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Inverse Agonist Action of Leu-Enkephalin at δ_2-Opioid Receptors Mediates Spinal Antianalgesia

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ABSTRACT

Dynorphin A(1-17) given intrathecally releases spinal cholecystokinin to produce an antianalgesic action against spinal morphine in the tail-flick test in CD-1 mice. The present study showed that following the cholecystokinin step, a δ_2-opioid inverse agonist action of Leu-enkephalin (LE), was involved. Pretreatment with intrathecal LE antiserum eliminated dynorphin and cholecystokinin-8s antianalgesia. A small dose of LE intrathecally produced antianalgesia that like that from dynorphin A(1-17) and cholecystokinin was eliminated by naltriben but not 7-benzylidenenaltrexone (δ_2- and δ_1-opioid receptor antagonist, respectively). This LE step was followed by *N*-methyl-D-aspartate (NMDA) receptor activation. MK801, an NMDA receptor antagonist, eliminated the antianalgesia from dynorphin A(1-17), cholecystokinin-8s, and LE. Furthermore, none of the three were effective against morphine analgesia in 129S6/SvEv mice possibly because of their deficiency in NMDA receptor response. In 129S6/SvEv mice, [D-Ser^2]-Leu-enkephalin-Thr analgesia was not attenuated by LE; thus, this δ_2-analgesic agonist and LE inverse agonist action did not occur through competition at the same δ_2-receptor in CD-1 mice. In CD-1 mice, a linear sequence of dynorphin A(1-17) → cholecystokinin → LE → NMDA receptors was indicated: cholecystokinin antiserum inhibited cholecystokinin but not LE; naltriben inhibited LE but not NMDA. The uniqueness of LE in linking dynorphin A(1-17), cholecystokinin, δ_2-opioid, and NMDA receptor activation may unify the separate known mechanisms involved in the antiopioid actions of these components against morphine.

Intrathecally (i.t.) administered morphine produces antinociception in the mouse tail-flick test. The i.t. administration of 5 fmol of dynorphin A(1-17) (Dyn), an endogenous opioid peptide, produces an antianalgesic action against morphine through the release of spinal cholecystokinin (CCK) (Rady et al., 1999). Dyn activates an ascending pathway to the brain, which in turn activates a descending pathway to the spinal cord to produce CCK release; spinal transection eliminates the antianalgesic action of Dyn (Wang et al., 1994). Dyn-induced antianalgesia is also eliminated by intracerebroventricular (i.c.v.) administration of flumazenil, a benzodiazepine receptor antagonist, into the brain (Rady et al., 1998a). Flumazenil administered i.c.v. also inhibits i.c.v. pentobarbital (Wang and Fujimoto, 1993) and neurotensin (B. B. Holmes and J. M. Fujimoto, unpublished) antianalgesia, which results from activation of the same descending portion of the pathway and release of CCK.

The present investigation was initiated based on the preliminary observation that the antianalgesic action of i.t. CCK8s was eliminated by the i.t. administration of naltriben, a δ_2-opioid receptor antagonist. This observation suggested that there was an opioid intermediate involved in producing the CCK effect. This opioid was not Dyn because i.t. CCK8s does not release spinal Dyn (Rady et al., 1998b). The purpose of the present work was to establish the initial observation and to characterize and possibly identify the opioid intermediary. The sequence of steps in the antianalgesic action of Dyn to release CCK is illustrated in Fig. 1. In the present study, the further steps labeled 1, 2, and 3 were investigated by administration of the antagonists indicated at each step. Experiments will be presented that provide evidence that Leu-enkephalin (LE) is the probable δ_2-opioid receptor agonist that produces antianalgesia. This antianalgesic response produced by LE indicated an inverse agonist action opposite to the classical analgesic function ascribed to LE. LE administration will in turn be shown to activate an *N*-methyl-D-aspartate (NMDA) receptor response to produce antianalgesia. Evidence will also be presented for the linear sequence of the steps as given in Fig. 1. Placing these components in a linked sequence is discussed in relation to implications of their known antiopioid actions in explaining some of the

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ABBREVIATIONS: i.t., intrathecal; Dyn, dynorphin A(1-17); CCK, cholecystokinin; CCK8s, sulfated CCK8; LE, Leu-enkephalin; NMDA, *N*-methyl-D-aspartate; % MPE, percentage maximum possible effect; BNTX, 7-benzylidenenaltrexone; MK801, dizocilpine; ME, Met-enkephalin; DPDPE, [D-Pen^{2,5}]-enkephalin; DSLET, [D-Ser^2]-Leu-enkephalin-Thr; DOR-1, δ-opioid receptor-1; LE-Arg^6, Leu-enkephalin-arginine^6.

