, NJ) were used for this study. All dilutions for morphine and midazolam were done in saline solution. Rats were randomly divided into the groups, containing 8 to 57 per group.

In an acute study, rats received two injections only. Rats were first administered with saline (1 ml/kg, b.wt., IP) or midazolam (0.03, 0.3, 3.0, or 30.0 mg/kg, b.wt., IP), and 30 min later with a second injection of saline or morphine (1.0, 3.0, 10.0, 30.0, or 100.0 mg/kg, b.wt., SC). The catalepsy was determined 60 min after the second injection. The rat was grasped gently around the back, and gently inverted so that the legs were pointed towards the ceiling. A rat was considered cataleptic if it maintained completely a rigid posture for 15 s, and did not attempt to right, as described by Trujillo and Akil (20). Results are presented as percent of rats cataleptic 60 min after the second injection. The lethality was expressed as the percent of rats found dead within 24 h following the second injection.

In chronic treatment, rats received two injections daily for 11 days. Rats were first injected with saline or midazolam (0.03, 0.3, or 3.0 mg/kg, b.wt., IP), and 30 min later with a second injection of saline or morphine (10.0 mg/kg, b.wt., SC). This schedule of injection has previously been shown to produce a tolerance to the analgesic effects of morphine (14,18). The lethality results are presented as the percent of rats found dead in the 11 day period. The antinociception (tail-flick and hot-plate tests) and body weight were measured as described in the legends to the figures. The tail-flick test was conducted with a Tail-Flick Analgesia Meter (IITC, Woodland Hills, CA). The rheostat-controlled light (sensitivity 5, beam at 90%) was focused on the tail (2.5–3.0 cm from the tip), and the time interval from the onset of the heat stimulus to the flick of the tail was recorded automatically by the instrument. A cut-off time of 10 s was used to avoid tissue damage caused by intense heat. The hot-plate test was performed with a model 38D Analgesia Meter (IITC, Woodland Hills, CA). This test involves keeping the rat on a metal plate surface maintained at 52°C ± 0.5°C. The surface was surrounded by Plexiglas walls to form an area of 12.50 × 11.25 inches. The end point in this test was generally taken as licking of a hind paw or jumping, whichever came first in response to thermal stimuli. In the absence of a response, hot-plate trials were terminated at 60 s (12,14,18). The analgesic data shown here were collected on the eleventh day. Rats were weighed daily and the results are presented as the gain in body weight in 11 days.

The lethality and catalepsy in rats were compared by using a nonparametric test, i.e., Fisher's exact probability test. The tail-flick and hot-plate test comparisions between various drug treatments were carried out with one-way ANOVA followed by the Newman-Keuls t-test. p-Values less than 0.05 were considered to be statistically significant.

RESULTS

Animals injected with midazolam (0.03 to 30.0 mg/kg) in the acute treatment, followed by saline injection, showed no catalepsy (0/8, 0%, data not shown). In saline-pretreated rats, no catalepsy was observed with doses of morphine up to 10.0 mg/kg. With 30.0 mg/kg morphine, 10/16 animals (62.5%) were cataleptic, while with 100.0 mg/kg, 16/16 (100%) were cataleptic (Fig. 1). Strong interaction was observed when both the drugs were injected together. Midazolam (0.3, 3.0, and 30.0 mg/kg) caused an increase in the cataleptic action of morphine (Fig. 1).

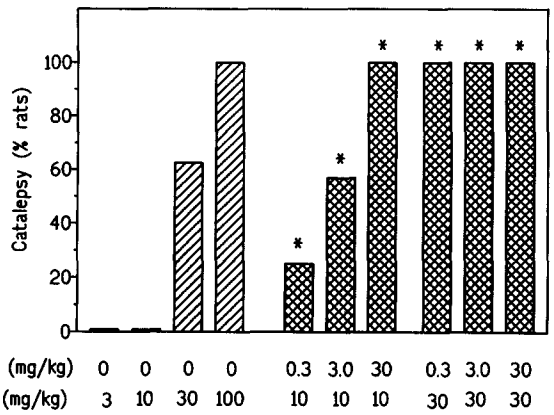

FIG. 1. Enhancement of morphine-induced catalepsy by different doses of midazolam. Rats were first treated with saline or midazolam (0.3, 3.0, or 30.0 mg/kg, b.wt., IP), followed 30 min later with saline or morphine (10 or 30 mg/kg, b.wt., SC). Values are presented as the percent of rats that became cataleptic 60 min after the last injection (n = 8–16). Statistical comparisions were done by Fisher's exact probability test. A value of $*p < 0.05$ was considered to be statistically significantly different from the saline-morphine group.

