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Opioid Antagonists: Indirect Antagonism of Morphine Analgesia by Spinal Dynorphin A

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AKSU, F., B. B. HOLMES AND J. M. FUJIMOTO. *Opioid antagonists: Indirect antagonism of morphine analgesia by spinal dynorphin A.* PHARMACOL BIOCHEM BEHAV 45(2) 409–418, 1993.—Naloxone and norbinaltorphimine when given ICV to mice can antagonize IT morphine-induced analgesia indirectly by releasing spinal dynorphin A(1-17) (Dyn A). Dyn A produces an antianalgesic action against IT morphine. In the present study, drugs with varying amounts of opioid antagonist to agonist action (nalbuphine, levallorphan, naltrexone, and naltrindole) were given ICV to determine whether they antagonized IT morphine-induced inhibition of the tail-flick response as an indication of spinal Dyn A release. Additional pharmacological tests were used as criteria for Dyn A release: a) Small doses of the opioid antagonists naloxone and norbinaltorphimine administered IT inhibited the antagonistic action; b) dynorphin antiserum given IT blocked the action of Dyn A; c) desensitization to the effect of Dyn A was produced by 3-h pretreatment with morphine, 10 mg/kg SC, or by pretreatment with the agents themselves. When given ICV, nalbuphine, levallorphan, and naltrexone released Dyn A in the spinal cord to produce an antianalgesic effect. Naltrindole, a δ-receptor antagonist, did not release Dyn A. Dyn A release did not appear to involve δ-receptors. Thus, a number of opioid antagonists inhibit the analgesic action of opioid agonists indirectly through Dyn A release.

Opioid antagonists Spinal dynorphin A release Spinal morphine

OPIATE antagonists such as naloxone are highly efficacious in treating opiate overdose. They are remarkable in that they produce few side effects in themselves in drug-free subjects but in opiate-dependent subjects are potent in precipitating opiate withdrawal signs. Naloxone is used as an experimental tool to assess the involvement of endogenous opioids in physiological processes. The interaction between opiate antagonists and agonists occurs competitively at opioid receptors although interactions at allosteric sites and nonequilibrium antagonism are possible (52–55). Other types of interactions such as receptor and competitive dualism may be implicated (34,35). Recently, we described an indirect mechanism of antagonism (25). ICV administration of naloxone (a μ-opioid receptor antagonist that acts on δ- and κ-receptors at higher doses) and norbinaltorphimine (N-BNI), a κ-receptor antagonist, indirectly antagonize IT morphine-induced analgesia in the mouse tail-flick test by activating an antianalgesic system in the brain to release dynorphin A (1-17) (Dyn A) spinally. Spinal Dyn A antagonizes morphine-induced analgesia; such diverse agents as clonidine (14,15), physostigmine (18), and midazolam (42) given ICV release spinal Dyn A. This antagonistic action of Dyn A is described as an "antianalgesic" action and activation of an antianalgesic system in the brain is thought to lead to the release of Dyn A in the spinal cord. Seminal work by others has shown that dynorphin (1-13) (31) and des-tyrosine-dynorphin (57) have antianalgesic actions. In our hands, the antianalgesic potency of Dyn A (fmol doses) was much greater than these agents (nmol doses) (43).

In the present study, we considered the possibility that agents other than naloxone and N-BNI, which possess varying amounts of opioid antagonist to agonist activity, might release spinal Dyn A when given ICV. For this purpose, the opioid antagonists levallorphan, naltrexone, and naltrindole were chosen to see if they produce an indirect antagonistic action. Nalbuphine, a mixed-agonist-antagonist but used primarily as an analgesic agent, was included.

Levallorphan is an opioid antagonist (1). Despite its predominant opioid antagonist properties at μ-receptors, it has some analgesic agonist action (32). However, the analgesic action is not useful because of it can produce dysphoric effects that may be due to κ-agonist action (34). Levallorphan is equipotent in antagonizing μ- and κ-agonists in a shock titration procedure in monkeys (12).

Naltrexone, an oxymorphone derivative, is an opioid an-

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tagonist that is more potent than naloxone and has a longer duration of action (5). Naltrexone has high affinity for μ-receptors with lesser affinity for κ- and δ-receptors (19,45,53). Naltrindole is a potent and δ-opioid receptor antagonist and is a derivative of the antagonist naltrexone (2,41,40,55).

Nalbuphine is an analog of oxymorphone and is a mixed agonist-antagonist (13,47). At equianalgesic doses, nalbuphine shows greater antagonistic activity than the other mixed agonist-antagonists such as pentazocine, butorphanol, and buprenorphine (47). Nalbuphine produces antagonistic effects at μ-receptors and agonistic effects at κ-receptors (9,12,39,47). Even though it is a potent analgesic agent, it is remarkable in that dysphoria is minimal despite its antagonistic properties.

In the present study, the experimental approach involved ICV administration of the test agents in mice using the tail-flick test. If spinal Dyn A were released, then this Dyn A would antagonize the analgesia induced by IT morphine (Fig. 1). Further presumptive tests for spinal Dyn A release involved antagonism of the action of Dyn A by IT administration of naloxone, N-BNI, or dynorphin antiserum. Also, pretreatment with morphine SC was used to produce desensitization to the antianalgesic action of Dyn A. The results of this study indicated that levallorphan, naltrexone, and nalbuphine given ICV released spinal Dyn A; however, naltrindole did not.

METHOD

Animals and Measurement of Antinociceptive Response

Male ICR mice, weighing 25–35 g, from Sasco Laboratories (Omaha, NE) were used. Each mouse was used only once. The antinociceptive response to drugs under various experimental conditions was measured with the radiant heat tail-flick test (TFT) (8). The lamp intensity was set to provide a predrug response time of 2–4 s. A cutoff time of 10 s was used to prevent damage to the tail. The percent maximum possible effect (% MPE) was calculated for each mouse using the following formula (10):

$$\% \text{ MPE} = (\text{postdrug time} - \text{predrug time})\, 100/(10 - \text{predrug time})$$

The mean % MPE was calculated for each group and compared statistically.