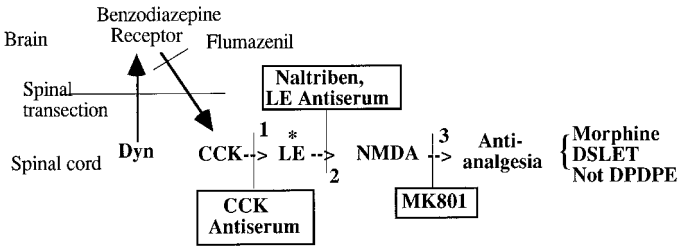

Fig. 1. Components involved in the production of antianalgesia in CD-1 mice. The spinal action of Dyn activates a neuronal pathway to the brain where benzodiazepine receptors (which can be inhibited by flumazenil, a benzodiazepine receptor antagonist) are activated and in turn produce spinal CCK release (Rady et al., 1998a, 1999). The present investigation (components in bold) proposes that a δ_2-opioid receptor mediated step involves LE, a point of major emphasis marked by an asterisk, followed by NMDA receptor activation to produce antianalgesia. Steps 1, 2, and 3 were investigated by use of inhibitory agents (enclosed in rectangles), the results of which also specify the sequence as illustrated. Note that LE acted as an inverse agonist to produce antianalgesia instead of the classically assigned role of an analgesic agent.

diverse observations regarding the development of tolerance to morphine.

Materials and Methods

Animals and Measurement of Antinociception. Male CD-1 mice weighing 25 to 30 g were purchased from Charles River (Wilmington, MA). For one later set of experiments 129S6/SvEv inbred mice (9 weeks old) were obtained from Taconic Farms (Germantown, NY). Each animal was used for only one experiment. In the radiant heat tail-flick test for antinociception, the predrug control tail-flick latency (the average of two trials) was determined to be 2 to 4 s. A cut-off time set at 10 s was used as the maximal antinociceptive response. In the single dose experiments, the percentage maximum possible effect (% MPE) for each mouse was calculated by the following formula:

$$\%\ \mathrm{MPE} = \frac{(\mathrm{Postdrug} - \mathrm{Predrug}) \times 100}{(10 - \mathrm{Predrug})}.$$

To establish dose-response relationships for i.t. morphine antinociception as affected by various treatments, the quantal response to morphine was determined. Tail-flick latencies greater than three standard deviations over the mean predrug time were considered to be antinociceptive. At each dose of morphine the mice showing antinociception were expressed as a percentage of the number of animals (usually eight but in a few cases seven) in that group. Three or more dose levels were used to construct each of the dose-response curves. The percentage responding values were transformed to probit values plotted against the log of the morphine dose, ED_{50} values and 95% confidence intervals were derived, and comparisons of the slope and ED_{50} values were made (Litchfield and Wilcoxon, 1949).

Drug Administration. All drugs were administered i.t. in a volume of 5 μl by the method of Hylden and Wilcox (1980) 5 min (unless stated otherwise) before the tail-flick test. Morphine, 7-benzylidenenaltrexone (BNTX), naltriben, MK801 (dizocilpine), and NMDA were dissolved in a 0.9% saline solution and Dyn, CCK8s, LE, met-enkephalin (ME), [D-Pen^{2,5}]-enkephalin (DPDPE), and [D-Ser^2]-Leu-enkephalin-Thr (DSLET) were dissolved in a 0.9% saline solution containing 0.01% Triton X-100. Control serum, LE antiserum, ME antiserum, and CCK antiserum were diluted with 0.9% saline. When drugs were given together, the solutions were premixed so that the drugs were given in a single 5-μl volume. Doses and times of administration of drugs are given with each experiment and based on previous publications (Rady et al., 1994, 1999). Appropriate vehicle solutions were administered when a drug was not given. All studies were done in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

Statistical Analysis. Analysis of the single dose experiment results were as follows. Those involving a comparison between the mean % MPE for two groups was by Student's t test. Those involving more than two groups were first analyzed by analysis of variance and comparison of all the groups to each other was by Newman-Keuls test or comparison of the other groups to one control group was by Dunnett's test. In the case of Newman-Keuls analysis, the results for only the main comparisons are given even though all possible comparisons were made. In all analyses, $P \leq 0.05$ was taken to indicate a significant difference between groups.

Source of Drugs. The drug sources were as follows: morphine sulfate·5H_2O (Mallinckrodt Chemical Works, St. Louis, MO), and Dynorphin A(1-17) and DSLET (Peninsula Laboratories, Inc., Belmont, CA). DPDPE, NMDA, and MK801 (dizocilpine maleate) were obtained from Sigma (St. Louis, MO) and CCK8 antiserum from Chemicon International, Inc. (Temecula, CA). LE and ME antiserum were produced in rabbits in our laboratory and were the same batch as used previously (Tseng et al., 1985; Vanderah et al., 1996). Control serum was obtained from rabbits. LE and ME were from Bachem, Inc. (Torrance, CA). The BNTX and naltriben were from previous stocks (Rady et al., 1994). The δ-receptor oligonucleotides, as previously used (Tseng et al., 1994), were synthesized by Dr. John Richard (Molecular Research Laboratories, Durham, NC). The antisense oligonucleotide is a phosphorothioate of the following sequence: 5′-AGG GCA CCA GCT CCA TGG CG-3′ and the sequence of the mismatch oligonucleotide used for control purposes is 5′-GGC GTC GAC CTA CTT CGG CG-3′. The doses of the drugs, given with the experiments, were for the forms stated above.

