Pharmacology Biochemistry and Behavior, Vol. 46, pp. 331-339, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Dynorphin A(1-17) Mediates Midazolam Antagonism of Morphine Antinociception in Mice[1]

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Received 26 August 1992

RADY, J. J. AND J. M. FUJIMOTO. *Dynorphin A(1-17) mediates midazolam antagonism of morphine antinociception in mice.* PHARMACOL BIOCHEM BEHAV 46(2) 331-339, 1993.—Studies have shown that midazolam acts in the brain to antagonize the antinociception produced by morphine. The purpose of this study was to determine if spinal dynorphin A(1-17) (Dyn) was involved in the antagonistic effects of midazolam. A number of drugs when administered intracerebroventricularly (ICV) to mice release Dyn in the spinal cord to antagonize morphine-induced antinociception. In the present study using the mouse tail-flick test, midazolam administered ICV produced a dose related reduction of the antinociception induced by morphine given intrathecally (IT). The antagonistic action of midazolam against morphine-induced antinociception involved the release of Dyn in the spinal cord, as evidenced by the following results. 1) Administration of naloxone, nor-binaltorphimine and dynorphin antiserum, IT, eliminated the antagonistic effect of midazolam, given ICV, against morphine. Treatment with these opioid antagonists and dynorphin antiserum is known to inhibit the action of spinally released Dyn. 2) Production of desensitization to the effect of spinal Dyn by pretreating with morphine, 10 mg/kg subcutaneously 3 h before the tail-flick test, abolished the antagonistic action of midazolam given ICV. A 3-h pretreatment with midazolam, ICV, also produced desensitization to the antianalgesic action of Dyn given IT. 3) Elimination of the Dyn component of action of midazolam by administration of naloxone, nor-binaltorphimine and dynorphin antiserum, IT, uncovered slight antinociceptive activity of midazolam, given ICV. Coadministration of flumazenil (a benzodiazepine antagonist), bicuculline (a GABA antagonist) and picrotoxin (a chloride ion channel blocker) inhibited the midazolam effect. Thus, activation of the supraspinal GABA/benzodiazepine receptor complex by midazolam appeared to be involved in the antagonistic action of midazolam and the release of spinal Dyn.

Dynorphin antibody Intrathecal naloxone nor-binaltorphimine Antianalgesia Antinociception
Morphine Midazolam Benzodiazepine Dynorphin A(1-17)

BENZODIAZEPINES are administered along with opiates as preoperative or postoperative medication. Thus interactions between opiates such as morphine and benzodiazepines are of great interest. Even though Shannon et al. (34) reported that the antinociceptive activity of morphine is not changed by oxazepam and diazepam administered subcutaneously (SC) varying results regarding interactions between benzodiazepines and morphine are found in the literature. For instance, administration of chlordiazepoxide orally enhances morphine-induced antinociception (8). Midazolam administered intrathecally (IT) produces antinociception (25,30,42) and increases morphine-induced antinociception (42). On the other hand, administration of diazepam SC (28) and administration of diazepam, clonazepam, medazepam, nitrazepam, and flurazepam orally (8) decreases morphine-induced antinociception. Also, intracerebroventricularly (ICV) administered diazepam and midazolam decrease morphine-induced antinociception (22,30,43). These latter observations along with the report by Rosland and Hole (33) indicate that the antagonistic action of the benzodiazepines against morphine-induced analgesia arise from benzodiazepines acting on higher centers in the central nervous system. Rosland and Hole found that the antagonistic action of intraperitoneally administered diazepam on the antinociception induced by morphine, administered SC, is abolished by spinal transection in mice (33). The present studies investigate the possibility of dynorphin A(1-17) (Dyn) involvement in the antagonistic action of midazolam, given ICV, on morphine-induced antinociception.

Dyn when administered IT at a dose of 5 femtomoles in the mouse antagonizes the antinociception produced by a

[1] Supported by VA medical funds and NIDA grant DA00451.
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number of agents administered ICV (12) and morphine given IT (9). Also, a variety of agents (physostigmine, clonidine, morphine, naloxone, nor-binaltorphimine) given ICV release Dyn in the spinal cord, which can produce an antianalgesic action (9,10,13,17). For example, release of spinal Dyn explains the ability of clonidine (an α_2-adrenergic agonist), administered ICV, to antagonize the antinociception induced by morphine, given IT, in the mouse tail-flick test. The release of Dyn acts to limit the antinociceptive component of action of clonidine given by itself, ICV (9). This latent antinociceptive action can be uncovered by attenuating the action of the Dyn (9–11). The apparent similarity between the actions of clonidine and midazolam to antagonize morphine-induced antinociception raised the possibility that midazolam like clonidine may release spinal Dyn.

METHODS

Animals and Tail-flick Response

Male ICR mice, weighing 25–35 g, from Sasco Inc. (Omaha, NE) were used in all experiments. Each mouse was only used once. Antinociceptive activity of morphine and midazolam under various experimental conditions was measured using the radiant heat tail-flick test described by D'Amour and Smith (3). The lamp intensity was set to provide a predrug response time of 2–4 s (this intensity was adjusted once to allow for the predrug response time to be 2–4 s for all mice and then this one intensity was used for all experiments). The predrug time was determined on the day of the experiment. During the determination of the postdrug response time a cut-off time of 10 s was used to prevent damage to the tail. The antinociceptive response is reported here as percent maximum possible effect (% MPE) as calculated according to the formula of Dewey et al. (5):

$$\text{Percent MPE} = \frac{(\text{postdrug time} - \text{predrug time})\ 100}{(10 - \text{predrug time})}.$$

The mean % MPE ± SEM for the various groups were analyzed for differences using the Student's *t*-test (for comparisons of 2 groups) or one-way analysis of variance followed by Newman-Keuls' test (for comparisons of more than 2 groups) with $p \leq 0.05$ indicating a statistically significant difference (39).