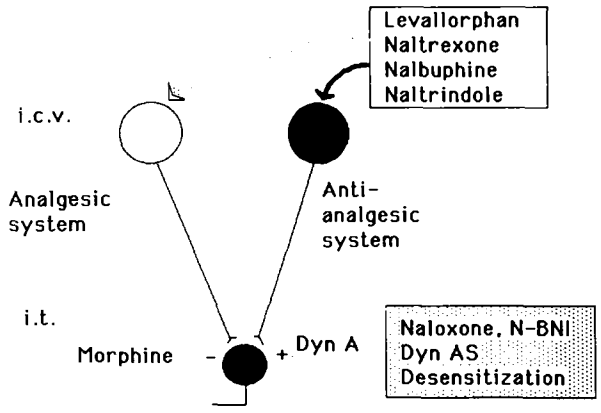

FIG. 1. Illustration of the conceptual approach used for the experiments. Test agents (opioid antagonists, top box) were given ICV. If they release Dyn A by activation (bold arrow) of an antianalgesic system, antagonism of IT morphine-induced analgesia occurred. The antianalgesic action of Dyn A can then be inhibited by small doses of naloxone, N-BNI, and dynorphin antiserum given IT (bottom stippled box) or by pretreatment with morphine SC, which produces desensitization to the subsequent action of Dyn A. Even though the antianalgesic system is pictured as a descending system, no corresponding anatomic evidence exists for such a representation. The opioid antagonists given ICV may also activate an analgesic system (dotted arrow) that represents multiple pathways such as descending noradrenergic and serotonergic controls involved in modulating the nociceptive response in the tail-flick test (4).

Drug Administrations and Statistical Analysis

Levallorphan, naltrexone, and naltrindole were administered ICV 10 min before the TFT to determine if these agents inhibited the antinociceptive effect of morphine given IT 5 min before the TFT. Inhibition of IT morphine-induced antinociception was taken as the first indication of spinal Dyn A release. In the case of ICV nalbuphine, IT methysergide was given to decrease the analgesic component, making it easier to determine if there was an antianalgesic component. The dose and the duration of action of the agents under test were determined in other experiments. Volumes of 4 and 5 ml were used, respectively, for ICV (21) and IT (27) injections. All nonpeptide drugs were dissolved in 0.9% sodium chloride solution. Dyn A was dissolved in 0.01% Triton X-100 in 0.9% sodium chloride solution. Appropriate vehicles were given in control groups.

The major approaches used to test for the presumptive release of spinal Dyn A are illustrated in Fig. 1 based upon concepts that were previously developed (16-18). Dynorphin antiserum reverses the antianalgesic action of spinal Dyn A by binding to the Dyn A (15,16). Dynorphin antiserum or control antiserum were administered IT 1 h before the TFT. Administration of a small dose of naloxone or N-BNI,IT,5 min before the TFT inhibits the antianalgesic action of Dyn A. Another means of eliminating the antianalgesic action of Dyn A is a 3-h pretreatment with morphine, 10 mg/kg SC, to produce desensitization to Dyn A (17,25). The adequacy of this pretreatment was tested by giving IT morphine and IT Dyn A. By the same token, if the agents under test (levallorphan etc., ICV) release spinal Dyn A pretreatment with that agent should also produce desensitization to the antianalgesic action of Dyn A. Doses for the standard IT agents (Dyn AS, naloxone, N-BNI, naltrexone, Dyn A, and morphine) were the same as in previous published studies (14–18).

The data were analyzed by Student's t-test for comparison of two group means; analysis of variance (ANOVA) followed by Newman-Keuls' test was used for multiple comparisons of means and Dunnett's test was used for comparisons of multiple group means to one group mean (50).

Sources of Drugs

Drugs were obtained from the following sources: nalbuphine HCl (Endo Laboratories, Garden City, NY); levallorphan tartrate (Hoffmann-La Roche, Nutley, NJ); naltrexone HCl (National Institute on Drug Abuse, Rockville, MD); naltrindole HCl and norbinaltorphimine dihydrochloride (Research Biochemical Inc., Natick, MA); morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO); dynorphin A (1-17) (Peninsula Laboratories, Inc., Belmont, CA); naloxone HCl (Dupont Pharmaceuticals, Garden City, NY); methysergide maleate (Sandoz Pharmaceuticals, East Hanover, NJ); [D-Pen^{2,5}]-Enkephalin (DPDPE) (Bachem California, Torrance, CA). Dynorphin antiserum (Dyn AS) and control serum (Con AS) were produced in rabbits (16,24) and were previously

characterized (16). Time, dose, and route of administration of the agents are stated with each experiment. The doses stated were for the form of the drugs as given above.

RESULTS

Dose-Response and Time Course of ICV Levallorphan- and Naltrexone-Induced Antagonism of Antinociception Induced by IT Morphine

Levallorphan given ICV antagonized IT morphine-induced antinociception in the tail-flick test in a limited manner (Fig. 2A). The greatest antagonism was obtained at 5 ng and increasing the dose to 10 ng and 1 μg did not increase the antagonism. Using a 5-ng dose of levallorphan IT but changing the times of administration, the antagonistic action was found to decrease rapidly. The antagonistic action of ICV levallorphan against IT morphine was not significant at 30 min (Fig. 2B). Levallorphan, 5 ng, alone had no effect on tail-flick latency; at higher doses (1, 10, 20, and 30 μg), minimal analgesia was seen that ranged from 25.6 ± 8.5 to 37.4 ± 3.7 %MPE (mean ± SE) using 9–10 mice per group.