Results

Naltriben but Not BNTX Eliminates the Antianalgesic Action of i.t. CCK8s and Dyn. The format for eliciting the antianalgesic action is illustrated in Fig. 2A where i.t. morphine, 1 μg, was given 5 min before the tail-flick test. The morphine antinociception was attenuated by the administration of CCK8s (5 ng) along with the morphine. The antianalgesic action of CCK8s was unaffected by the i.t. administration of BNTX, a δ_1-opioid receptor antagonist, but was eliminated by i.t. naltriben, a δ_2-opioid receptor antagonist. CCK8s by itself or in combination with naltriben did not produce analgesia (last two groups in Fig. 2A). The i.t. administration of naltrindole (10 μg, 5 min), which inhibits both δ_1- and δ_2-agonist action, eliminated CCK8s antianalgesia (data not shown). Figure 2B shows that the antianalgesic action of i.t. Dyn was also eliminated by naltriben but unaffected by BNTX.

The results shown in Fig. 3A indicated that naltriben eliminated CCK8s-induced antianalgesia in a dose-dependent manner. Figure 3B shows the result of pretreatment with DOR-1 antisense, which down-regulates the δ_2-receptor in the spinal cord (Tseng et al., 1994). The group treated for 3 days with the DOR-1 antisense oligodeoxynucleotide did not give an antianalgesic response to i.t. CCK8s, whereas the mismatch oligodeoxynucleotide-treated group did.

Parts of these single dose experiments were extended by performing dose-response studies for the i.t. morphine. The data in Table 1 shows the ED_{50} value of 0.07 μg (0.04–0.12 μg, 95% confidence interval) for i.t. morphine. The i.t. administration of Dyn and CCK8s shifted the dose-response curve for i.t. morphine to the right in a parallel manner to give ED_{50} values of 0.71 and 0.44 μg, respectively; both were significantly different from morphine alone. The addition of

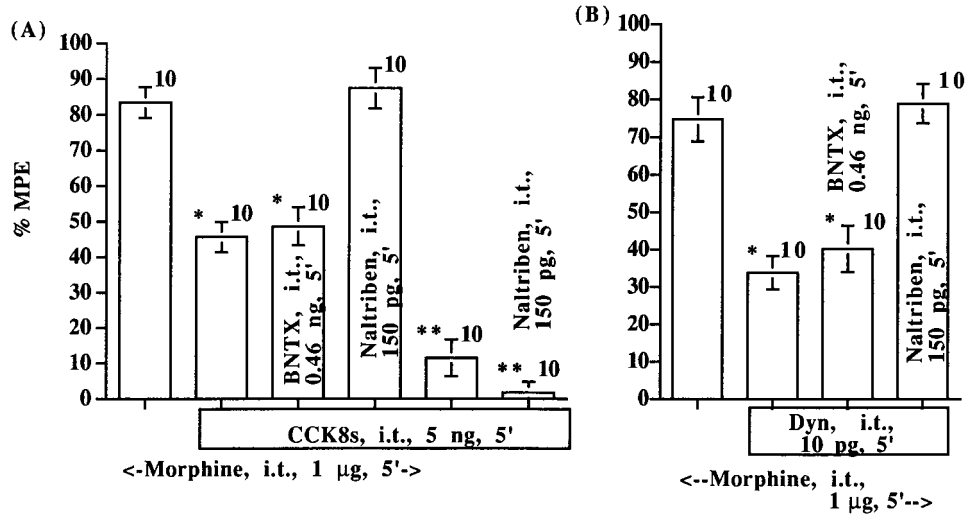

Fig. 2. Antianalgesic action in the tail-flick test of i.t. CCK8s (A) and Dyn (B) inhibited by naltriben, a δ_2-opioid receptor antagonist, but not by BNTX, a δ_1-antagonist. Starting from the bottom of the figure, groups given morphine i.t. to produce analgesia are overlapped by the arrow (so the last two groups in A did not receive morphine). The agents that produce antianalgesia, CCK8s and Dyn, are indicated within the rectangles; the treatments with antagonists, in this case BNTX and naltriben, are indicated within or above the columns. Other variables will be designated above the columns. The mean % MPE along with the S.E. and the numbers of mice in each group are indicated at the top of the column. Groups designated with the same asterisk were not different from each other but were significantly different from all groups not similarly marked by Newman-Keuls test ($P \leq 0.05$). All the agents were given in the same solution.

50 pg of naltriben shifted the ED_{50} values back to the control morphine value. Participation of δ_2-opioid receptors in antianalgesia was peculiar in that δ-receptor action is usually associated with analgesia. The next step was to find out what this δ-receptor ligand might be.