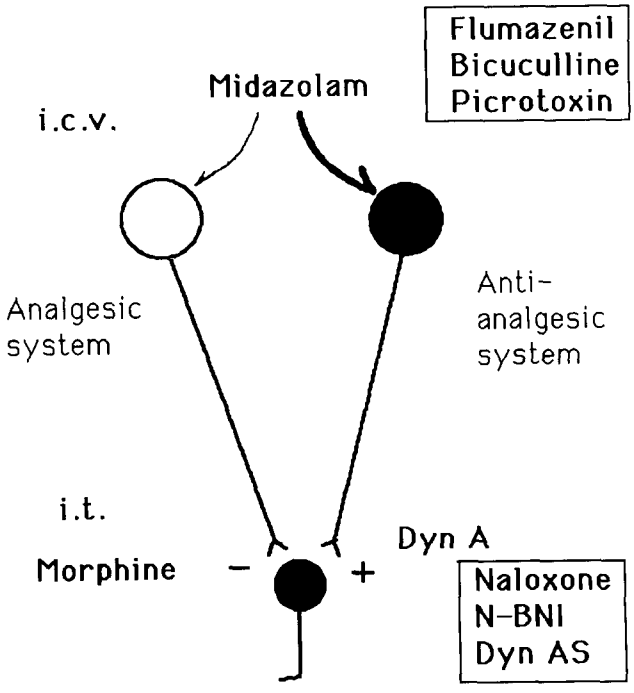

FIG. 1. Illustration of the experimental approach for determining the involvement of spinal Dyn in the antagonistic action of midazolam (Mid) were as follows. Midazolam, given i.c.v., antagonized the antinociception induced by morphine (Mor) given i.t. Reversal of this effect by agents given i.t. (bottom box nor-abinaltorphimine, N-BNI; dynorphin antiserum, Dyn AS) would indicate that Dyn A was released in the spinal cord. Experiments were included where a pretreatment with morphine, SC, produced desensitization to the antianalgesic effect of Dyn whereby the antagonistic action of midazolam was eliminated. A pretreatment with midazolam, i.c.v., was also given to determine if midazolam, could produce desensitization to the action of i.t. Dyn. Further experiments on reversal of the antagonistic effect of midazolam by agents given i.c.v. (top box) would indicate the involvement of the benzodiazepine receptor complex.

Antagonistic Effect of Midazolam and Test for Mediation by Dyn

The experimental approach is illustrated in Fig. 1. Midazolam was administered to mice ICV in a 4 μl volume by the method of Haley and McCormick (15) under light halothane anesthesia, generally 10 min before the tail-flick test. Morphine was administered IT in a 5 μl volume by the method of Hylden and Wilcox (19) 5 min before the tail-flick test. A control group given solvent vehicle ICV (in place of midazolam) and IT morphine, 1 μg at 5 min, was included for comparison. This type of protocol constituted the standard combination of treatments for showing the antagonistic action of midazolam against morphine.

To determine the dose-response relationship for midazolam antagonism of morphine the dose of midazolam given ICV was varied while the dose (1 μg) and time (5 min) of administration of morphine remained constant. The duration of the antagonistic action was determined by administration of midazolam (0.25 μg) ICV at various times before the tail-flick test while the dose and time of morphine administration remained as above.

Experiments to provide presumptive evidence for release of spinal Dyn as the basis for the antagonistic action of midazolam involved groups of mice to which naloxone, 1 pg, or nor-binaltorphimine, 10 ng, was coadministered with morphine IT. Also groups of mice were pretreated 1 h before the tail-flick test with dynorphin antiserum, 5 μg, IT. The response of these groups of mice were evaluated against the responses of groups given the standard combination of midazolam with morphine. The IT administration of naloxone, nor-binaltorphimine and dynorphin antiserum antagonizes the antianalgesic action of Dyn that is released in the spinal cord by various agents (11,13).

A further presumptive test for release of Dyn is production of desensitization to the antianalgesic action of Dyn by pretreatment with morphine, 10 mg/kg, SC (12). Groups of mice were pretreated 3 h before the tail-flick test with morphine and the antagonistic effect of ICV midazolam against IT morphine was evaluated against groups that were pretreated with saline and then given the standard combination of midazolam and morphine.

Clonidine given ICV releases Dyn and its latent antinociceptive action can be uncovered by eliminating the Dyn-mediated antianalgesic component of action by administering naloxone, nor-binaltorphimine or dynorphin antiserum IT (9,11). To test midazolam for latent antinociceptive activity, groups of mice were given midazolam, 0.5 μg, ICV at 10 min before the tail-flick test and naloxone, 1 pg, or nor-binaltorphimine, 10 ng, IT, 5 min before the tail-flick test or dynorphin antiserum, 5 μg, IT, 1 h before the tail-flick test. The responses of these groups were compared to the response of groups treated with midazolam, ICV, and solvent vehicle given IT at the appropriate time. Control serum was given in place of dynorphin antiserum.

Administration of Benzodiazepine/GABA Receptor Antagonists

Other experiments to implicate the involvement of the benzodiazepine/GABA receptor ionophore complex in the antagonistic action of midazolam were performed. Flumazenil, bicuculline, and picrotoxin were tested for their ability to eliminate the antagonistic action of midazolam against morphine. Groups of mice were given flumazenil, 0.5 μg, bicuculline, 1 μg, or picrotoxin, 0.25 μg, in the same solution as the ICV dose of midazolam. The responses of these groups were compared to responses to the standard combination of midazolam with morphine.

