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Endogenous Dynorphin Modulates Calcium-mediated Antinociception in Mice

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SMITH, F. L. AND W. L. DEWEY. *Endogenous dynorphin modulates calcium-mediated antinociception in mice.* PHARMACOL BIOCHEM BEHAV 45(2) 383-391, 1993.—We previously reported that calcium administered IT produces antinociception by stimulating spinal Met-enkephalin release. However, at times the antinociceptive effects of calcium in the tail-flick test are greatly diminished. The results of this study indicates that during these periods calcium also stimulates endogenous dynorphin release. Dynorphin has been reported to block opiate-induced antinociception. Calcium-injected mice (150-600 nmol, IT) pretreated with vehicle IP displayed a poor degree of antinociception. Alternatively, pretreating mice with pentobarbital (45 mg/kg, IP) restored the antinociceptive effects of calcium. Low doses of naloxone and nor-binaltorphimine (BNI) did not produce antinociception but restored the antinociceptive effects of calcium. Dynorphin (1-17) (Dyn 1-17), and Dyn (1-13), but not Dyn (1-8), blocked the antinociceptive effects of calcium restored with pentobarbital. These results indicate that calcium-mediated antinociception was sensitive to injected dynorphins. In additional experiments, antiserum to Dyn (1-13) was found to restore the antinociceptive effects of calcium, presumably by binding dynorphin released by calcium.

Calcium Intrathecal Endogenous opioid peptides Dynorphin Antianalgesia Antinociception Pentobarbital Mice

THE injection of calcium ions into the IT space of mice has been shown to result in dose-dependent antinociception in the tail-flick test (33,47,58,59). There are good indications that antinociception occurs as a result of endogenous opioid release from the spinal cord. Calcium potentiates the antinociceptive effects of IT-injected morphine (33), DAMGO (μ), DPDPE (δ), and U-50,488H (κ) (59). In opposite fashion, IT injection of the opiate receptor antagonists naloxone (μ) and naltrindole (δ), but not nor-binaltorphimine (BNI) (κ), block the antinociceptive effects of calcium (47,59). Further, data indicates that the endogenous opioid released by calcium is Met-enkephalin, which has been shown to activate spinal δ-opioid receptors (47). Antiserum to Met-enkephalin blocks calcium-mediated antinociception, while irreversible blockade of μ receptors with β-funaltrexamine (FNA) IT blocks antinociception induced by DAMGO but not DPDPE or calcium.

However, at times the antinociceptive effects of calcium in the tail-flick test are greatly diminished. This study was undertaken in an attempt to determine the mechanisms behind the reduced efficacy of calcium. Our results indicate that during periods of reduced antinociception calcium stimulated Met-enkephalin release but also stimulated the spinal release of the opioid peptide dynorphin. Ample evidence indicates the presence of high levels of immunoreactive dynorphin and κ-opioid receptors in the dorsal horn of the spinal cord (4,34,38,56). The dynorphins exhibit a higher affinity for κ- over μ- and δ-opioid receptors (6,8,20). The dynorphins have been shown to produce antinociception in certain nociceptive tests; however, they have also been shown to inhibit the antinociceptive effects of other opioids. The dynorphins injected centrally are effective in blocking visceral mechanical and cold-water nociception (24,45,49,52), whereas they are ineffective in blocking thermal nociception at doses that do not impair motor function (14,36,40,44,48,49,55). Further, in tests of thermal nociception the dynorphins have been shown to antagonize morphine-induced antinociception (15,18,32,46, 48,55). Fujimoto and associates accumulated an impressive volume of data demonstrating the existence of a dynorphin-containing "antianalgesic" system that can inhibit the antinociceptive properties of various agents, including morphine (15-19,25). Low doses of dynorphin (1-17) [Dyn (1-17)] were found to block physostigmine- and morphine-induced antinociception, while low doses of naloxone and nor-BNI were found to enhance antinociception (18). In addition, they reported that antiserum with selectivity for Dyn (1-17) and Dyn (1-13) enhanced the analgesic effects of physostigmine by binding to endogenous dynorphin released by physostigmine (16). A treatment strategy similar to that of Fujimoto and

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associates was employed in this study to determine if the diminished antinociceptive properties of calcium resulted from endogenous dynorphin release.

METHOD

Animals and Intrathecal Injections

Male ICR mice (25-35 g) were obtained from Dominion Laboratory Animals (Dublin, VA). Employing a modification of the methods of Hylden and Wilcox (27), drugs or vehicle were injected free-hand into the intrathecal space between lumbar L5 and L6 using a 30-ga needle connected to a 50-μl microsyringe. The injection volume in all cases was 5 μl/mouse. Mice were not anesthetized for the procedure, and correct placement of the needle was evidenced by a "flick" of the mouse's tail.

Tail-Flick Test for Antinociception

The tail-flick test used to assess for antinociception in mice was developed by D'Amour and Smith (9) and modified by Dewey et al. (11). Prior to injections, the baseline (control) latency for the mouse was determined. Only mice with a control reaction time between 2–4 s were used. The test latency following drug treatment was assessed at the appropriate time and a 10-s maximum cut-off time was used to prevent damage to the tail. Antinociception was quantified according to the method of Harris and Pierson (23) as the percentage of maximum possible effect (%MPE), which was calculated as [%MPE = (test − control/10 − control) × 100]. The %MPE was calculated for each mouse and the average %MPE ± SEM determined for each treatment group. Vehicle control group sizes consisted of 6–8 mice, while drug treatment groups consisted of 12–15 animals because treatment effects were tested twice to confirm the findings. Vehicle control and treatment results were graphed together for the sake of convenience The data were analyzed using analysis of variance (ANOVA) followed by Dunnett's test (12) for post-hoc comparisons of treatments with their respective control. Log-dose probit ED_{50} values for dose-response data were calculated according to the methods of Tallarida and Murray (51).