Antianalgesic Action of LE. The experiment depicted in Fig. 4A shows that a 1-h pretreatment with LE antiserum i.t. eliminated the antianalgesic action of CCK8s, suggesting that LE might be the antianalgesic ligand in the spinal cord. The control serum had no noticeable effect.

In Fig. 4B a fixed dose of morphine was given i.t. along with various doses of LE. LE antagonized the antinociceptive action of morphine in a dose-dependent manner. The i.t. administration of ME at 1, 10, and 50 ng had no effect on i.t. morphine analgesia (Table 2). ME antiserum at a dose similar to that for LE antiserum (200 μg) did not produce any effect (Table 2). Leu-enkephalin-Arg^6, a possible precursor of LE, given at a dose equivalent to LE (3.6 pmol) did not produce antianalgesia against i.t. morphine (Table 2). These results demonstrated that LE but not ME nor Leu-enkephalin-Arg^6 acted as an antianalgesic agent in the spinal cord. Because the results in Fig. 2 demonstrated that the antianalgesic action of CCK8s was eliminated by naltriben but not BNTX, the next step was to show the same selectivity for LE.

The antianalgesic action of LE was eliminated by i.t. naltriben but not by BNTX (Fig. 5A). The rightward parallel shift of the i.t. morphine dose-response curve produced by i.t. administration of LE was reflected by the change in the ED_{50} value (Table 1). The administration of naltriben along with the LE eliminated this rightward shift of the i.t. morphine dose-response curve.

Up to this point, the assumption had been that the CCK step preceded the δ_2-LE step. This assumption was addressed by the experiment depicted in Fig. 5B. The antianalgesic action of i.t. CCK8s was eliminated by 1-h pretreatment with i.t. CCK antiserum, a result in agreement with previous findings (Rady et al., 1998b). The antianalgesic action of i.t. LE was not affected by CCK antiserum treatment. These results suggested that the CCK step preceded the LE step.

Elimination of CCK8s-, Dyn-, and LE-Induced Antianalgesia by i.t. MK801. The i.t. administration of MK801, a nonequilibrium NMDA receptor inhibitor, along with i.t. CCK8s, Dyn, and LE eliminated the respective antianalgesic actions (Fig. 6). These results suggested that NMDA receptor stimulation might be involved in the antianalgesic action of these agents.

Antianalgesic Action of i.t. NMDA. Figure 7 shows that the administration of NMDA i.t. produced antianalgesia against morphine. Furthermore, the antianalgesic action of NMDA was not affected by the i.t. administration of CCK antiserum. Naltriben at a high dose did not antagonize the NMDA-induced antianalgesia. This result indicated that the CCK and LE steps preceded the NMDA step.

An Additional Aspect of the Selective Antianalgesic Action of LE and NMDA. LE i.t. was antianalgesic against i.t. DSLET, a δ_2-agonist (Fig. 8A). It was not antianalgesic against DPDPE, a δ_1-receptor agonist. NMDA had a similar selectivity of antianalgesic action against DSLET- but not DPDPE-induced antinociception (Fig. 8B). These results parallel those for Dyn and CCK8s (Rady et al., 1999).

Lack of Antianalgesic Response to NMDA, CCK8s, and LE in 129S6/SvEv Mice. Administration of NMDA, CCK8s, and LE i.t. did not inhibit i.t. morphine-induced analgesia in 129S6/SvEv mice (Fig. 9A). These results are consistent with the lack of responsiveness to NMDA in these mice (Kolesnikov et al., 1998). This lack of response was not due to absence of δ-receptors because i.t. DSLET (Fig. 9B) and DPDPE (data not shown) produced antinociception. The antianalgesic δ_2-receptor action of LE may involve NMDA responsiveness because LE did not produce antianalgesia against i.t. DSLET in 129S6/SvEv mice (Fig. 9B). An alternative explanation for the lack of antianalgesic action in these mice is presented under *Discussion*.

Discussion

The antianalgesic action of spinal CCK following endogenous release by i.t. administration of Dyn (Rady et al., 1999) or administration of CCK8s i.t. was inhibited by the δ_2-opioid receptor antagonist naltriben given i.t. Also, 3-day treatment with DOR-1 antisense oligodeoxynucleotide, which down-regulates spinal δ_2-receptors (Tseng et al., 1994), inhibited the antianalgesic action of CCK8s. The indication that a δ_2-receptor function was involved in these antianalgesic responses prompted consideration of ME and LE as the endog-