Experiments were also performed to confirm that midazolam given IT produces antinociception (25,30,42) and that the antagonistic action of ICV midazolam was not due to diffusion to the spinal cord.

Source of Drugs

Drugs were obtained from the following sources: morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO); midazolam free base and flumazenil (Ro15-1788) were gifts from Hoffmann-La Roche (Nutley, NJ); (+)-bicuculline and picrotoxin (Sigma Chemicals, St. Louis, MO); dynorphin A (1-17) (Peninsula Laboratories, Belmont, CA); naloxone hydrochloride (National Institute on Drug Abuse, Rockville, MD) and nor-binaltorphimine dihydrochloride (Research Biochemicals, Inc., Natick, MA). Dynorphin antiserum and control serum were produced by the method of Hollt et al. (16) and were characterized as previously published (10,11). All drugs except for midazolam and Dyn were dissolved in a 0.9% NaCl solution. Midazolam was partially dissolved in a 0.9% NaCl solution and adjusted to a final pH of 3.5 with hydrochloric acid to complete dissolving. Dyn was dissolved in a 0.01% Triton X-100 solution in 0.9% NaCl solution. Doses stated hereafter refer to the drugs in the forms above. Times and doses for drug administration were known from previous literature (9-13,17) or determined in preliminary experiments. Time, dose, and route of administration are stated with each experiment.

RESULTS

Antagonistic Effect of Midazolam Given ICV on the Antinociception Induced by Morphine Given IT

To determine the dose-response relationship of the antagonistic effect of midazolam, the dose (1 μg) and time (5 min) of administration of morphine IT were kept constant while the dose of midazolam administered ICV at 10 min was varied (Fig. 2A). Midazolam produced a dose-dependent, biphasic effect on morphine-induced antinociception. The greatest inhibition occurred with a 0.25-μg (0.77 nmol) dose of midazolam. At doses of 1 and 2 μg ICV the antagonistic effect against morphine decreased. The question of whether the dose of morphine used was too high to see antagonism by the lower doses of midazolam was addressed by using a lower dose of morphine (0.5 μg). This dose of morphine given IT, produced a % MPE of 65.2 ± 7.5%. When midazolam was given ICV at a dose of 0.05 μg, against this dose of IT morphine the % MPE was not altered (64.7 ± 10.0%). Therefore, the lower dose of midazolam did not produce antagonism even under a more favorable condition. To determine the time course of the antagonistic action of midazolam, the time of administration of midazolam (0.25 mg) was varied while the dose and time of administration of morphine were kept constant. The antagonistic activity of midazolam lasted for less than 45 min (Fig. 2B).

Vehicle was given in place of the agent in control experi-

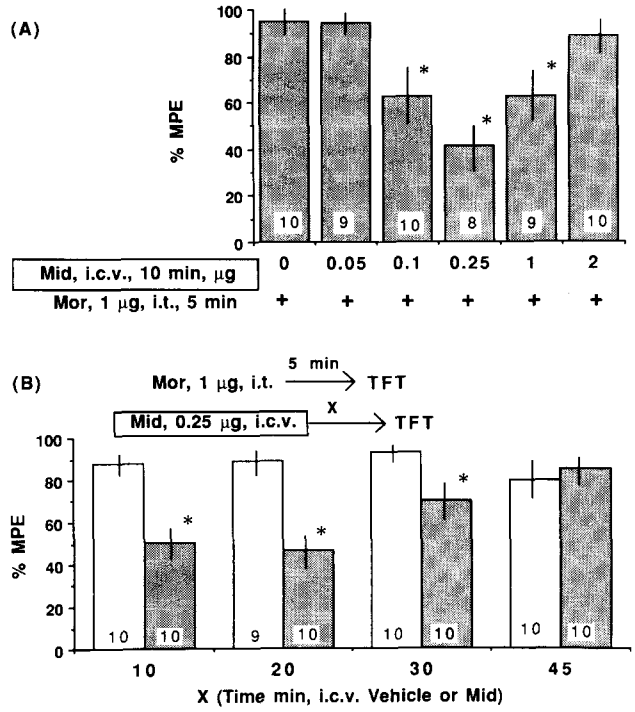

FIG. 2. Dose-response and time-response relationship for the antagonistic activity of i.c.v. midazolam (Mid) given against i.t. morphine (Mor) inhibition of the tail-flick test in mice. (A) The antagonism of morphine-induced antinociception by the administration of midazolam, i.c.v., was biphasic. *Significantly different from control group (group 1, morphine alone) using ANOVA and Newman-Keuls' test; $p \leq 0.05$. In this and subsequent figures the groups are numbered 1,2, . . . , n from left to right with the left-most bar as group 1; the vertical lines at the top of the bars denote the SEM; the number at the base of the bars indicates the number of mice; a + under the bar indicates that the drug stated on the left was administered. (B) The duration of antagonistic activity of midazolam was determined by altering the time (X) of administration of midazolam, i.c.v., while the time for administration of morphine i.t. remained constant. Open bars depict groups in which vehicle was administered i.c.v. with the morphine i.t. and shaded bars depict groups that received midazolam i.c.v. with the morphine i.t. *Significantly different from the control group (open bar to the immediate left) using Student's t-test; $p \leq 0.05$.

ments. Saline given IT in place of morphine and acidified saline (pH 3.5), the vehicle for midazolam, given ICV in place of midazolam, had no effect on the tail-flick latency (10.1 ± 6.7 % and 12.1 ± 3.6 %, respectively). Midazolam given alone ICV at the doses used in the above experiment produced minimal antinociception (Table 1).