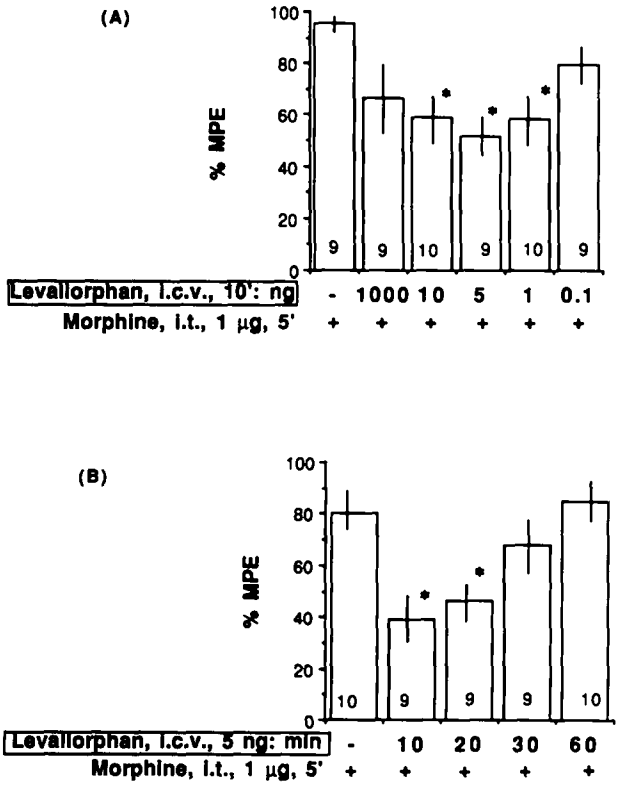

FIG. 2. Dose-response and time course for the antagonistic action of ICV levallorphan on morphine-induced antinociception. (A) Varying doses of levallorphan were given ICV 10 min before the TFT in mice and the dose (1 μg) and time of administration (5 min) of IT morphine were kept fixed. Note that complete antagonism of morphine-induced analgesia was not obtained. The greatest antagonism was obtained at 5 ng levallorphan. Hereafter a "+" indicates that the drug at the left was given while "−" indicates that the appropriate vehicle was given; inhibition of the tail-flick response was expressed as the percentage maximum possible effect (% MPE), mean ± SEM. The numbers within each bar are the numbers of animals used in each group. *Significant difference from the IT morphine group by Dunnett's test ($p < 0.05$). (B) Duration of action of the antagonistic effect of 5 ng levallorphan ICV was determined by varying the time of administration of levallorphan. Time and dose of IT morphine were kept fixed. The duration of action of levallorphan was less than 30 min. *Significant difference from the IT morphine group by Dunnett's test ($p < 0.05$).

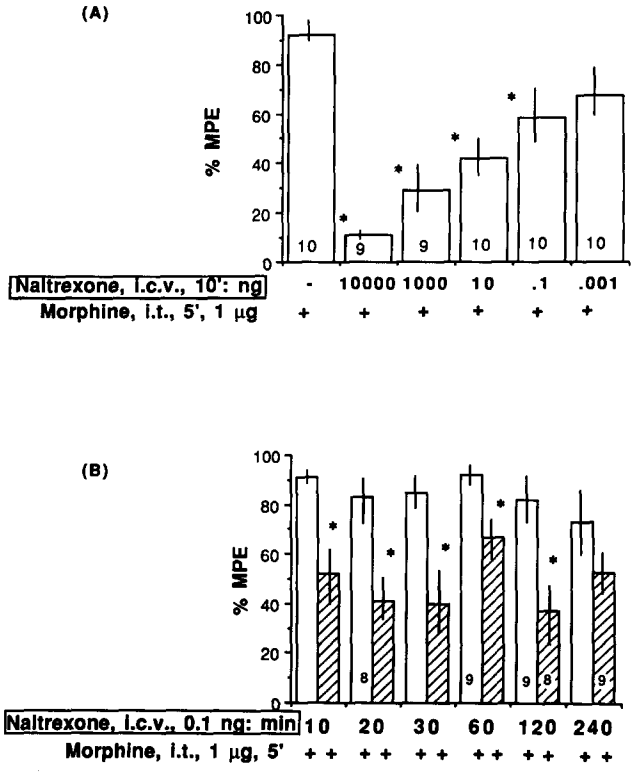

FIG. 3. Dose-response and time course for the antagonistic action of ICV naltrexone on IT morphine-induced antinociception. (A) Varying doses of naltrexone were given ICV 10 min before the TFT and the dose and time of IT morphine were kept fixed. Complete antagonism was obtained at the 10-mg dose of naltrexone. (B) The duration of action of naltrexone was determined by changing the time of administration of ICV naltrexone and keeping the time and dose of IT morphine fixed. The open and striped bars represent, respectively, ICV saline and naltrexone given at the designated times with IT morphine. Ten mice were used in those groups where the numbers are not given. *Significant difference from the IT morphine group ($p < 0.05$) by Dunnett's test in (A) and by Student's *t*-test in (B).

Naltrexone, ICV, antagonized morphine-induced analgesia over a large dose range, 0.1 ng–10 μg (Fig. 3A). This antagonism was not limited as in the case with levallorphan. Complete antagonism was obtained at 10 μg naltrexone. A time-response study showed that the antagonistic action of 0.1 ng naltrexone lasted 120 min (Fig. 3B). ICV naltrexone alone had no effect on the tail-flick latency (data not shown).

Elimination of the Antagonistic Effect of ICV Levallorphan and Naltrexone by IT Dynorphin Antiserum, Dyn AS

Dyn AS and control antiserum were administered IT in a pretreatment 1 h before the tail-flick test against ICV levallorphan and naltrexone (Figs. 4A and 4B). Pretreatment with IT Dyn AS eliminated the antagonistic actions presumably by binding the Dyn A in the spinal cord (16). Dyn AS had no effect on the antinociceptive action of IT morphine (groups 4 vs. 1), that is, the response to morphine was the same as in

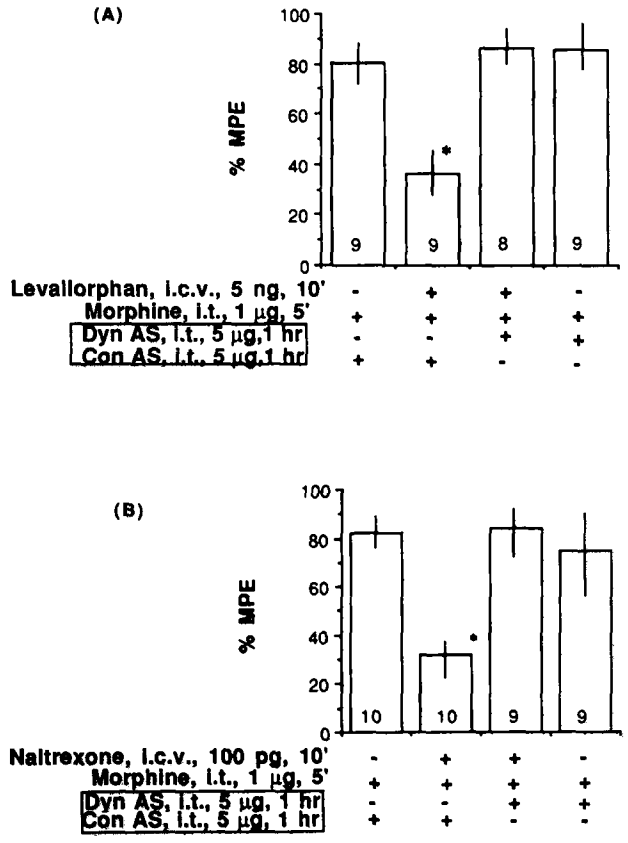

FIG. 4. Elimination of the antagonistic effect of ICV levallorphan and naltrexone on IT morphine-induced antinociception by dynorphin antiserum (Dyn AS). (A) The antagonistic effect of ICV levallorphan (5 ng) on morphine-induced antinociception was demonstrated in group 2 compared to 1 (groups are assumed to be numbered from left to right). An intrathecal pretreatment of Dyn AS (5 μg) 1 h earlier eliminated this antagonism (group 3 vs. 2). Dyn AS had no effect on the IT morphine response (group 4). Control antiserum, Con AS. (B) A 1-h pretreatment of Dyn AS eliminated the antagonistic effect of naltrexone (100 pg) against IT morphine. Dyn AS had no effect on the morphine response (group 4). *Significant difference from all other groups in both panels using ANOVA followed by Newman-Keuls' test ($p < 0.05$).