Experimental Design

Initial experiments were designed to determine if the dose-dependent effects of calcium injected IT could be restored with pentobarbital. Peak calcium-mediated antinociception in the past has been shown to occur 10–15 min following IT injection (33,59). Therefore, in this study mice were tested 15 min after calcium injection. Mice received pentobarbital (45 mg/kg, IP) 15 min before injection of vehicle or incremental doses of calcium (150–600 nmol). Experiments were also designed to determine if IT injection of low doses of opioid receptor antagonists would restore the antinociceptive effects of calcium. Mice received vehicle, naloxone, or nor-BNI at various times before and after IT injection of vehicle or calcium (600 nmol). In additional studies, groups of mice injected with calcium also received incremental doses of naloxone and nor-BNI. Other experiments were designed to determine if the dynorphins injected IT would block the antinociceptive effects of calcium that had been restored by pentobarbital (45 mg/kg, IP). Mice received vehicle, Dyn (1-17), Dyn (1-13), or Dyn (1-8) 5–75 min before and 5–10 min after IT injection of vehicle or calcium (600 nmol). These same mice also received an IP injection of vehicle or pentobarbital (45 mg/kg) 15 min before vehicle or calcium (600 nmol). In addition, other mice received incremental doses of Dyn (1-17), Dyn (1-13), or Dyn (1-8) 10 min before pentobarbital. Mice then received vehicle or calcium (600 nmol) 15 min after pentobarbital administration. Experiments were also conducted to determine during periods of reduced antinociception if calcium stimulates the release of an endogenous opioid to inhibit the antinociceptive effects of Met-enkephalin also released by calcium. Control rabbit antiserum or antisera containing antibodies to [Met^5]-enkephalin, [Leu^5]-enkephalin, Dyn (1-13), or β-endorphin were injected IT 15–105 min before calcium (600 nmol). In additional experiments, mice received incremental doses of the antisera 45 min before calcium. Other groups reported that these antisera exert maximal effects on opioid-induced antinociception when injected 75 min before testing (18,54). We speculated if calcium releases an opioid peptide that blocks the antinociceptive effects of Met-enkephalin released by calcium then antiserum might bind that inhibitory peptide and restore the antinociceptive effects of calcium.

Drugs and Chemicals

Calcium chloride (anhydrous) and naloxone HCl were obtained from Sigma Chemical Co. (St. Louis, MO). nor-BNI and U-50,488H were obtained from Research Biochemicals, Inc. (Natick, MA). These agents were dissolved in distilled water. Dyn (1-17), Dyn A (1-17), Dyn (1-13), and Dyn (1-8) were obtained from Bachem, Inc. (Torrance, CA) and prepared according to the method of Fujimoto and Rady (18). Rabbit antisera to [Met^5]-enkephalin, [Leu^5]enkephalin, Dyn (1-13), and β-endorphin were produced and characterized according to the methods outlined in Tseng and Suh (54) and provided as a generous gift from Drs. L. L. F.-Tseng and J. M. Fujimoto (Medical College of Wisconsin, Milwaukee, WI).

RESULTS

Calcium-injected mice (150–600 nmol, IT) pretreated with vehicle IP displayed a poor degree of antinociception (Fig. 1). Calcium at all three doses failed to produce a significant effect above the vehicle (IP + IT) control %MPE value of 10 ± 5. Alternatively, pretreating mice with pentobarbital (45 mg/kg, IP) restored the antinociceptive effects of calcium. A significant increase in antinociception occurred in mice injected with the 300- and 600-nmol doses of calcium compared to similarly injected mice pretreated with vehicle IP. Pentobarbital pretreatment produced no significant antinociception in IT vehicle-injected mice (%MPE = 6 ± 2). Because the response curves were significantly different from parallel, comparison of ED_{50} values for calcium-induced antinociception between the vehicle (IP) and pentobarbital (IP) groups was not possible. However, the ED_{50} value of 369 (95% C.L. 309–477) nmol in the pentobarbital-treated group was not significantly different from that found in previous studies of 344 (95% C.L. 251–469) nmol (47,59). Pentobarbital produced a peak restoration of antinociception when mice were tested 30 min later, whereas the effects of calcium were diminished when mice were tested at 45 min (data not shown). Therefore, mice were tested 30 min after pentobarbital injection for the remainder of the study, where indicated.