In the remainder of the experiments, the primary hypothesis tested was that midazolam antagonized morphine-induced antinociception by activating the release of Dyn from the spinal cord.

Modification of the Antagonistic Effect of Midazolam by Administration of Naloxone, Nor-binaltorphimine and Dynorphin Antiserum, IT

The antianalgesic effect of Dyn released spinally after administration of a variety of agents ICV is abolished by the administration of naloxone or nor-binaltorphimine IT (9,13). The antagonistic effect of midazolam, given ICV, was again obtained against morphine given IT (Fig. 3A, group 1 vs. 2). This effect of midazolam was abolished by the administration of either naloxone or nor-binaltorphimine IT (group 3 and 4 vs. 2). The antagonistic effect of midazolam against morphine was also eliminated by administration of dynorphin antiserum IT (Fig. 3B, group 3 vs. 2). It has been shown previously that control serum and dynorphin antiserum attenuated by prebinding in vitro with Dyn have no effect on Dyn activity (11). Thus, the results indicated that spinal Dyn release was involved in the antagonistic actions of midazolam administered ICV.

Another feature of the action of naloxone, nor-binaltorphimine and dynorphin antiserum administered IT is that they may uncover a latent antinociceptive action of certain agents, like clonidine, which have biphasic actions (9–11). That is, by antagonizing the antianalgesic action of the released Dyn, naloxone, nor-binaltorphimine and dynorphin antiserum administered IT may uncover the latent antinociceptive action, which occurs simultaneously with the antianalgesic action. The results illustrated in Fig. 4A indicated that IT administered naloxone and nor-binaltorphimine uncovered the latent antinociceptive action of midazolam given by itself ICV. Note that the dose of midazolam used in this experiment (0.5 μg) is higher than that used in the previous experiments (0.25 μg). When the lower dose of midazolam was used no antinociceptive activity was found (data not given). A 4-μg dose of midazolam given ICV produced a % MPE of approximately 11.1 ± 6.0 %, however, when naloxone was given IT along with the midazolam the % MPE increased to 56.0 ± 10.8 %.

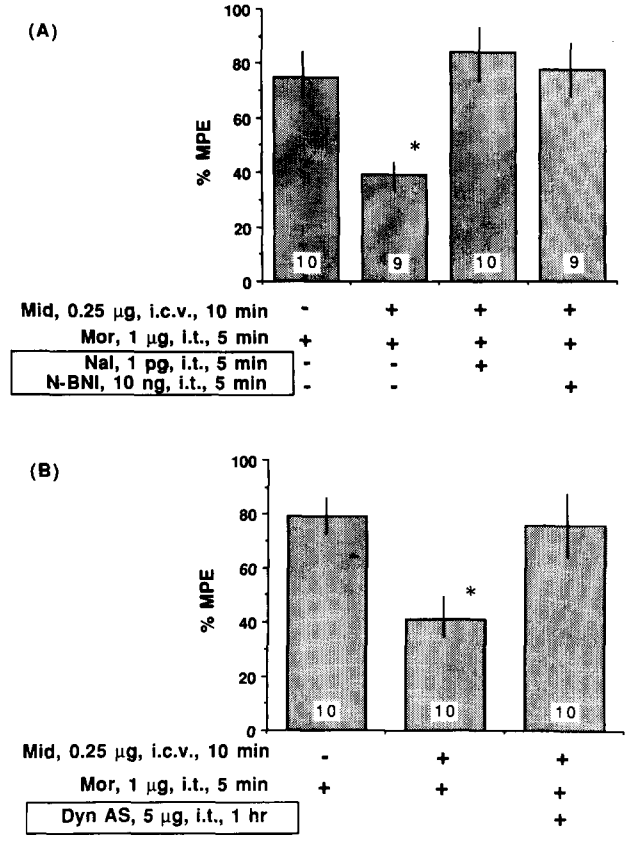

FIG. 3. Inhibition of the Dyn-mediated antianalgesic effect eliminated the antagonistic actions of midazolam administered i.c.v. (A) Midazolam administered i.c.v. decreased the antinociceptive activity of morphine given i.t. (group 2 vs. 1). This antagonism was eliminated by coadministration of naloxone (Nal; group 3 vs. 2) and nor-binaltorphimine (N-BNI; group 4 vs. 2) with morphine. *Significantly different from all other groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$. In this and subsequent figures a – under the bar indicates that the appropriate vehicle was administered. (B) The antagonism of morphine antinociception by midazolam, i.c.v., (group 2 vs. 1) was also eliminated by administration of dynorphin antiserum (Dyn AS) i.t. 1 h before the tail-flick test (group 3 vs. 2). *Significantly different from other groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$.

TABLE 1

THE ANTINOCICEPTIVE EFFECT OF VARIOUS DOSES OF MIDAZOLAM GIVEN ALONE ICV 10 MIN BEFORE THE TAIL-FLICK TEST

Dose μg	%MPE ± SEM*	n
0.5	16.2 ± 4.7	10
1	16.0 ± 3.9	9
2	15.8 ± 3.1	10
4	27.0 ± 4.6	10

*Percent maximum possible effect as calculated according to Dewey et al. (5).

Therefore, ICV midazolam did produce a significant antinociceptive response when the antianalgesic system was blocked. Figure 4B presents an experiment in which dynorphin antiserum administered IT as a 1-h pretreatment also was able to uncover the latent antinociceptive action of midazolam given ICV. These results further indicated that midazolam administered ICV released spinal Dyn. These results are similar to those observed with clonidine given ICV (9–11).