the group given control antiserum. The effectiveness of Dyn AS indicated that the antagonistic action of ICV levallorphan and naltrexone against IT morphine was an indirect action mediated by spinal Dyn A.

Desensitization to the Antagonistic Effect of ICV Levallorphan and Naltrexone by Pretreatment with SC Morphine and the Agents Themselves

Morphine, 10 mg/kg SC, 3 h, presumably releases spinal Dyn A to produce desensitization to the subsequent antianalgesic action of Dyn A (17). The antagonistic effect of ICV levallorphan and ICV naltrexone on IT morphine-induced antinociception was eliminated by a 3-h pretreatment of SC morphine (Figs. 5A and 5B). Morphine pretreatment had no effect on IT morphine analgesia (group 4). If ICV levallorphan and naltrexone treatments release spinal Dyn A, a pretreatment with these agents should also produce desensitization to the antianalgesic action of Dyn A. Levallorphan given ICV 5 ng (1 h) (Fig. 6A) and naltrexone given ICV 0.1 ng (4 h) (Fig. 6B) produced desensitization to the antianalgesic action of IT Dyn A. Levallorphan and naltrexone pretreatments had no effect on IT morphine analgesia (group 4, Figs. 6A and 6B).

Dose- and Time-Response Relationship for the Antinociceptive Action of ICV Nalbuphine

In an initial experiment, we attempted to show that ICV nalbuphine would antagonize the antinociceptive action of IT morphine. However, this attempt failed because ICV nalbuphine had significant analgesic activity that made it difficult to show its antagonistic activity against IT morphine. As shown in Fig. 7A, ICV administration of various doses of nalbuphine 10 min before the tail-flick test produced antinociceptive action in a dose-dependent manner. Using a dose of 30 μg nalbuphine, the analgesic action peaked at 10 min and was back to baseline levels by 40 min. (Fig. 7B).

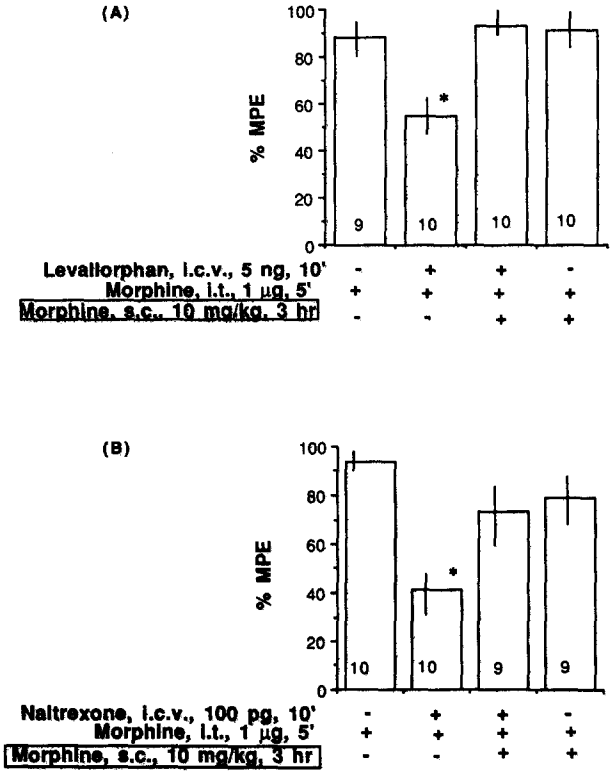

FIG. 5. Elimination of the antagonistic effect of ICV levallorphan and naltrexone on IT morphine-induced antinociception by 3-h pretreatment with SC morphine. (A) A 3-h pretreatment with morphine, 10 mg/kg SC, eliminated the antagonism by levallorphan of IT morphine-induced response (groups 1, 2, and 3). This pretreatment with morphine produces desensitization to the antianalgesic action of Dyn A (17). (B) Similarly, the antagonistic effect of ICV naltrexone was eliminated by morphine pretreatment. The response of group 4 in both (A) and (B) showed that morphine pretreatment had no effect on a subsequent response to IT morphine. *Significant difference from all other groups using analysis of variance (ANOVA) followed by Newman-Keuls' test ($p < 0.05$).