Low doses of opiate receptor antagonists have been shown to block the inhibitory effects of endogenous dynorphin and potentiate the antinociceptive effects of physostigmine (18). If calcium released dynorphin, then blockade of the effects of dynorphin would be predicted to restore the antinociceptive effects of calcium. Naloxone and nor-BNI were injected IT at

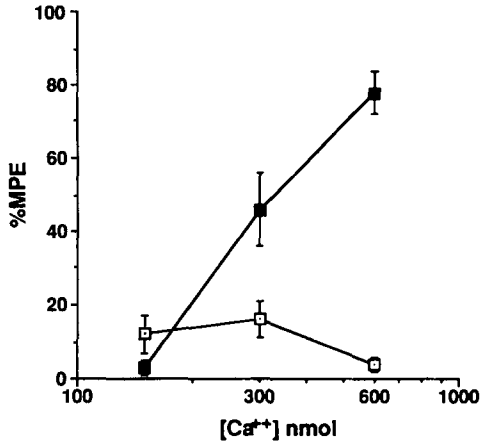

FIG. 1. Restoration of the antinociceptive effects of calcium with pentobarbital injected IP. Pentobarbital (45 mg/kg) was injected 15 min before IT injection of vehicle or calcium. Fifteen minutes after injection of calcium, mice were tested for the percentage of maximum possible effect (%MPE) ± SEM in groups consisting of six to eight mice. The %MPE for the IP vehicle + IT vehicle group was 4 ± 2, while the %MPE for the IP pentobarbital + IT vehicle group was 6 ± 2. (□), IP vehicle + IT calcium; (■), IP 45 mg/kg pentobarbital + IT calcium.

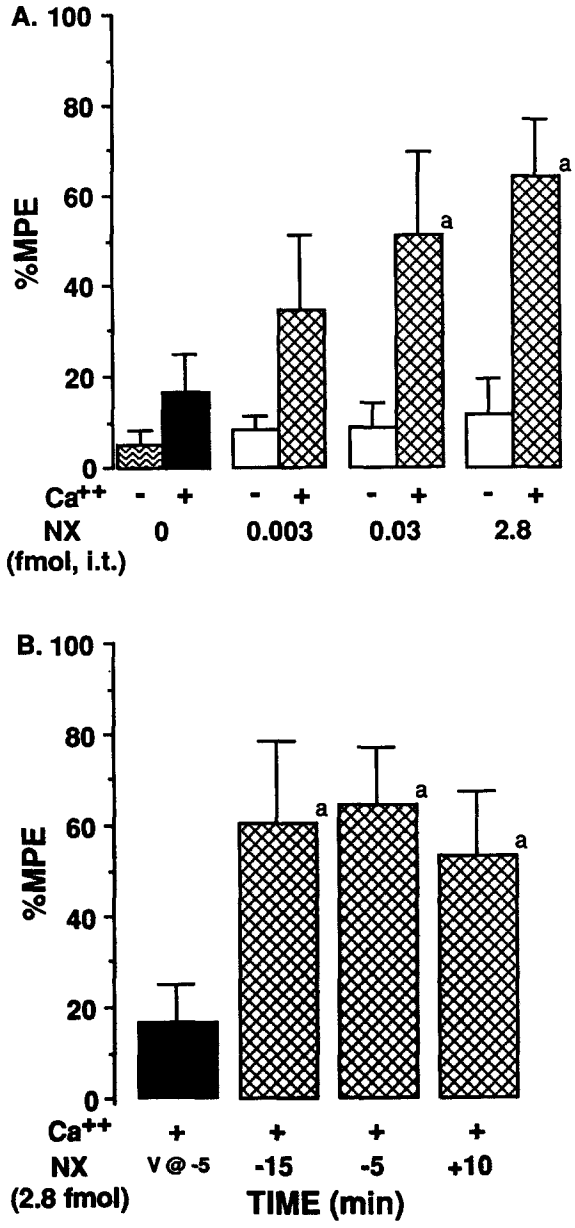

FIG. 2. Restoration of calcium-mediated antinociception with low doses of naloxone injected IT. For dose-response studies (A), mice were injected IT with vehicle or incremental doses of naloxone 5 min before IT injection of vehicle or calcium (600 nmol). Mice were tested 15 min after IT injection of vehicle or calcium. In time course studies (B), vehicle or naloxone were injected IT at the indicated times on the ordinate before or after IT injection of vehicle or calcium (600 nmol). For a, $p < 0.05$, compared to vehicle + calcium value. (▨), IT vehicle + IT vehicle; (■), IT vehicle + IT calcium; (□), IT naloxone + IT vehicle; (▩), IT naloxone + IT calcium.

doses that reportedly do not antagonize DAMGO (μ)- and U50,488 (κ)-induced antinociception (15,16). Naloxone (0.003–28.0 fmol) alone produced no significant antinociception but restored the antinociceptive effects of calcium (Fig. 2a). Time course studies revealed that antinociception was restored when naloxone (2.8 fmol) was injected before and after calcium (Fig. 2b). In a similar fashion, the κ-receptor antagonist nor-BNI (1.24 fmol -12.4 nmol) (50) alone produced no significant antinociception but restored the antinociceptive effects of calcium (Fig. 3a). Time course studies revealed that antinociception was restored when nor-BNI (124 pmol) was administered after calcium (Fig. 3b). However, the δ-opioid antagonist naltrindole (0.1 pmol–1.0 nmol) (37), tested at a peak time effect of 40 min before the tail-flick test, failed to restore calcium-mediated antinociception (data not shown). The doses of naltrindole were below the 11- to 22-nmol doses reported to block DPDPE-induced antinociception (47).