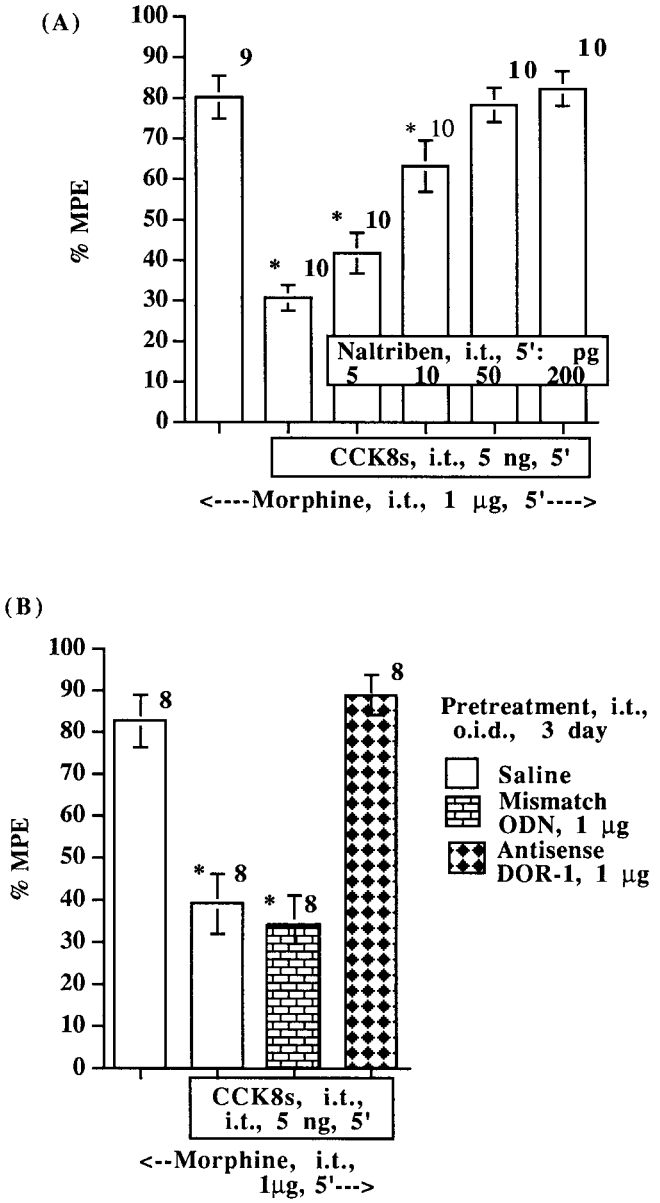

Fig. 3. A, elimination of the antianalgesic action of i.t. CCK8s by increasing doses of naltriben. The asterisk indicates a significant difference from the control group using Dunnett's test ($P \leq 0.05$). B, effect of oligodeoxynucleotide antisense to DOR-1 on CCK8s antianalgesia. Each group of mice was treated once a day with either saline/Triton solution, mismatch ODN (oligodeoxynucleotide), or antisense ODN for 3 days. On the 4th day, the groups were given i.t. morphine and CCK8s. Groups designated with the same asterisk were not different from each other but were significantly different from all groups not similarly marked by Newman-Keuls test ($P \leq 0.05$).

TABLE 1

ED_{50} values for morphine alone and in combination with Dyn, CCK8s, or LE and naltriben (NTB) all given i.t. 5 min before the tail-flick test

	ED_{50} (95% CI)
	μg
Morphine alone	0.07 (0.04–0.12)
+Dyn, 10 pg	0.71 (0.52–0.97)[a]
+Dyn + NTB, 50 pg	0.06 (0.04–0.08)
+CCK8s, 5 ng	0.44 (0.29–0.67)[a]
+CCK8s + NTB, 50 pg	0.14 (0.07–0.27)
+LE, 2 ng	0.59 (0.30–1.15)[a]
+LE + NTB, 50 pg	0.08 (0.04–0.14)

[a] ED_{50} value significantly different from morphine alone value, $P \leq 0.05$.

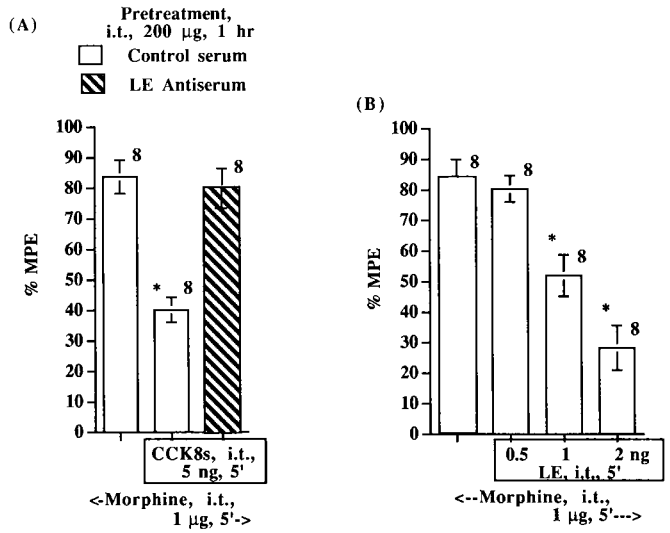

Fig. 4. A, effect of pretreatment with LE antiserum on CCK8s antianalgesia. The LE antiserum or control serum was given i.t., 1 h before the tail-flick test. Asterisk indicates significant difference by Newman-Keuls test ($P \leq 0.05$). B, antianalgesic action of LE. Various doses of LE were given along with i.t. morphine. Asterisk indicates significant difference from control group by Dunnett's test ($P \leq 0.05$).