Desensitization to Dyn and Elimination of the Antagonistic Action of Midazolam, Given ICV, on Morphine-induced Antinociception

A 3-h pretreatment with 10 mg/kg of morphine, SC, produces desensitization to the antianalgesic action of Dyn and thus to the antianalgesic component of the action of agents which release Dyn (12). In the present experiment, the inhibi-

tion of morphine-induced antinociception by administration of midazolam ICV was eliminated by pretreating the mice with morphine, 10 mg/kg SC (Fig. 5A). Note that this morphine pretreatment did not affect the activity of the morphine which was subsequently administered. Thus, the effect of the morphine pretreatment was attributed to an elimination of the antagonistic action of midazolam.

The results given in Fig. 5B demonstrated the ability of midazolam given ICV to produce desensitization to the antianalgesic action of Dyn administered IT. Comparison of groups 1 vs. 2 indicated that Dyn, 10 pg (5 femtomole) given IT antagonized morphine-induced antinociception. A 3-h pretreatment with midazolam ICV produced desensitization to the antagonistic effect of Dyn. This desensitization to Dyn was consistent with midazolam pretreatment releasing Dyn in the spinal cord.

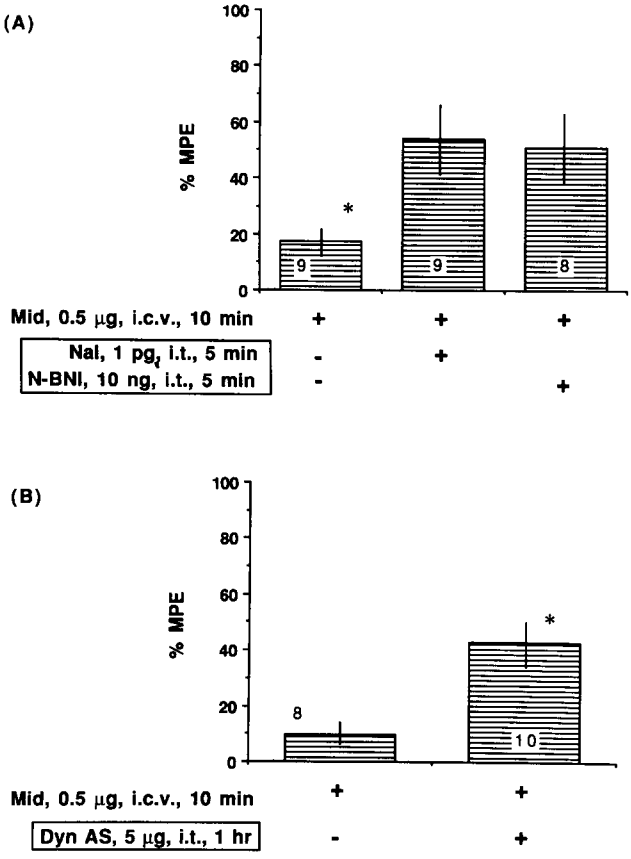

FIG. 4. Elimination of the Dyn-mediated antianalgesic effect uncovered the apparent antinociceptive activity of i.c.v. midazolam. Administration of midazolam alone produced minimal antinociception. (A) The response to i.c.v. midazolam plus i.t. saline is shown by group 1. Compared to this group, an increased antinociceptive response was obtained with midazolam when opioid antagonists (naloxone, Nal and nor-binaltorphimine, N-BNI) were administered i.t. (group 1 vs. 2 and 3). *Significantly different from other two groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$. (B) Similarly, an antinociceptive response to midazolam administered i.c.v. was also produced by the administraion of dynorphin antiserum i.t. 1 h before the tail-flick test. *Significantly different from the i.c.v. midazolam plus i.t. saline group using Student's t-test; $p \leq 0.05$.

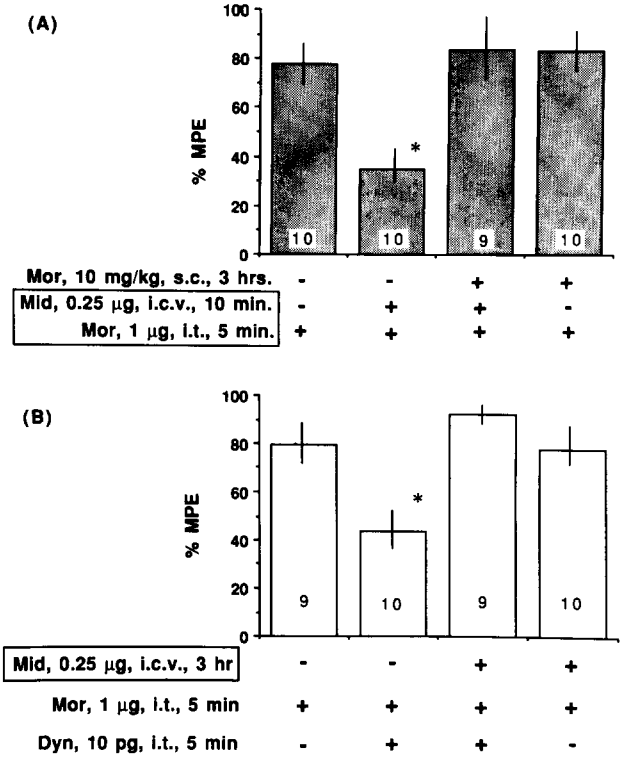

FIG. 5. Production of desensitization to the antianalgesic effect of Dyn eliminated the antagonistic activity of midazolam. (A) Midazolam, i.c.v., antagonized the antinociceptive activity induced by i.t. morphine (group 2 vs. 1). This antagonism was eliminated by pretreatment with morphine, s.c., 3 h before the tail-flick test (group 3 vs. 2). Morphine pretreatment did not alter the antinociceptive activity of the morphine administered i.t. (group 4 vs. 1). *Significantly different from the other groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$. (B) Coadministration of Dyn with morphine, i.t. reduced morphine-induced antinociception (group 2 vs. 1). The activity of the morphine was restored by pretreating with midazolam, i.c.v., 3 h before the tail-flick test (group 3 vs. 2). The pretreatment with midazolam did not alter the antinociceptive action of morphine given i.t. (group 4 vs. 1). *Significantly different from other groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$.