Other experiments examined whether exogenously administered dynorphin would block the antinociceptive effects of calcium restored with pentobarbital. Low doses of Dyn (1-17) (0.047 and 4.7 fmol) blocked the antinociceptive effects of calcium (Fig. 4a). Dyn (1-13) (623 pmol) also blocked calcium-mediated antinociception, although this necessitated using much higher doses than Dyn (1-17) (Fig. 4b). Dyn (1-8) (51 pmol–10.2 nmol) was ineffective in blocking calcium mediated antinociception (Fig. 4c). The 10.2-nmol Dyn (1-8) dose alone produced a 37 ± 20 %MPE, which may reflect the stimulation of μ-receptors. These data indicated that the antinociceptive effects of calcium might be inhibited by κ-opioid receptor stimulation. However, U-50,488H (0.1–40 pmol) failed to block the antinociceptive effects of calcium. In addition, higher doses of U-50,488H (14–54 nmol, IT) alone produced a limited degree of antinociception, presumably through κ-receptor stimulation (data not shown). Time course studies revealed that Dyn (1-17) (4.7 fmol, IT) blocked (25-5 min before calcium) but did not reverse (5–10 min after calcium) the antinociceptive effects of calcium (Fig. 5a). Dyn (1-13) (623 pmol, IT) also blocked (25-5 min before calcium) but did not reverse (5 min after calcium) antinociception (Fig. 5b). Dyn (1-8) (5.1 nmol, IT) was ineffective in blocking (45-5 min before calcium) or reversing (5 min after calcium) antinociception (Fig. 5c). U-50,488H (0.4 pmol, IT) also failed to block (5–45 min before calcium) or reverse (10 min after calcium) the antinociceptive effects of calcium (data not shown).

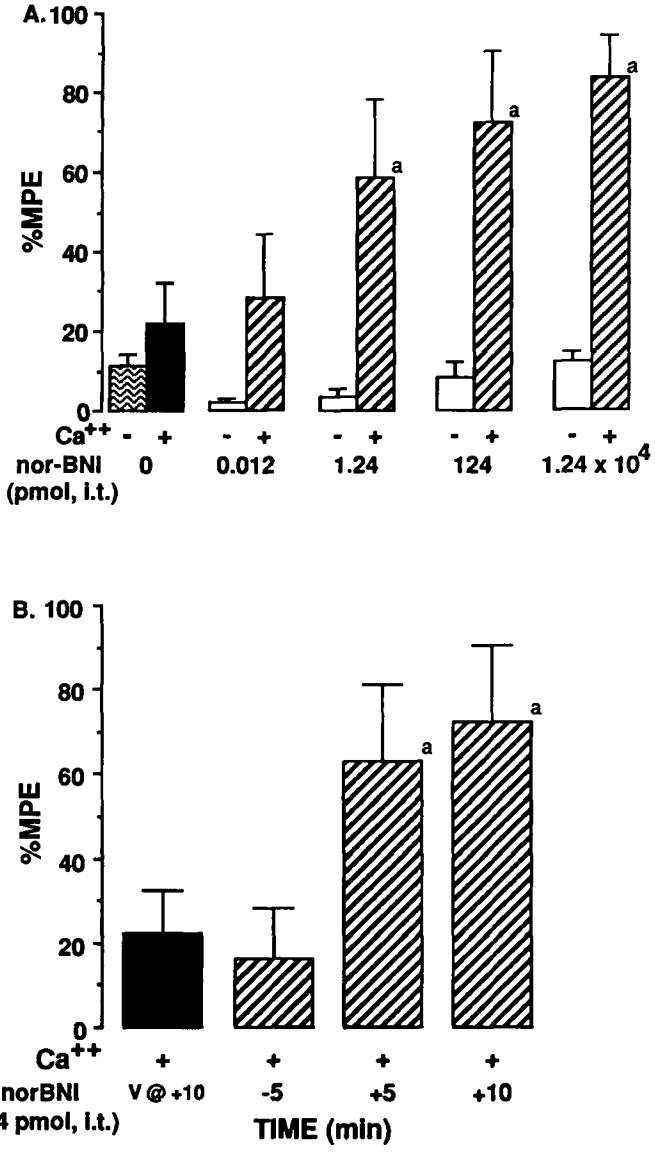

FIG. 3. Restoration of the antinociceptive effects of calcium using low doses of nor-binaltorphimine (BNI) injected IT. For dose-response studies (A), mice were injected IT with vehicle or calcium (600 nmol) 10 min before IT injection of vehicle or incremental doses of nor-BNI. Mice were tested 15 min after IT injection of vehicle or calcium. In time course studies (B), vehicle or nor-BNI were injected IT at the indicated times on the ordinate before or after IT injection of vehicle or calcium (600 nmol). For a, $p < 0.05$, compared to vehicle + calcium value. (▨), IT vehicle + IT vehicle; (■), IT vehicle + IT calcium; (□), IT nor-BNI + IT vehicle; (▨), IT nor-BNI + IT calcium.

The finding that calcium-mediated antinociception was sensitive to exogenously administered dynorphins suggested that calcium at times may stimulate the release of endogenous dynorphin to inhibit its own antinociceptive effects. Therefore, we speculated that injection of antibodies directed against dynorphin might bind released dynorphin and prevent the inhibition of calcium-mediated antinociception. Dyn (1-13) antiserum alone failed to alter tail-flick latencies but was active in restoring the antinociceptive effects of calcium (Fig. 6a). Cross-reactivity of the Dyn (1-13) antiserum to Dyn (1-