Animals injected with midazolam (0.03 to 30.0 mg/kg) in the acute treatment, followed by saline, showed no lethality (0/8, 0%, data not shown). In saline-pretreated rats, no lethal-

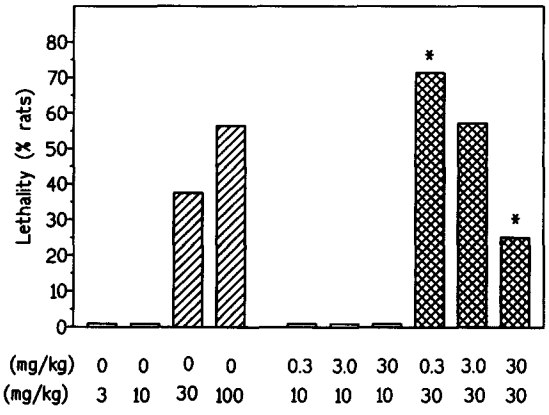

FIG. 2. Enhancement of morphine-induced lethality by different doses of midazolam. Rats were first treated with saline or midazolam, followed 30 min later with saline or morphine. Details of doses of midazolam and morphine are same as described in legend to Fig. 1. Values are presented as the percent of rats found dead within 24 h following injections. Statistical comparisions were done by Fisher's exact probability test. A value of $*p < 0.05$ was considered to be statistically significantly different from the saline-morphine group.

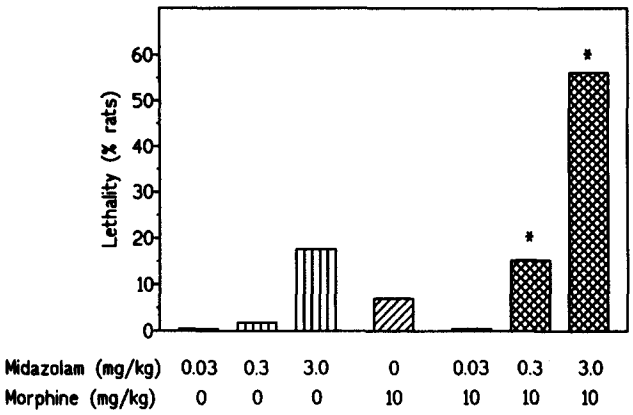

FIG. 3. Enhancement of morphine-induced lethality by different doses of midazolam in morphine-tolerant rats. Rats were first treated with saline or midazolam (0.03, 0.3, or 3.0 mg/kg), followed 30 min later with saline or morphine (10.0 mg/kg), daily for 11 days. Values are presented as the percent of animal found dead after 11 days (n = 16–59). Statistical comparisions were done by Fisher's exact probability test. A value of $*p < 0.01$ was considered to be statistically significantly different from the saline-morphine group.

ity was observed with doses of morphine up to 10.0 mg/kg. With 30.0 mg/kg, 6/16 animals (37.5%) were dead, while with 100.0 mg/kg, 9/16 (56.7%) were dead (Fig. 2). There was no lethality when different doses of midazolam (0.3, 3.0, and 30.0 mg/kg) were administered prior to morphine (10.0 mg/kg) treatment (Fig. 2). However, when the morphine (30.0 mg/kg) dose was injected with different concentrations of midazolam of 0.3 mg/kg (5/7, 71.4%), 3.0 mg/kg (4/7, 57.1%), and 30.0 mg/kg (2/8, 25.0%), animals were dead. As we increased the dose of midazolam, the lethal effect of morphine at 30.0 mg/kg was antagonized by midazolam (Fig. 2).

In order to investigate whether chronic treatment of midazolam had any effect on morphine-induced lethality, rats were injected with midazolam and morphine for 11 days. Animals treated with midazolam (0.03 mg/kg) showed no lethality (0/16, 0%), but lethality was observed at higher doses of midazolam 0.3 mg/kg (1/56, 1.8%), and 3.0 mg/kg (3/17, 17.6%, Fig. 3). Saline-pretreated rats treated with 10.0 mg/kg morphine showed some lethal effect (4/57, 7.0%). When midazolam-pretreated animals were injected with 10.0 mg/kg morphine, the lethal effect was increased. Midazolam increases the lethal effect at 0.3 mg/kg (9/59, 15.2%) and 3.0 mg/kg (9/16, 56.2%) in 11 days (Fig. 3).

Animals treated chronically with midazolam or morphine alone showed no antinociception in the tail-flick and hot-plate test on day 11 (Fig. 4A, B). The animals treated with 0.03 to 3.0 mg/kg midazolam along with the 10.0 mg/kg morphine group exhibited significant antinociception, $F(7, 56) = 68.7$, $p < 0.01$, when compared with the saline-morphine group in the tail-flick test (Fig. 4A), but in the hot-plate test, only 3.0 mg/kg midazolam with 10.0 mg/kg morphine showed significant antinociception, $F(7, 26) = 18.6$, $p < 0.01$, on day 11 (Fig. 4B).