Experiments to Implicate the Involvement of the Benzodiazepine Receptor Ionophore Complex in the Antianalgesic Action of Midazolam

The activation of the antianalgesic system by midazolam may result from interaction of midazolam with the benzodiazepine/GABA receptor chloride ion channel complex. As shown in the previous experiments, midazolam administered ICV reduced the antinociceptive activity of morphine given IT (Fig. 6A, 6B, and 6C). Midazolam-induced antagonism of morphine was inhibited when flumazenil (Ro15-1788), a benzodiazepine receptor antagonist, was coadministered with midazolam ICV (Fig. 6A). Reversal of the antagonistic effect of midazolam was also seen when bicuculline, a $GABA_A$ receptor antagonist, (Fig. 6B) and picrotoxin, a chloride ion channel blocker, (Fig. 6C) were administered along with the midazolam ICV. Thus, blocking the various parts of the benzodiazepine/GABA receptor ionophore complex inhibited the antagonistic activity of ICV midazolam.

Antinociceptive Activity of Midazolam Administered IT

Midazolam administered IT produces a dose-dependent reduction in the tail-flick response (Fig. 7A). This is in contrast to the lack of overt antinociceptive activity observed with administration of midazolam ICV. A 0.15-μg dose of morphine given IT and a 1-μg dose of midazolam given IT produced antinociceptive responses that were equal (Fig. 7B, groups 1 and 2). When midazolam and morphine are coadministered IT, at half the dose of each given alone, the antinociceptive response was much greater than the response observed for each drug given by itself at the whole dose (Fig. 7B, group 3). Therefore, the interaction between morphine and midazolam when both are given IT was synergistic (more than simply additive), which contrasted the antagonistic interaction observed between ICV midazolam and IT morphine. The antinociceptive response to IT administered midazolam, 1 μg (38.5 ± 7.2%) was not altered by the administration of 1 pg of naloxone (49.0 ± 10.5%) and 10 ng of nor-binaltorphimine IT (57.0 ± 10.9%). Thus, midazolam given IT was antinociceptive and did not release Dyn spinally.

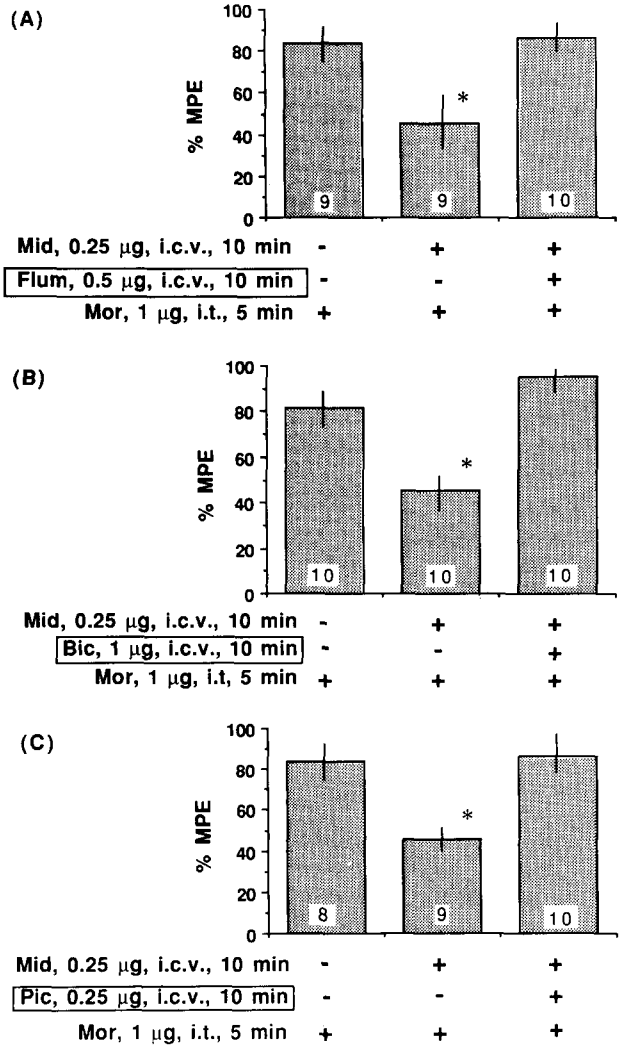

FIG. 6. The antagonistic action of i.c.v. midazolam involved the participation of the benzodiazepine/GABA receptor ionophore complex. Antagonism of morphine-induced antinociception by midazolam depicted in panel A, B, and C (group 2 vs. 1) was inhibited (group 3 vs. 2) by coadministration, with i.c.v. midazolam, of (A) a benzodiazepine antagonist, flumazenil (Flum, Ro15-1788); (B) a GABA antagonist, bicuculline (Bic); and (C) a chloride ion channel blocker, picrotoxin (Pic). *Significantly different from the other two groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$.