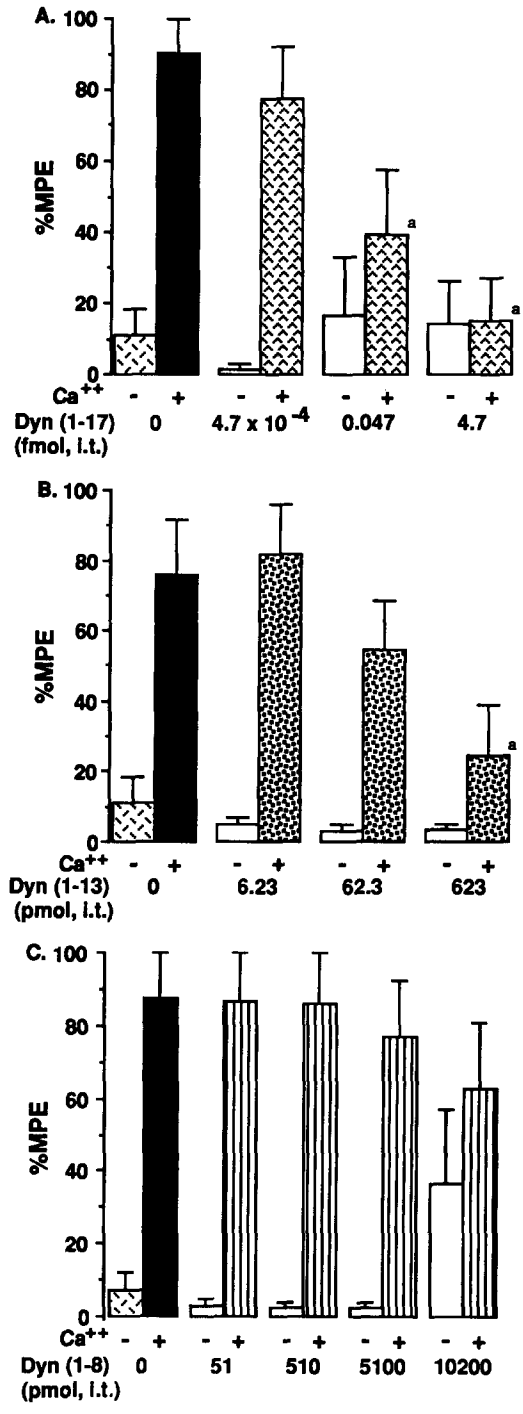

FIG. 4. Inhibition of the antinociceptive effects of calcium with Dyn (1-17), Dyn (1-13), or Dyn (1-8) injected IT. In (A), (B), and (C), mice were injected IT with vehicle, Dyn (1-17), Dyn (1-13), or Dyn (1-8) 10 min before IP injection of vehicle or pentobarbital (45 mg/kg). Fifteen minutes after injecting pentobarbital, mice were injected IT with vehicle or calcium (600 nmol) and tested 15 min later. For a, $p \alpha 0.05$, compared to pentobarbital + vehicle (IT) + calcium (IT) value. (▨), IP vehicle + IT vehicle + IT calcium; (■), IP pentobarbital + IT vehicle + IT calcium; (□), IP pentobarbital + IT dynorphin + IT vehicle; (▨), IP pentobarbital + IT Dyn (1-17)+ IT calcium; (▨), IP pentobarbital + IT Dyn (1-13)+ IT calcium; (▥), IP pentobarbital + IT Dyn (1-8)+ IT calcium.