We also recorded the body weight in one of the groups. Animals treated chronically with morphine (10.0 mg/kg) for 11 days gained 37% less weight compared to control animals injected with saline alone. This decrease in gain in body weight was abolished in the morphine-treated animals given midazolam (0.3 mg/kg) injections (Fig. 5).

DISCUSSION

The present study demonstrates that the benzodiazepine receptor agonist midazolam significantly potentiates morphine-induced catalepsy, lethality, and analgesia. Acute treatment with midazolam did not produce catalepsy and lethality, but when combined with morphine, the effect was potentiated by the interactions of these two drugs. This interaction was observed relatively at all doses of midazolam ranging from 0.3 to 30.0 mg/kg, except the 30 mg/kg midazolam decreases the lethal effects of 30 mg/kg morphine. In acute treatment, we have already shown that midazolam does not produce any antinociception by itself and had no effect on antinociception produced by morphine in the tail-flick and hot-plate test

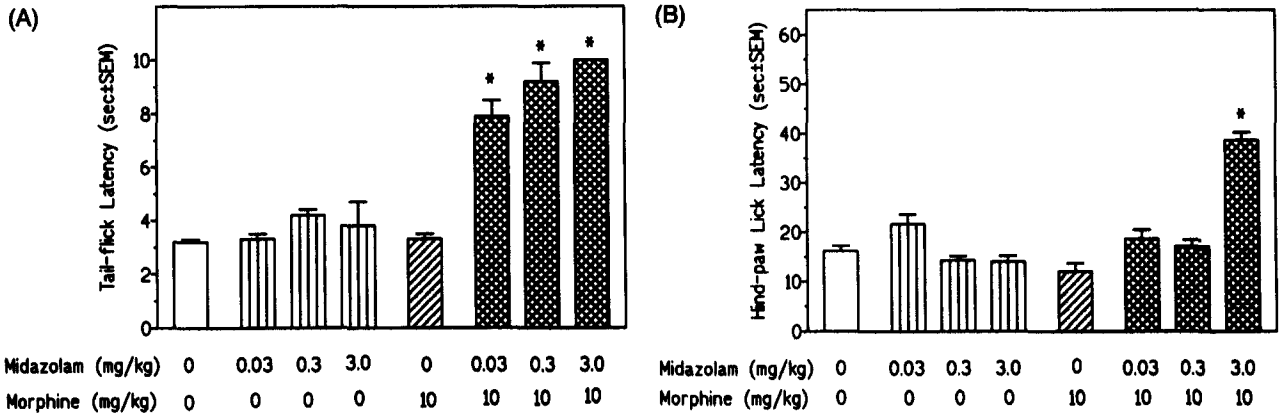

FIG. 4. Antinociception exhibited on 11th day after chronic injections of saline, midazolam, and morphine, and together assessed by measuring the tail-flick (A) and hind-paw lick (B) latency in the rat. Rats were first treated with saline or midazolam (0.03, 0.3, or 3.0 mg/kg), followed 30 min later with saline or morphine (10.0 mg/kg). Values are expressed as mean ± SEM (n = 6–8). Statistical comparisions were done by one-way ANOVA followed by Newman-Keuls t-test. A value of $*p < 0.05$ was considered to be statistically significantly different from the saline-saline or saline-morphine group.

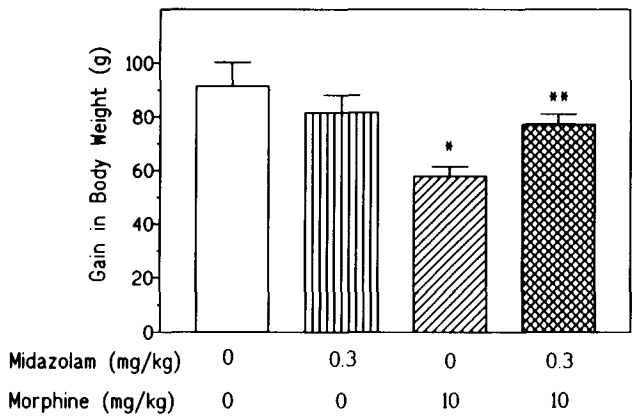

FIG. 5. Prevention by midazolam of a decrease in gain in the body weight of rats caused by chronic injection of morphine. Gain in the body weight was calculated for 11 days. Rats were first treated with saline or midazolam (0.3 mg/kg), followed 30 min later with saline or morphine (10.0 mg/kg). Statistical comparisions were done by one-way ANOVA followed by Newman-Keuls *t*-test. A value of $*p < 0.01$ and $**p < 0.01$ was considered to be statistically significantly different from the saline-saline and saline-morphine group, respectively.