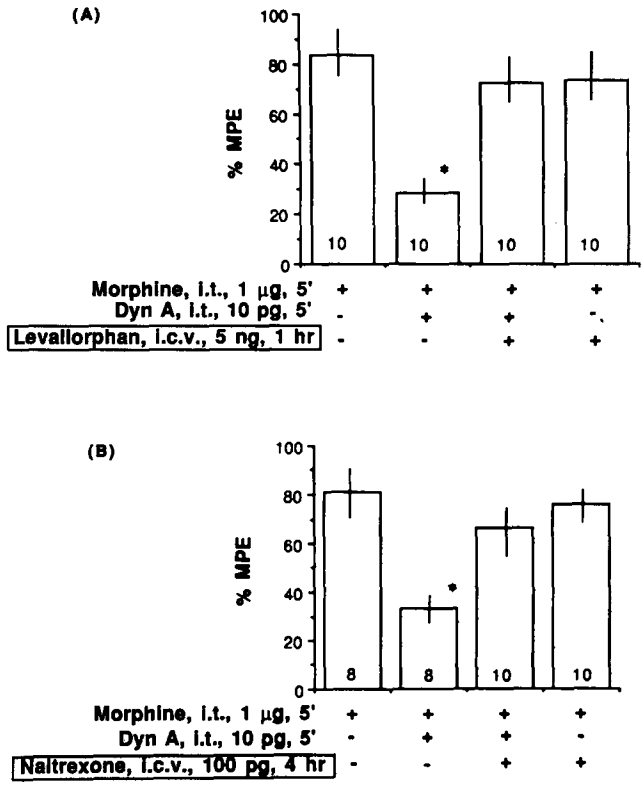

FIG. 6. Pretreatment with levallorphan or naltrexone produced desensitization to the action of Dyn A. Morphine antinociception (1 μg, 5') was antagonized by Dyn A (10 pg) given IT 5 min before the tail-flick test (TFT) (group 1 vs. 2, A and B). (A). A pretreatment with ICV levallorphan 5 ng 1 h before the TFT produced desensitization to Dyn A in that the antianalgesic response to Dyn A was not obtained (group 3). (B). Similarly, ICV naltrexone pretreatment eliminated the antianalgesic action of Dyn A. *Significant difference from all other groups using analysis of variance (ANOVA) followed by Newman-Keuls' test ($p < 0.05$).

Enhancement of the Antinociceptive Action of ICV Nalbuphine with Opioid Antagonists and Dynorphin Antiserum in the Presence of Methysergide

Morphine given ICV can be shown to release spinal Dyn A if the spinal descending noradrenergic analgesic component of morphine action (4) is attenuated (16). It is known that nalbuphine acts on κ-receptors in the brain to produce analgesia and activation of descending serotonergic pathways are involved in κ-agonist-induced antinociception (23,56). Thus, for the experiments depicted in Figs. 8A, 8B, and 8C the analgesic action of ICV nalbuphine, 30 μg, was attenuated by IT administration of a serotonin antagonist, methysergide, 1 μg. Administration of small doses of either naloxone (10 femtog) or N-BNI (10 ng) at 5 min restored nalbuphine-induced antinociception (Figs. 8A and 8B, group 3 vs. 2). This effect of naloxone and N-BNI was consistent with inhibition of the action of spinally released Dyn A, which resulted in an apparent increase in the antinociceptive action of nalbuphine. Dynorphin antiserum was given as a pretreatment to determine if Dyn A was more specifically involved. Administration of 5 μg dynorphin antiserum 1 h prior to the TFT had the same effect (Fig. 8C, group 3 vs. 2) as IT administration of naloxone or N-BNI. By attenuating the descending analgesic control system with methysergide, an increase in the antinociceptive response of IT nalbuphine was produced by pretreatment with the dynorphin antiserum.

Morphine Pretreatment Produced Desensitization to the Antianalgesic Action of Dyn A Released by ICV Nalbuphine

Dyn A, 10 pg IT, significantly decreased the antinociceptive response to ICV nalbuphine (Fig. 9A). Dyn A alone had no activity on the TFT (data not shown). A 3-h pretreatment with 10 mg/kg SC morphine produced desensitization to the antianalgesic action of Dyn A so that IT Dyn A no longer affected the nalbuphine-induced analgesia (Fig. 9A, group 3). This morphine pretreatment had no effect on ICV nalbuphine analgesia (group 4 vs. 1). In Fig. 9B, where ICV nalbuphine-induced analgesia was attenuated by IT administration of methysergide (groups 1 vs. 2), morphine pretreatment restored the analgesic action of nalbuphine (group 3). Morphine pretreatment alone at 3 h does not affect the TFT (17). The combination of SC morphine pretreatment (3 h) and methysergide IT (1μg) produced no effect in a control group (data not shown). These results provided further evidence indicating that nalbuphine released Dyn A in the spinal cord.

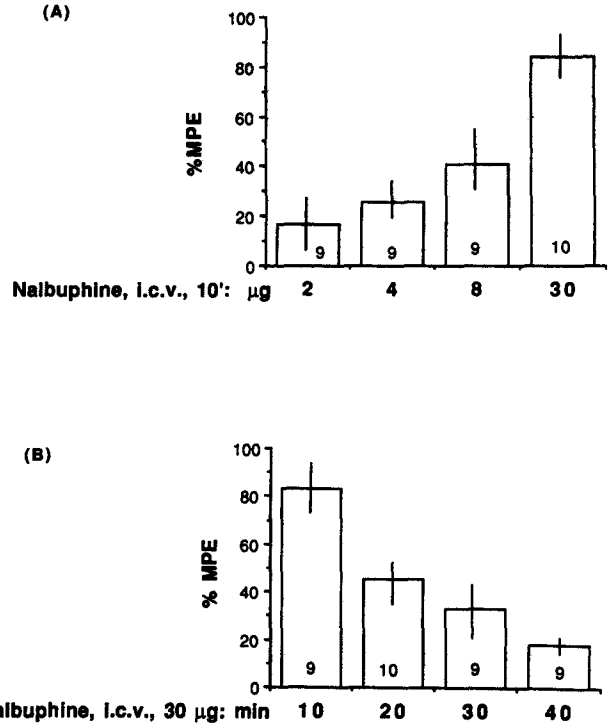

FIG. 7. Dose-response and time course for the antinociceptive activity of nalbuphine given ICV. (A) Nalbuphine given ICV at various doses 10 min before the tail-flick test (TFT) showed almost a maximal analgesic effect at 30 μg. (B) Duration of action of the 30-μg dose of nalbuphine was determined by varying the time of administration of nalbuphine before the TFT. The effect was gone by 30 min.