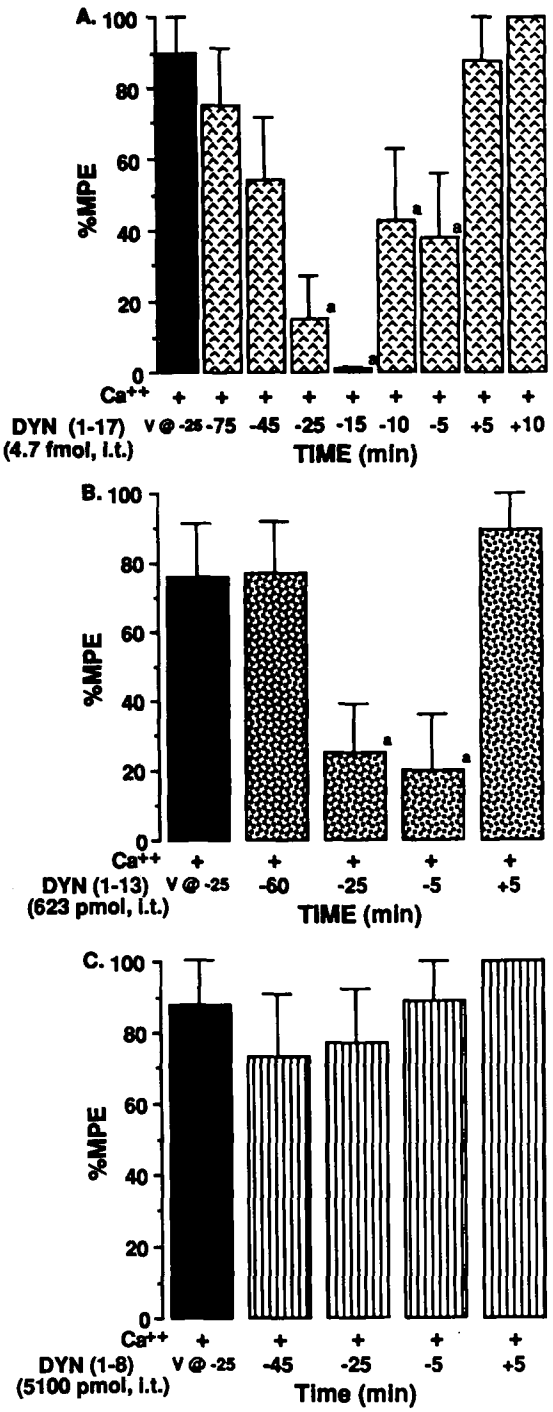

FIG. 5. Time-dependent inhibition of the antinociceptive effects of calcium with Dyn (1-17), Dyn (1-13), or Dyn (1-8) injected IT. In (A), (B), and (C), mice were injected IT with vehicle, Dyn (1-17), Dyn (1-13), or Dyn (1-8) 5–75 min before or 5–10 min after IT injection of vehicle or calcium (600 nmol). These mice had also received an IP injection of pentobarbital (45 mg/kg) 15 min before IT injection of vehicle or calcium. For a, $p \alpha 0.05$, compared to pentobarbital + vehicle (IT) + calcium value. (■), IP pentobarbital + IT vehicle + IT calcium; (▨), IP pentobarbital + IT Dyn (1-17)+ IT calcium; (▩), IP pentobarbital + IT Dyn (1-13)+ IT calcium; (▥), IP pentobarbital + IT Dyn (1-8)+ IT calcium.

17), Dyn (1-13), and Dyn (1-8) is 100, 83.7 and >0.002%, respectively (16). In addition, maximal restoration of antinociception occurred when Dyn (1-13) antiserum was injected 45–75 min before calcium (Fig. 6b). Antisera to other opioid peptides were tested to determine their potential for restoring the antinociceptive effects of calcium. Antisera to Leu-enkephalin (50–200 μg) and β-endorphin (50–200 μg) failed to restore calcium-mediated antinociception (Table 1). However, antiserum to Met-enkephalin (50–200 μg) significantly blocked the low level of antinociception produced by calcium on that test day (Table 1). We previously established that Met-enkephalin antiserum blocks the antinociceptive effects of calcium (47).

DISCUSSION

Pentobarbital appeared to restore the antinociceptive effects of calcium to that observed when calcium alone was active (33,47,58,59). This led us to speculate that pentobarbital removed the influence of some endogenous system that was blocking the effects of calcium. Our results support the possibility that calcium stimulated the release of dynorphin to inhibit its own antinociceptive effects and that pentobarbital restored antinociception by blocking the influence of dynorphin. The ability of injected dynorphins to block antinociception restored with pentobarbital (Figs. 4 and 5) suggests that pentobarbital restored antinociception by blocking the release of endogenous dynorphin. If dynorphin was tonically released, then pentobarbital itself might have produced a paradoxical antinociception following inhibition of dynorphin release. However, pentobarbital failed to produce antinociception and follows other reports of barbiturates producing no effects (2) or making animals hyperresponsive to noxious stimuli (3,35). There is evidence that tonic spinopetal noradrenergic (26,28,39), serotonergic (29,57,61), and purinergic (10,43) antinociceptive systems contribute to the antinociceptive effects of opioids. Barbiturates reportedly inhibit these tonic systems, resulting in a reduction in the analgesic potency and efficacy of morphine (1,2,35). Barbiturate-induced reductions in opiate analgesia have been demonstrated to not occur as a result of dynorphin release (19).

Naloxone and nor-BNI restored the antinociceptive effects of calcium, and this suggests that these agents antagonized the inhibitory effects of an endogenous opioid released by calcium (Figs. 2 and 3). Naloxone and nor-BNI could have antagonized μ- and κ-opioid receptors; however, the range of doses were below those found to block DAMGO (μ)- or U50,488 (κ)-mediated antinociception (18). In addition, much higher doses of naloxone have been shown to block the antinociceptive effects of Met-enkephalin released by calcium (47, 59). Thus, the paradoxical restoration of the effects of calcium implies that naloxone and nor-BNI were acting in a manner that could not be explained by the usual blocking effects on μ- and κ-receptors. As mentioned earlier, dynorphin effectively blocks the antinociceptive effects of morphine and other agents (15,18,32,46,48,55). In addition, Fujimoto and associates accumulated evidence to indicate the existence a dynorphin-containing antianalgesic system that can block antinociception induced by morphine and other agents (15–19,25). There are indications that many analgesic agents stimulate both antinociceptive and antianalgesic processes and that blockade of the antianalgesic component with low doses of opioid receptor antagonists enhances the degree of antinociception. For example, naloxone and nor-BNI enhance the antinociceptive properties of physostigmine and morphine