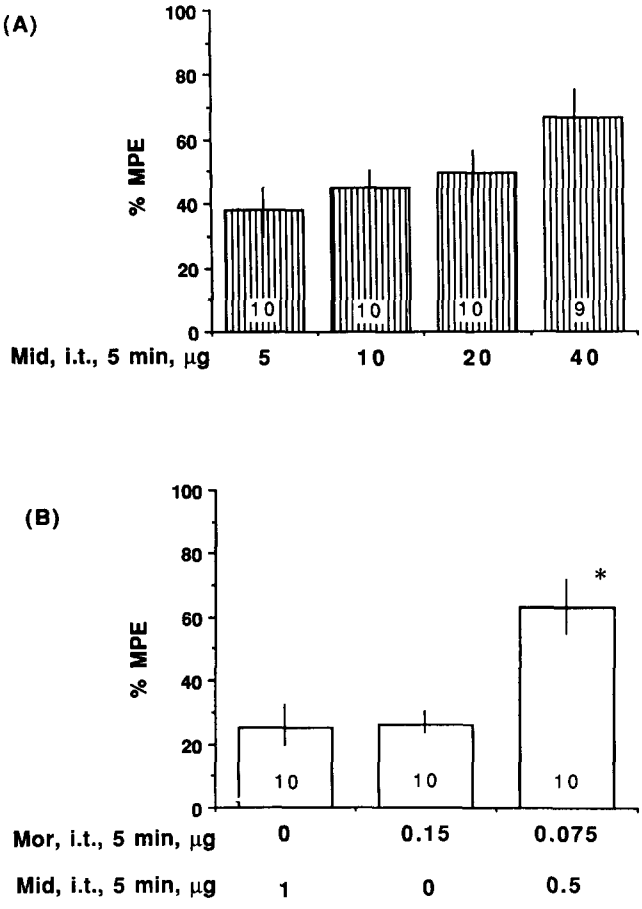

FIG 7. Midazolam administered i.t. produced antinociceptive activity. (A) Administration of midazolam i.t. produced a dose-dependent antinociceptive response. (B) Administration of morphine i.t., 0.15 μg, (group 1) or midazolam, 1 μg, (group 2) produced a small but equal antinociceptive effect. Coadministration of half of the dose of each compound (0.075 μg of morphine and 0.5 μg of midazolam) produced an antinociceptive response, which was greater than either compound given alone at the full dose (groups 1 and 2 vs. 3). *Significantly different from other groups using ANOVA and Newman-Keuls' test; $p \leq 0.05$.

DISCUSSION

Evidence that the Antagonistic Action of ICV Administered Midazolam Against IT Administered Morphine Involves Spinal Dyn Action

As mentioned in the introduction, midazolam produces little antinociception and possesses antagonistic activity after administration into the brain. Spinal administration, however, results in antinociception. These similarities to clonidine led to the hypothesis that midazolam, like clonidine, produced

the antagonistic actions by activating the Dyn-mediated antianalgesic system. The experiments to determine spinal Dyn involvement in the antagonistic activity of midazolam were performed in this study.

Evidence for release of spinal Dyn induced by midazolam, given ICV, was provided first by experiments with opioid antagonists and dynorphin antiserum. The opioid antagonists, naloxone and nor-binaltorphimine, administered IT, at low doses, abolished the antagonistic action of midazolam against morphine-induced antinociception. This finding is in line with inhibition of Dyn-induced antianalgesic action by doses of naloxone and nor-binaltorphimine, which respectively do not inhibit spinal mu and kappa receptors (9,13).

Next, a 1-h pretreatment with dynorphin antiserum was effective in attenuating the antagonistic action of midazolam against morphine-induced antinociception. The antibody presumably binds the Dyn so that the Dyn is inactivated (10,11). Also, both IT administered naloxone and nor-binaltorphimine and IT administered dynorphin antiserum treatment uncovered an antinociceptive action of midazolam, given ICV Inhibiting the activity of Dyn released in the spinal cord resulted in the antinociceptive activity of ICV administered midazolam to be manifested, a characteristic similar to clonidine (9–11). That is, even though midazolam, like clonidine, had little antinociceptive action by itself, ICV (Table 1), inhibition of the antianalgesic component of action increased the antinociceptive activity (Fig. 4). The antinociceptive activity may not be large, however, there was a two- to threefold increase in the antinociceptive response induced by midazolam when the antianalgesic system was blocked. The antagonists alone given IT do not affect the tail-flick response (13). Also, naloxone, nor-binaltorphimine and dynorphin antiserum, as used here, do not enhance the antinociceptive action of [D-Pen^2-D-Pen^5]-enkephalin and β-endorphin because these agonists do not release spinal Dyn (10,11, unpublished data). Therefore, the results were interpreted as demonstrating a latent antinociceptive action of ICV midazolam.

In addition, ICV midazolam, like clonidine, acted on the antinociception induced by IT morphine with a biphasic dose-response curve. Thus, the biphasic dose-response relationship for midazolam, given ICV, is consistent with midazolam possessing both an antinociceptive and antianalgesic component of action like that found previously for clonidine, given ICV (9–11). Even though the antinociceptive activity of midazolam may be small, it could be enough to interact in an additive or more than additive fashion with morphine. Therefore, as the dose of midazolam increases the interaction between morphine and midazolam may become more of an additive or more than additive interaction allowing the antinociception to overcome the antianalgesic activity of the lower doses of midazolam. More work is necessary to show that midazolam has an antinociceptive effect by acting in the brain. The magnitude and the relationship between the antianalgesic and antinociceptive components of action require further delineation.

A second explanation for this biphasic effect is that at the larger doses midazolam may interact with morphine to produce a major depressant effect rather than reversal of antianalgesia. However, at the doses of midazolam used in the experiments, the mice appeared to behave normally and did not display any outward signs of central nervous system depression.