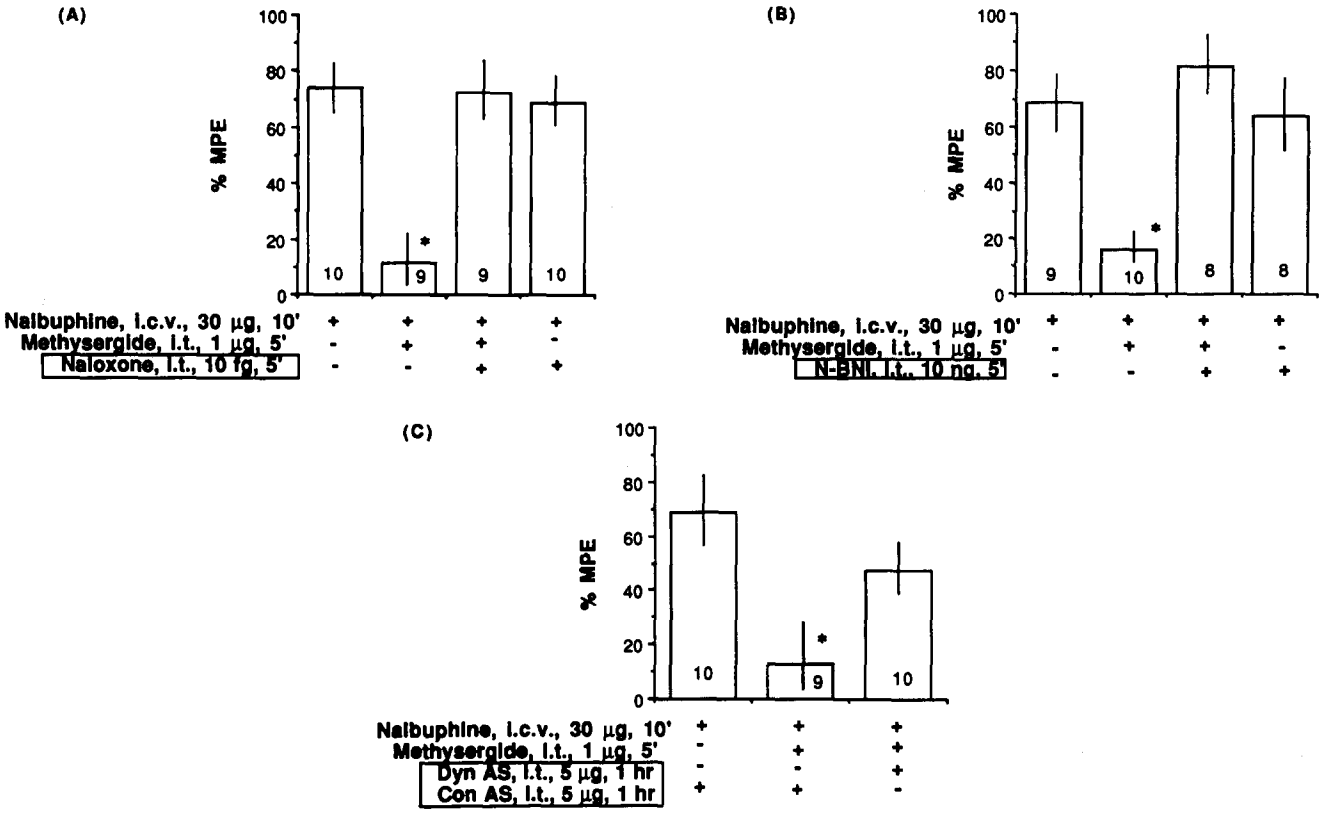

FIG. 8. Enhancement of the antinociceptive action of nalbuphine with intrathecal naloxone and norbinaltorphimine (N-BNI) and Dyn AS. (A) The antinociceptive action of nalbuphine was attenuated by IT administration of methysergide (group 1 vs. 2), consistent with the mediation of nalbuphine analgesia by a descending serotonergic system. Concurrent intrathecal injection of naloxone along with the methysergide restored the analgesic response to nalbuphine to the original level (group 3). Presumably, IT naloxone antagonizes the antianalgesic Dyn A component (18) of nalbuphine action. (B) N-BNI given IT similarly restored the analgesic action of nalbuphine to its original level, presumably by the same mechanism as IT naloxone (18). (C) Dyn AS restored the analgesic action of ICV nalbuphine. The fourth group of (A) and (B) showed that naloxone and N-BNI had no effect on 30 μg nalbuphine antinociception alone. *Significant difference from other groups using analysis of variance (ANOVA) followed by Newman-Keuls' test ($p < 0.05$).

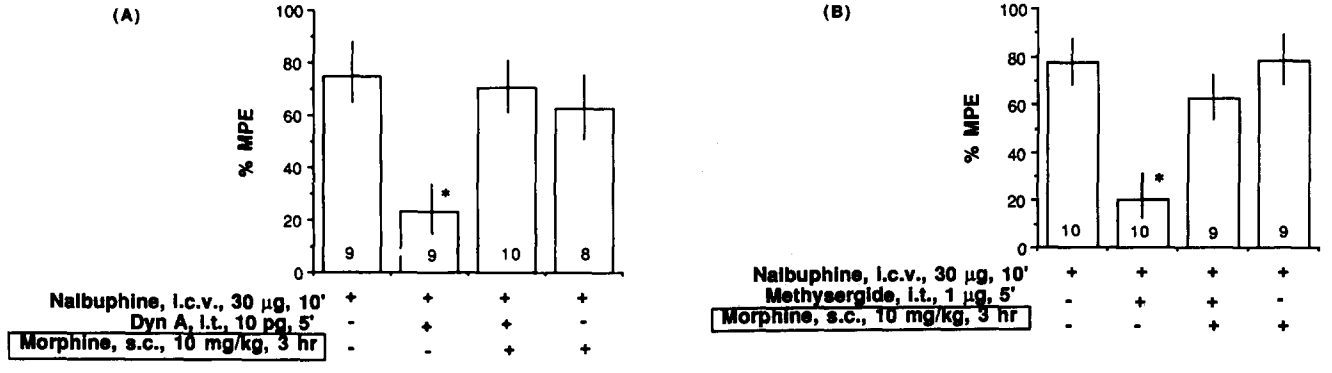

FIG. 9. (A) The analgesic effect of ICV nalbuphine was antagonized by IT Dyn A (group 1 vs. 2). Morphine pretreatment eliminated this response to Dyn A (group 3). (B) In a different protocol, nalbuphine-induced analgesia was antagonized by IT administration of methysergide. Under this condition, pretreatment with morphine also restored the analgesic response to nalbuphine (group 3), presumably by producing desensitization to the Dyn A component of nalbuphine action. The fourth group in each panel showed that morphine pretreatment had no effect on ICV nalbuphine-induced antinociception. *Significant difference from all other groups using analysis of variance (ANOVA) followed by Newman-Keuls' test ($p < 0.05$).

Administration of Naltrindole, a δ-opioid Receptor Antagonist, ICV to Determine if it Released Spinal Dyn A

At various doses, naltrindole ICV did not antagonize IT morphine-induced antinociception (Fig. 10). This lack of action was taken to indicate that naltrindole did not release spinal Dyn A.