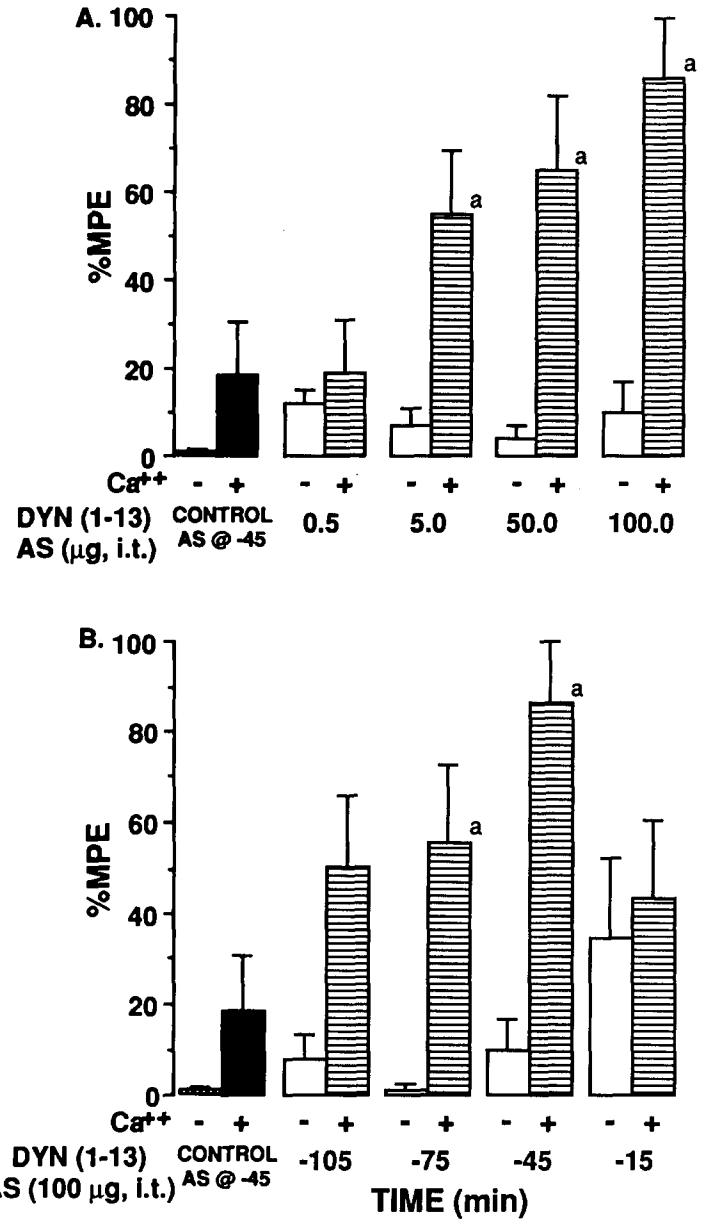

FIG. 6. Restoration of the antinociceptive effects of calcium with antiserum to Dyn (1-13). In (A), mice were injected IT with control antiserum or Dyn (1-13) antiserum 45 min before IT injection of vehicle or calcium (600 nmol). Mice were tested 15 min after IT injection of vehicle or calcium. In time course studies (B), control antiserum or Dyn (1-13) antiserum were injected IT at the indicated times on the ordinate before IT injection of vehicle or calcium (600 nmol). For a, $p \alpha 0.05$, compared to control antiserum + calcium. (□), IT control antiserum + IT vehicle; (■), IT control antiserum + IT calcium; (▨), IT Dyn (1-13) antiserum + IT vehicle; (▤), IT Dyn (1-13) antiserum + IT calcium.

(15,17,18). We also showed that similar doses of naloxone and nor-BNI restored the effects of calcium, possibly by blocking the actions of dynorphin released by calcium. However, naloxone has been shown to produce antinociception under certain conditions, and it might be argued that naloxone contributed to the effects of calcium. Low doses of naloxone elicit analgesia in certain pain states in man and animals (21,30,42) by disinhibiting the release of endogenous opioids (30). In addition, Wu and associates reported the presence of a tonic medullary κ-receptor-mediated hyperalgesic system (60), which naloxone might block to produce a paradoxical antinociception. However, we found that naloxone and nor-BNI produced no antinociception that might have contributed to the effects of calcium. Fujimoto and coworkers also failed

TABLE 1

EFFECT OF IT PRETREATMENT OF ANTISERA TO [met⁵]-ENKEPHALIN (A/S met-Enk), [leu⁵]-ENKEPHALIN (A/S leu-Enk), AND β-ENDORPHIN (A/S β-EP) ON THE ANTINOCICEPTIVE PROPERTIES OF IT CALCIUM ADMINISTRATION IN THE TAIL-FLICK TEST

Antisera (IT)	%MPE* Distilled water (IT)	%MPE* Calcium (600 nmol, IT)
Control serum (200 μg)	4.0 ± 4.0	38.8 ± 16.6
A/S met-Enk (50 μg)	2.8 ± 2.8	52.3 ± 18.1
A/S met-Enk (100 μg)	2.8 ± 2.8	4.4 ± 2.9†
A/S met-Enk (200 μg)	5.5 ± 3.5	1.3 ± 1.3†
Control serum (200 μg, −60 min)	4.0 ± 4.0	38.8 ± 16.6
A/S met-Enk (200 μg, −105 min)	1.4 ± 1.0	52.6 ± 18.0
A/S met-Enk (200 μg, −60 min)	5.5 ± 3.5	1.3 ± 1.3†
A/S met-Enk (200 μg, −45 min)	5.6 ± 4.4	17.6 ± 12.3
A/S met-Enk (200 μg, −15 min)	4.8 ± 2.6	50.9 ± 18.6
Control serum (200 μg)	4.0 ± 3.3	37.3 ± 18.1
A/S leu-Enk (50 μg)	0.2 ± 0.2	34.1 ± 16.0
A/S leu-Enk (100 μg)	16.8 ± 16.6	40.1 ± 17.3
A/S leu-Enk (200 μg)	0.7 ± 0.7	39.3 ± 17.9
Control serum (200 μg, −60 min)	4.0 ± 3.3	37.3 ± 18.1
A/S leu-Enk (200 μg, −105 min)	4.5 ± 2.1	42.0 ± 17.1
A/S leu-Enk (200 μg, −45 min)	0.7 ± 0.7	39.2 ± 17.9
A/S leu-Enk (200 μg, −15 min)	0.2 ± 0.2	52.3 ± 15.5
Control serum (200 μg)	7.7 ± 7.1	34.9 ± 16.9
A/S β-EP (50 μg)	4.7 ± 2.9	41.4 ± 17.6
A/S β-EP (100 μg)	4.2 ± 2.5	30.0 ± 15.8
A/S β-EP (200 μg)	0.8 ± 0.5	26.4 ± 15.5
Control serum (200 μg, −60 min)	7.7 ± 7.1	34.9 ± 16.9
A/S β-EP (200 μg, −105 min)	5.3 ± 4.0	31.9 ± 15.5
A/S β-EP (200 μg, −45 min)	0.8 ± 0.5	26.4 ± 15.5
A/S β-EP (200 μg, −15 min)	5.6 ± 4.4	25.0 ± 16.4