Results from experiments on production of desensitization to Dyn provided further support for possible Dyn release induced by ICV administered midazolam. We have shown previously that a 3-h pretreatment with morphine, 10 mg/kg, SC, presumably releases spinal Dyn and produces desensitization to Dyn (12). A 3-h pretreatment with Dyn IT also produces desensitization to subsequently administered Dyn (12). Therefore, agents that release Dyn should produce desensitization to the antianalgesic effects of Dyn. In the present experiment, a 3-h pretreatment with morphine SC eliminated the antagonism produced by ICV administered midazolam against IT administered morphine. In parallel fashion, a 3-h pretreatment with midazolam ICV produced desensitization to the antianalgesic action of Dyn, administered IT. All of the results were consistent with the possibility of ICV administered midazolam releasing Dyn in the spinal cord to antagonize the antinociceptive action of morphine administered IT. We have referred to this type of antagonistic action as the antianalgesic action of Dyn.

These results somewhat parallel the results of Rosland and Hole (33). Their work demonstrates that disconnecting the spinal cord from supraspinal communication eliminated the antagonistic effect of systemically administered benzodiazepines against morphine. This suggests that a descending pathway from the brain to the spinal cord may be necessary to produce the antagonism. Therefore, it seems possible that the descending antianalgesic system mediated by Dyn could be this pathway.

Benzodiazepine/GABA Receptor Complex Involvement in the Antianalgesic Action of Midazolam

Benzodiazepine receptors are present in the brain (1,23,38). The benzodiazepine receptor is part of a receptor complex along with the GABA receptor (2,29,35,40). This complex also contains a picrotoxin sensitive chloride channel (21,26). In the present experiments, manipulation of the function of this complex with antagonists helped to demonstrate that midazolam interacted with this complex to produce the antianalgesic effects. The coadministration of flumazenil, a benzodiazepine antagonist (18,24), with midazolam ICV eliminated the antagonistic effect of midazolam against morphine-induced antinociception. Similarly, the effect of midazolam was inhibited by coadministration of bicuculline, a GABA antagonist, and picrotoxin, a chloride channel antagonist, ICV. Thus, midazolam administered ICV interacted with the benzodiazepine/GABA receptor ionophore complex in the brain to trigger the antianalgesic action.

Evidence exists for activation of the benzodiazepine/GABA receptor complex being involved in an antagonistic action against morphine-induced analgesia. Sivam and Ho (36) have concisely summarized the interactions that occur between morphine and GABA agonists and antagonists. Generally, increases in GABA concentration in the brain whether induced by inhibitors of GABA degradation or GABA agonists inhibit morphine analgesia. Furthermore, Drower and Hammond (7) report that 4,5,6,7-tetrahydroisoxazolo (5,4-c) pyridin-3-ol (THIP), a $GABA_A$ receptor agonist, produces hyperalgesia in rats when microinjected into the nucleus raphe magnus and nucleus reticularis gigantocellularis pars alpha of rats. Similarly, the periaqueductal gray matter and the nucleus raphe dorsalis are sites at which antagonism can be elicited (4,32). Barbiturates also antagonize morphine-induced antinociception (20,27) and this antagonism also occurs through activation of supraspinal GABA receptors (6).

Other Possible Mechanisms for Midazolam Antagonism of Morphine as Suggested by the Literature

Niv et al. (25) reported that systemic administration of midazolam produces hyperalgesia and Rattan et al. (30) could

produce hyperalgesia with large doses of midazolam given IT in rats. Therefore, hyperalgesia as a mechanism of action for midazolam was considered. From our dose-response relationship for the effect of midazolam, given ICV against morphine-induced antinociception, the greatest amount of antagonism was observed at the 0.25-μg dose of midazolam. It would seem reasonable that this would be the dose at which hyperalgesia would be found. We attempted to find an overt hyperalgesic effect in the tail-flick test by presetting the control tail-flick latency response time to 6–8 s. We were not able to show a reduction in tail-flick latency to midazolam administered ICV (unpublished data). Also, at doses as high as 40 μg, midazolam given IT produced antinociception. At a smaller dose, 0.5 μg, midazolam produced synergism with IT administered morphine much like IT administered clonidine (31,37,41). (At the 0.25-μg dose used ICV, it seems unlikely that sufficient midazolam would reach the spinal cord to produce a latent hyperalgesia.) Thus, we felt that hyperalgesia was not an explanation for the antagonistic action of midazolam.

Rattan et al. (30) suggest that midazolam binds to opioid receptors and displacement of the morphine may be a mechanism for antagonism. In our experiment, the midazolam was given ICV and the morphine IT, at different sites, so that such an interaction would be unlikely.

In summary, midazolam administered ICV interacted with a benzodiazepine/GABA receptor ionophore complex in the brain. This interaction activated an antianalgesic system, which resulted in Dyn release in the spinal cord. The antianalgesic action of Dyn in the spinal cord appears to be the main mechanism for the antagonism of morphine-induced antinociception.

This knowledge may be helpful for clinical settings where midazolam and opiates are given together as preoperative or postoperative medication. It may allow for the blockade of midazolam antagonsim of morphine antinociception without altering the other actions of midazolam or the antinociceptive effect of morphine, resulting in better pain management. Another aspect is that the number of agents that activate the Dyn-mediated antianalgesic system is increasing. The types of agents that activate this system are quite varied and involve many different neuronal pathways to produce their main effects. However, activation of the Dyn-mediated antianalgesic system produces a common link between these various drug systems implying that a common pathway may be stimulated by all of the particular agents to induce antianalgesia. Preliminary studies also suggest that supraspinal benzodiazepine activity may be involved in the antianalgesic action of Dyn (14). These aspects require further investigation and make the interaction between opiates and benzodiazepines very intriguing.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Jerry Sepinwall, Hofman-La Roche Inc., Nutley New Jersey and Dr. I. K. Ho, University of Mississippi Medical Center, Jackson, MS for their helpful suggestions regarding the work.

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