Lack of Antagonism by ICV Levallorphan, Naltrexone, Naltrindole, and Nalbuphine Against ICV Morphine-Induced Antinociception

When these four agents were administered concurrently with ICV morphine, no effect on ICV morphine-induced analgesia was seen (Fig. 11). The doses of the antagonists were the same used as in the previous studies. Thus, the lack of ability of ICV levallorphan and naltrexone to antagonize ICV morphine-induced antinociception but their ability to antagonize IT morphine action was consistent with an indirect mechanism of antagonism of IT morphine-induced analgesia by Dyn A release.

DISCUSSION

In evaluating whether an agent given ICV releases spinal Dyn A, the test agent was administered at a site remote (ICV) from the spinal site at which Dyn A was released. The ability of this Dyn A to attenuate the analgesic action of IT morphine was an indication of Dyn A release. Further pharmacological tests for the putative release of Dyn A depended upon inhibition of the antianalgesic action of Dyn A by IT administration of naloxone, N-BNI, or dynorphin antiserum (16). Desensitization to the action of Dyn A at the spinal site was also produced by systemic morphine pretreatment (17). Application of a combination of these tests indicated that three of the four agents tested (levallorphan, naltrexone, nalbuphine, but not naltrindole) released Dyn A. The receptor selectivity of these agents is not an issue that can be clarified by the present results because a wide variety of agents such as clonidine (an α_2-adrenergic agonist), physostigmine (a cholinesterase inhibitor), midazolam (an anxiolytic, sedative benzodiazepine) can release Dyn A. In a more limited sense, the present results indicate that several other opioid antagonists [in addition to naloxone and nor binaltorphimine (25)] release Dyn A. In the following discussion, certain complications associated with demonstrating Dyn A release will be emphasized. One general

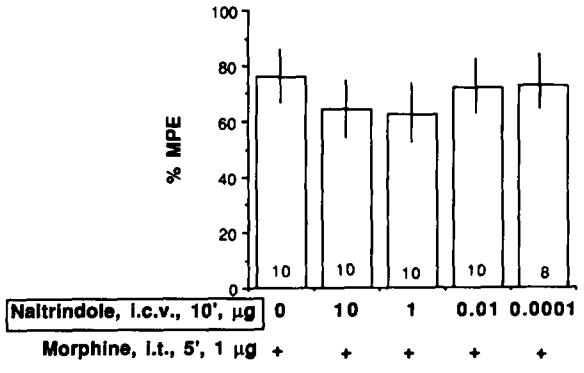

FIG. 10. The ICV administration of various doses of naltrindole 10 min before the tail-flick test (TFT) did not have an effect on the antinociception induced by IT morphine. Thus, this result was taken to indicate that ICV naltrindole did not release spinal Dyn A.

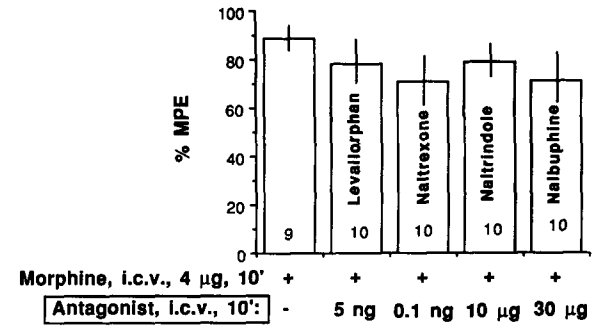

FIG. 11. The ICV administration of nalbuphine (30 μg), levallorphan (5 ng), and naltrexone (100 pg) did not antagonize ICV morphine-induced antinociception. The same doses and routes of administration of the antagonists were used as shown in the previous experiments to antagonize IT morphine-induced analgesia. This result indicated that these doses of these three antagonists were not sufficient to antagonize morphine-induced analgesia by a direct action on μ-opioid receptors in the brain. Naltrindole given at a 10-μg dose did not antagonize ICV morphine-induce analgesia. This dose of naltrindole inhibited the analgesic action of a δ-opioid receptor agonist, DPDPE (10 μg, given ICV with naltrindole 10 min before TFT) (data not given), but did not release Dyn A (previous figure).

complication is that the Dyn AS was not purified nor was a monoclonal antibody used. Nonspecific attenuation of Dyn A action, for instance, by an inflammatory reaction is therefore possible. However, Dyn AS that is prebound in vitro with Dyn A does not antagonize the in vivo action of Dyn A (16).

Levallorphan, when given ICV, antagonized IT morphine-induced analgesia. Because levallorphan was given ICV and morphine IT, the present result was similar to that in a previous article, where ICV naloxone and N-BNI indirectly antagonize IT morphine analgesia by release of spinal Dyn A (25). Thus, treatment with dynorphin antiserum eliminated the antagonistic effect of ICV levallorphan (Fig. 4). Pretreatment with systemic morphine, which produces desensitization to the antianalgesic action of Dyn A, also eliminated the antagonistic effect of ICV levallorphan. One-hour pretreatment with 5 ng levallorphan also produced desensitization to Dyn A (Fig. 6), consistent with levallorphan releasing spinal Dyn A. In this regard, pretreatment with IT Dyn A itself produces desensitization to the subsequent antianalgesic action of Dyn A (17). A notable feature was that the antagonistic action of ICV levallorphan against IT morphine was biphasic and maximal at the 5-ng dose. This finding might have several explanations. First, levallorphan is primarily an opioid antagonist, although it has been described as having agonist properties (12,32,34). If levallorphan were to have analgesic activity at higher doses, increasing the dose of levallorphan might oppose the antianalgesic action of spinally released Dyn A. In the phenylquinone test (a chemically induced nociceptive response) and the low-temperature hotplate test, but not in the tail-flick test, levallorphan shows analgesic activity in the dose range of 1–30 mg/kg systemically (32,35,37,39). In the present study, higher doses of ICV levallorphan by itself produced minimal analgesic action. The chemical-induced nociceptive tests and low-temperature hotplate test are more sensitive to agonist properties of the opioid antagonists than the tail-flick (37,44). It must be noted that, just like naloxone, levallorphan is used

primarily as an antagonist, but with manipulation of the test paradigm naloxone can also be shown to possess analgesic activity (20,30,51). A second alternative explanation might be that levallorphan had a limit beyond which it did not release any more Dyn A. Last, it might be that at the larger doses ICV some of the levallorphan redistributed down to the spinal cord to partially inhibit the action of Dyn A.