*The percentage of maximum possible effect (%MPE) was calculated using the method of Harris and Pierson (23).

†$p < 0.05$, compared to control serum + calcium treatment.

to demonstrate an antinociceptive effect of naloxone and nor-BNI when using a similar range of doses (18).

The ability of Dyn (1-17) and higher doses of Dyn (1-13) to block calcium-mediated antinociception (Fig. 4) is consistent with reports that other analgesic agents are blocked by the dynorphins (15,18,32,46,48,55). Our results indicate that antinociceptive effects of Met-enkephalin released by calcium (47) were sensitive to blockade by the dynorphins. In addition, amino acid segments 14–17 appeared to increase the blocking effect of Dyn (1-17) over that of Dyn (1-13). Fujimoto and coworkers similarly reported greater degree of inhibition with Dyn (1-17) (18). The receptor that mediates the inhibitory effects of dynorphin remains to be determined, although the logical choice would be κ-opioid receptors. However, the prototypic κ-agonist U-50,488H failed to block the antinociceptive effects of calcium. Further, U-50,488H possess no antianalgesic properties and has been reported to potentiate physostigmine- and morphine-induced antinociception (18, 41). Both U-50,488H and Dyn (1-17) have high affinity for κ_1 receptors, but Dyn (1-17) also binds to U-50,488H-resistant κ_2 and $\kappa_3$receptors (7). Future studies may demonstrate that κ_2 or κ_3 receptors mediate the inhibitory effects of dynorphin. Alternatively, Clark and associates reported that Dyn (1-17) displays a 100-fold greater affinity for δ-opioid receptor sites than for κ_3 sites (7). U-50,488H displays negligible binding to δ-receptors. Thus, dynorphin (but not U-50,488H) might have acted on δ-receptors and blocked the antinociception produced by Met-enkephalin released by calcium. This interpretation is possible because dynorphin, κ-receptors (34), and U-50,488H-resistant κ-receptors (7,22,53) are located in spinal laminae I–III, the same region containing Met-enkephalin-labeled perikarya and fibers (34).

Time course experiments reveal that Dyn (1-17) and Dyn (1-13) blocked but did not reverse the antinociceptive effects of calcium (Fig. 5). Because calcium releases spinal Met-enkephalin, we speculate that the dynorphins may have altered the release or the postsynaptic expression of the effects of Met-enkephalin. However, the inability of the dynorphins to reverse calcium-mediated antinociception suggests that previously activated processes were resistant to the dynorphins. Further, the finding that antinociception was blocked when Dyn (1-17) was administered 40 min before the tail-flick test (25 min before calcium) indicates that Dyn (1-17) stimulated long-acting intracellular processes. Metabolism studies of Dyn (1-17) in vivo show at time zero that more than 50% of the sample has been metabolized to des-tyrosine dynorphin A (Dyn 2-17) (62). It is unlikely that metabolites of dynorphin were inhibiting antinociception. Dyn (2-17) is ineffective in blocking physostigmine-induced antinociception (Fujimoto, personal communication). Agents that enhance or block second messenger systems may provide insight into the intracellular characteristics of this dynorphin-sensitive system.

The finding that antiserum to Dyn (1-13) restored the antinociceptive effects of calcium suggests that antiserum antagonized the inhibitory effects of endogenous dynorphin released by calcium (Fig. 6). Antiserum to other opioid peptides failed to restore antinociception, suggesting that the effects of calcium were selectively inhibited by dynorphin (Table 1). Dyn (1-13) antiserum has been reported to enhance the antinociceptive effects of physostigmine, morphine, DAMGO, and U-50,488H by binding endogenous dynorphin released by these agents (16). Thus, IT administration of the dynorphins blocked the antinociceptive effects of calcium, while Dyn (1-13) antiserum appeared to bind released endogenous dynorphin and restore the effects of calcium. It is also possible that the Dyn (1-13) antiserum blocked the actions of dynorphin by binding the receptors that dynorphin stimulated. However, if dynorphin inhibited the effects of calcium by stimulating κ-opioid receptors then the antiserum would have had to bind κ_2 or κ_3 receptors because the antinociceptive effects of U-50,488H (which acts at κ_1 receptors) have been shown to be potentiated by Dyn (1-13) antiserum (16). The slow onset of action for Dyn (1-13) antiserum to restore the effects of calcium suggests that the antibody had to penetrate to the site of dynorphin release rather than binding to dynorphin released into the cerebrospinal fluid. Other groups reported a similar slow onset of action for the Dyn (1-13) and Met-enkephalin antisera (5,16,47,54). Such slow penetration might follow the physical principle of extracellular tortuosity in which hydrophilic molecules such as morphine and peptides diffuse slowly in brain tissue (13).

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