TABLE 2

Lack of involvement of ME and LE-Arg^6 in antianalgesic action

Morphine was given to all groups, i.t., 5 min before the tail-flick test. CCK8s, 5 ng, ME, at the doses stated, and LE-Arg^6, 2.5 ng, were given at 5 min in the same solution as morphine. Control serum and ME antiserum, 200 *μg*, were given i.t., 1 h before the tail-flick test. Each group had eight CD-1 mice.

	% MPE	S.E.
Control serum + morphine	76.9	5.7
Control serum + morphine + CCK8s	41.7[a]	7.3
ME antiserum + morphine + CCK8s	40.3[a]	6.9
Morphine	85.4	4.1
Morphine + ME, 1 ng	82.2	6.5
Morphine + ME, 10 ng	82.9	3.5
Morphine + ME, 50 ng	78.8	6.1
Morphine	81.9	6.2
Morphine + LE-Arg^6	86.8	5.4

[a] % MPE significantly different from group not similarly marked, $P \leq 0.05$.

enous antianalgesic intermediary. Vaught and Takemori (1979) had shown differential interactions of these peptides on morphine analgesia and our earlier experiments with these peptides as mediators of analgesia provided antisera (Tseng et al., 1985). Also, Vanderah et al. (1996) used LE antiserum and found that LE is the agent responsible for enhancement of morphine analgesia by a CCK receptor antagonist given in the brain of mice. In the present study, LE antiserum inhibited the CCK8s-induced antianalgesia and spinal administration of a small dose of LE (2 ng, 3.6 pmol) produced an antianalgesic action. An action for LE opposite in function than that of enhancement of morphine analgesia was found. This antianalgesic action of LE was inhibited selectively by naltriben as were the antianalgesic actions of Dyn and CCK8s.

The antianalgesic action of Dyn, CCK8s, and LE were eliminated by the i.t. treatment with MK801 and i.t. administration of NMDA produced antianalgesia against i.t. morphine. Also, NMDA and LE had similar selectivity in producing antianalgesia against i.t. DSLET but not DPDPE. This

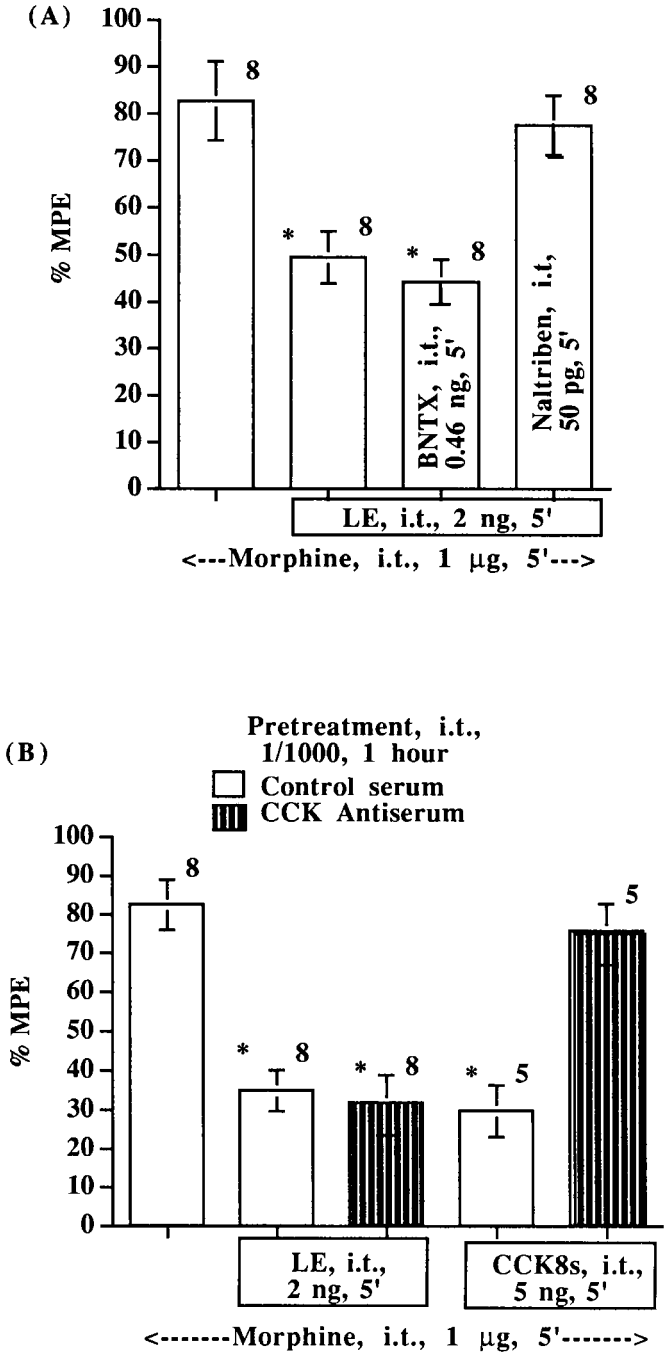

Fig. 5. A, elimination of the antianalgesic action of LE by naltriben but not BNTX. Asterisk indicates significantly different from other groups by Newman-Keuls test, $P \leq 0.05$. B, lack of effect of CCK antiserum on LE antianalgesia. Asterisk indicates significant difference by Newman-Keuls test ($P \leq 0.05$).