The evidence similarly indicated that the mechanism of the antagonistic action of ICV naltrexone against IT morphine was like that of ICV levallorphan, namely, dynorphin antiserum treatment and morphine pretreatment eliminated the antagonistic action of ICV naltrexone against IT morphine-induced analgesia. Also, 4-h pretreatment with ICV naltrexone produced desensitization to the Dyn A component of action of naltrexone. The difference from levallorphan was that the antagonistic action of naltrexone increased with increasing doses. At 10 μg, ICV naltrexone inhibited IT morphine-induced analgesia almost completely. The absence of a biphasic dose-response effect to naltrexone appeared to indicate the absence of an analgesic component of action to naltrexone. Naltrexone is two or three times more potent as an antagonist than naloxone and has a longer duration of action than naloxone in man (5,34). Naltrexone has no analgesic activity in the mouse phenylquinone test but has limited analgesic activity in other studies (20,34). Further, Bossut et al. (6,7) found that naltrexone and naloxone given IP in rats enhance electroacupuncture analgesia. They suggest two alternatives: Naloxone and naltrexone may act as analgesic agents or inhibit an antianalgesic system. According to our concept, naloxone and certain other opioid antagonists given IT (or systemically to act on the spinal cord) can inhibit an antianalgesic system involving spinal Dyn A release. The second alternative suggested by Bossut et al. above implies an opioid-mediated (sensitive to naloxone and naltrexone) antianalgesic system. Might this be the Dyn A system?

Based upon the spinal inflammatory and nerve ligation models, a role for Dyn A modulating nociceptive response in the dorsal horn of the spinal cord has been suggested (11). Substance P is proposed as one of the mediators involved in activating the Dyn A interneuron in the dorsal horn. Our evidence indicates that the antianalgesic effect of spinal Dyn A is separate from the action of substance P in antagonizing morphine-induced analgesia (3). A preliminary report indicates that IT calcium stimulates spinal Dyn A release to antagonize IT morphine analgesia in mice (48).

Nalbuphine given ICV could not be shown to antagonize the analgesic action of IT morphine in the tail-flick test. We attributed this failure to ICV nalbuphine possessing analgesic activity. In contrast to ICV levallorphan and naltrexone, the analgesic action of nalbuphine was substantial by itself (Fig. 7). We have shown previously (16) that a highly efficacious analgesic agent, morphine given ICV, can be shown to release spinal Dyn A when the descending noradrenergically mediated analgesic system is attenuated by IT administration of yohimbine (16). By analogy, as shown in the results for ICV nalbuphine, attenuation of the descending serotonergic system with IT methysergide provided the means for unmasking the activation of the Dyn A antianalgesic system. Methysergide treatment attenuated the analgesia produced by the 30-mg dose of ICV nalbuphine down from about 70% to about 20% MPE. Then, IT administration of naloxone, N-BNI, and dynorphin antiserum restored the analgesic action of nalbuphine back to its original level. In addition, morphine pretreatment restored nalbuphine analgesia. All of these results were consistent with ICV nalbuphine releasing spinal Dyn A. Only the single dose of nalbuphine was used, but with a change in dose the situation may change because the relative amounts of agonist to antagonist action would change. For example, IT morphine-induced analgesia is antagonized by SC nalbuphine (33); IT nalbuphine at lower doses antagonizes high doses of IT morphine but high doses of nalbuphine have no effect (28,46). The complexity of responses evoked by different ratios of agonist to antagonist actions was first demonstrated with mixtures of nalorphine and morphine (26). Nalorphine in different combinations with morphine produces a biphasic rather than monotonic curve of mean analgesic relief scores in patients. We did not study further the relationship between agonist and direct antagonistic action and indirect antagonism through release of Dyn A by ICV nalbuphine. In the present study, ICV levallorphan, naltrexone, and nalbuphine released spinal Dyn A at doses that did not produce direct antagonism of μ-receptors in the brain [ICV morphine-induced analgesia was not affected at these doses (Fig. 11)].

Even though the mechanism of antagonism of IT morphine by ICV nalbuphine is related to Dyn A release, one other interesting alternative should be mentioned. Ethylketocyclazocine, a mixed agonist/antagonist, produces hyperalgesia when microinjected into specific sites in the brain of rats (22). The medullary sites are similar to those in which administration of nicotine elicits hyperalgesia. Earlier studies indicate that the hyperalgesic action of ethylketocyclazocine is produced through activation of κ-receptors (29,59). Thus, it is possible in the present study that nalbuphine and levallorphan, which have κ-agonist activity, antagonize IT morphine-induced analgesia through production of hyperalgesia. However, we have not been able to demonstrate any hyperalgesic effect for spinal Dyn A in the tail-flick test (3,25). One important contrasting difference between the medullary hyperalgesic and Dyn A systems is that intracerebral naltrexone administration at the hyperalgesic sites produces analgesia (22) while in our study ICV administration of naltrexone produced an antianalgesic action through the release of spinal Dyn A. The possibility exists that the sites involved in producing hyperalgesia might be involved in the release of spinal Dyn A. On the other hand, ICV pentobarbital produces hyperalgesia and antagonizes morphine-induced analgesia without involving the release of spinal Dyn A (58).

Various doses of ICV naltrindole did not antagonize IT morphine-induced antinociception. We concluded that naltrindole did not release spinal Dyn A. However, this conclusion was based upon only one experiment. Naltrindole given ICV has been shown to have analgesic activity at higher doses in both the chemical nociceptive and tail-flick tests through activation of δ-receptors (49). DPDPE, a δ-receptor agonist, given ICV does not release spinal Dyn A (16). Therefore, it may be that δ-receptors are not involved in the activation of spinal Dyn A release. However, the caveat is that if we knew what mediator was involved in the descending analgesic pathway activated by DPDPE attenuation of that system might provide the condition necessary to test for the release of Dyn A.

Miaskowski et al. (36) have shown recently that ICV administration of ICI 174,864 (a δ-opioid receptor antagonist) and CTOP (a μ-receptor antagonist) antagonize antinociception induced in the rat by IT DAMGO, Tyr-D-Ala^2-Gly-NMePhe^4-Gly-ol^5 (a μ-agonist). The same mechanism as we reported here and previously with ICV naloxone and norbinaltorphimine may be involved in their findings. The antago-

nists may release spinal Dyn A to antagonize the analgesic effects of certain IT agonists such as DAMGO.

In conclusion, nalbuphine, levallorphan, and naltrexone administered ICV activates a Dyn A-mediated antianalgesic system. Intrathecal administration of naloxone, N-BNI, and dynorphin antiserum or production of desensitization to Dyn A action inhibited the antianalgesic effect, thereby implicating release of spinal Dyn A.

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