(12,18). Also, morphine-induced antinociception in the animals treated chronically with midazolam was similar to those animals that were injected chronically with saline in the tail-flick and hot-plate test (12,18). The chronic administration of midazolam on morphine-tolerant animals increases lethality, as seen in the present study. It also increases the antinociception in the midazolam-morphine group significantly, as we have already shown that this drug antagonizes morphine tolerance and dependence (12,18). This drug interaction also abolishes the loss of body weight produced by morphine alone.

Many opiate agonists have been shown to produce catalepsy, including morphine (11), β-endorphin (16), dermorphin (6,21), alfentanil (10), fentanyl (8), etorphine (8), and methadone (3). The nicotinic antagonist mecamylamine potentiated and prolonged morphine's catalepsy, but it inhibited that of methadone, possibly through striatal dopamine metabolism (3). Dopamine might also mediate β-endorphin-induced catalepsy, because the severity of the rigidity was related to release of dopamine in the nucleus accumbens (16). Alfentanil-induced muscle rigidity was antagonized by β-funaltrexamine and naloxonazine, implicating μ receptor in the response (10). Naloxone (6) or SK&F 86466 (21) reversed the effect of dermorphin, suggesting the role of μ receptor in opiate catalepsy.

Ketamine have been shown to increase the effect of general anesthetics (23), and also by μ agonist, μ/κ agonist, and a μ antagonist, indicating multiple receptors involvement in catalepsy (1). Muscular rigidity, caused by a high dose of alfentanil, was prevented by pretreatment with an adrenergic agonist (22) so that there might be an interaction between the opiate system and adrenoceptors. Catalepsy induced by morphine is also blocked by the dopamine receptor agonist (4). Administration of morphine into the periaqueductal gray produced periods of catalepsy, alternating with periods of explosive motor behavior (11), suggesting opiate mediation of both response, also suggesting that the periaqueductal gray region is the primary centre for the cataleptic action of opiate compounds through the opiate receptors. We have recently observed that midazolam inhibits binding of rat spinal, μ, δ, and κ opioid receptors. The affinity of midazolam for μ, δ, and κ receptor is 22,993, 591, and 49 times less, respectively, than the affinity of morphine for these receptors (12,13). This also suggests that benzodiazepines, including midazolam, can modulate effects of opioids by interacting at periaqueductal gray region or caudate-putamen to potentiate the cataleptic effects of opioids compounds. This cataleptic effect might be due to the μ receptor or κ receptor, or both together.

For animals in the present study, the combinations of morphine and midazolam in acute and chronic treatments increases lethality, thus suggesting that low doses of midazolam might be potentiating the respiratory depressant effects of morphine, producing deaths at doses of morphine that are not normally lethal, but at higher doses of midazolam is antagonizing the respiratory depressant effects. This is also supported by McDonald et. al. (9) who report that lorazepam antagonized the respiratory depressant effect of dimorphine in intensive care patients and of morphine in mice. Dose is also important, because midazolam had a biphasic effect, with low doses increasing respiratory effect and high doses supressing it. Morphine has been shown to bind to two different receptor sites. At low concentration it binds only to central nervous system opioid receptors, but at a high concentration, it blocks $GABA_A$ receptors (7,19) involved in GABA- and benzodiazepine-mediated effects. Thus, it is possible that opposite effects produced by midazolam and morphine on GABA receptor ionophore complex are responsible for the observed antagonistic effects. In the present study, chronic treatment of midazolam prolonged the antinociceptive effects of morphine by delaying the development of morphine-induced tolerance to antinociception in both the tail-flick and hot-plate test (Figs. 4A and B). A tail-flick test measures spinal as well as central analgesia, whereas the hind paw lick test used in the hot-plate method is indicative of central analgesia, which can be observed in spinalized animals. Therefore, it is possible that midazolam may be effective in attenuating the spinal opioid tolerance but not the central opioid tolerance produced by morphine (14,18). Chronic treatment for 11 days suggests that with an increase in the midazolam dose, the lethality is increased. We also observed that lethality caused by chronic treatment of midazolam and morphine was not due to loss in the body weight, because the loss in the body weight by chronic morphine was also antagonized by midazolam. It has been shown that chronic morphine reduces body weight of animals (5). However, a larger study is needed to conclude that midazolam and morphine act at a common or different brain structures, which are involved for the cataleptic and lethal interactions between these drugs.

In conclusion, we found that midazolam and morphine show strong interaction, supporting our earlier findings (12-14,18). The interactions occur in both the acute and chronic treatment, depending upon the relative concentration of midazolam to morphine and vise versa. Combinations of midazolam and opiates are commonly used intravenously for induction of general anesthesia, due to its rapid onset and relatively short duration of action. So appropriate caution should be taken when midazolam, a benzodiazepine receptor agonist, is used in combination with the prototypic opioid agonist, morphine, as both the benzodiazepine and opiates are used for their abused reasons.

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