selectivity matched that of Dyn- and CCK8s-induced antianalgesia (Rady et al., 1999). The series of results support the sequence of steps 1, 2, and 3 illustrated in Fig. 1. In step 1, CCK precedes LE because CCK antiserum did not inhibit the antianalgesic action of LE. In step 2, LE precedes NMDA because i.t. naltriben treatment inhibited the antianalgesic action of LE but not NMDA. Thus, Dyn and CCK but not NMDA functioned through LE and δ_2-opioid receptors. It should be noted that the presentation of the sequence here does not preclude more direct actions of any of these components as demonstrated for the excitatory actions of opioids (Crain and Shen 1990, 1998, 2000a; Chakrabarti et al., 1998). This provision may apply in situations, such as neuropathic and inflammatory pain models, where large amounts of pain modulators are released (Draisci et al., 1991; Lai, 2000).

In 129S6/SvEv mice, the presence of the analgesic action of DSLET and absence of the antianalgesic action of LE in the present study supports the possibility that NMDA receptor stimulation may be necessary for antianalgesic but not analgesic δ-receptor action. The antianalgesic action of LE through NMDA receptors would suggest that the signal transduction pathway involved in producing antianalgesia is different from those for analgesia (Crain and Shen, 1998, 2000b; Fundytus and Coderre, 1999). In the antianalgesic action of LE against DSLET analgesia in CD-1 mice, it is unlikely that this interaction was at the same δ_2-receptor between LE and DSLET. NMDA receptor activation produced antianalgesia against DSLET-, and morphine-, but not DPDPE-induced analgesia, a selectivity matching the other components in the linear system.

The lack of antianalgesic action to the series of agents in 129S6/SvEv mice fits the rationale that these mice are deficient in responsiveness to NMDA. An alternative possibility is a deficiency in GM_1 ganglioside function. Crain and Shen (2000b) find that GM_1 ganglioside given i.p. produces antianalgesia against systemic morphine in the 129S6/SvEv mice. Their concept is that GM_1 ganglioside is involved in the enhancement of the excitatory actions of opioids through Gs regulatory proteins (Crain and Shen, 2000a). Thus, the alternative is that the antianalgesic action to the components in our system does not occur when GM_1 ganglioside function is deficient. Another caveat is that chemical identification of the LE and glutamate (for the NMDA receptor) purported to be released in the spinal cord was not performed.

The question arises as to the source of the LE especially since Dyn is one of the components involved. Silberring et al. (1992) described a dynorphin convertase in the spinal cord that degrades Dyn into LE-Arg^6. A carboxypeptidase then acts on LE-Arg^6 to form LE. The convertase is highly specific to Dyn in that the liberation of LE-Arg^6 from α-neoendorphin and dynorphin B is very slow. The antianalgesic dose of Dyn (5 fmol) would yield 5 fmol of LE; this is much less than the antianalgesic dose of LE (2 ng, 3.6 pmol). The LE precursor LE-Arg^6 had no antianalgesic action at the 3.6-pmol dose. Also, CCK does not release Dyn (Rady et al., 1999) so it seems unlikely that Dyn is the source of the LE. The possibility of spinal Dyn becoming a source for LE does arise when there is a high concentration of Dyn in the spinal cord such as has been observed in certain pain models (Draisci et al., 1991). The i.t. administration of dynorphin (1-13) antiserum restores the sensitivity to morphine in a rat model of neuropathic pain (Nichols et al., 1997). On the other hand, endogenous LE-like material functions as an analgesic agent (Ossipov et al., 1996) where i.t. administration of LE antiserum increases the nociceptive flinching response to formalin administered into the paw of rats. Dynorphin (1-13) antiserum has the same effect. LE could have come from either dynorphin A(1-17) or preproenkephalin. Similarly, LE is implicated in the enhancement of morphine analgesia produced by a CCK receptor antagonist in the brain of mice (Vanderah et al., 1996). Administration of 2.5 μg of LE i.c.v. in mice enhances morphine analgesia. In the present case, i.t. LE

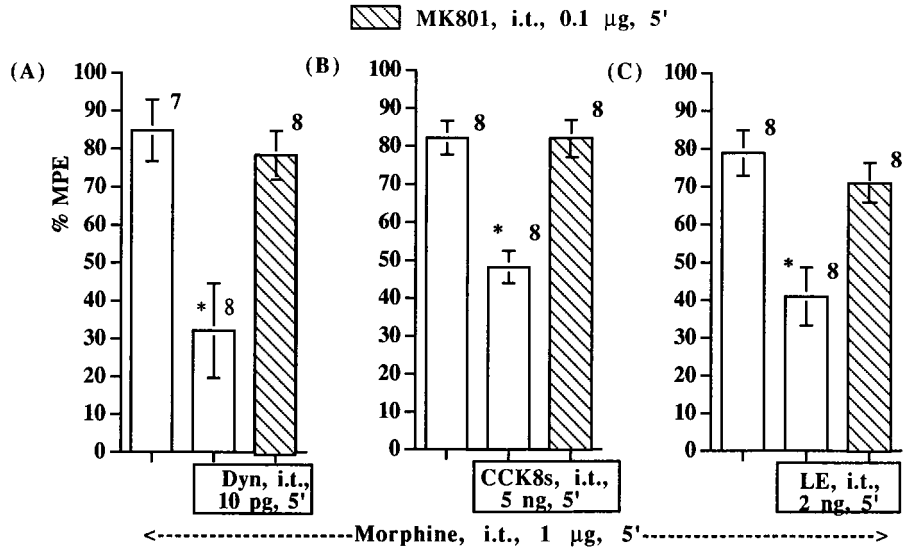

Fig. 6. Antianalgesic action of i.t. Dyn (A), CCK8s (B) and LE (C) as modified by i.t. MK801 administration. Asterisk indicates significant difference by Newman-Keuls test, $P \leq 0.05$.

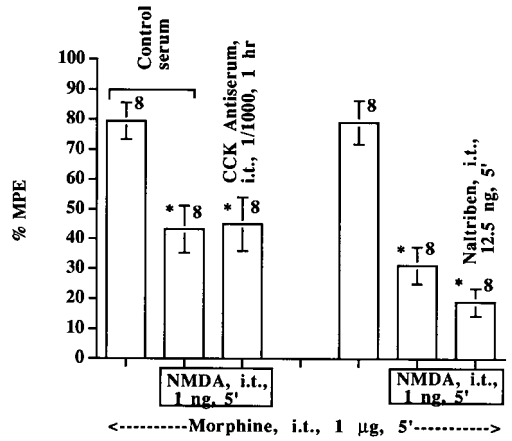

Fig. 7. NMDA-induced antianalgesic action in CD-1 mice action not eliminated by CCK antiserum pretreatment nor naltriben treatment. Asterisk indicates significant difference by Newman-Keuls test, $P \leq 0.05$.

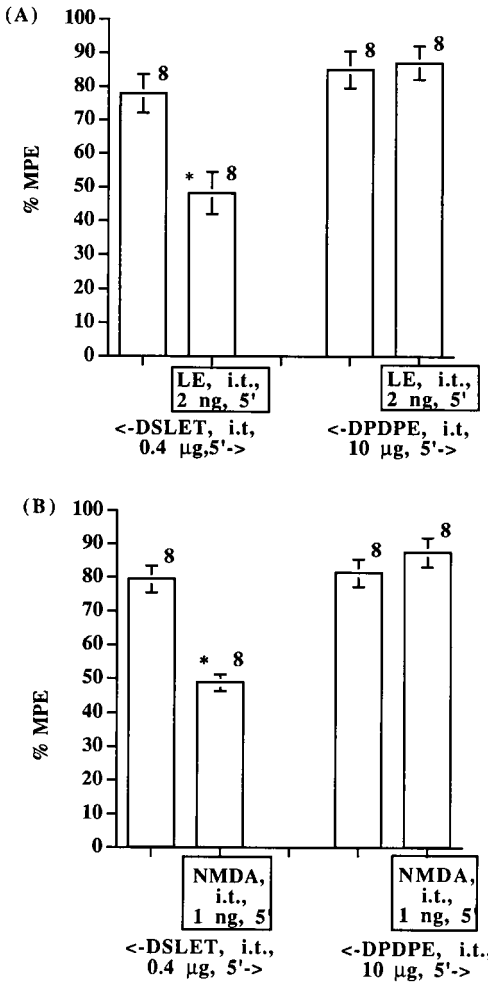

Fig. 8. Antianalgesic action of LE and NMDA against analgesic action of i.t. DSLET and DPDPE. A, LE given i.t. attenuated the analgesic action of i.t. DSLET but not DPDPE. B, NMDA given i.t. attenuated the analgesic action of i.t. DSLET but not DPDPE. Asterisk indicates significant difference by Student's t test, $P \leq 0.05$.

had the opposite effect of producing antianalgesia when interacting with morphine. For this reason, LE acted as an inverse agonist; more specifically, a δ_2-opioid receptor inverse agonist. This inverse agonist action was obtained at 2 ng, which is approximately 1/1000 the amount necessary to produce analgesic synergism with morphine (Vanderah et al., 1996). The concept of inverse agonist action has been applied to opioid receptor antagonists (among others, Szekeres and Traynor, 1997) but the ability of opioid agonists and antagonists to have such dual actions has been established by Crain and Shen (1990, 1998, 2000a). In the dorsal root ganglion preparation, they find that opioids (including Dyn, LE, and morphine) at low concentrations have an excitatory action to prolong the action potential duration. At higher concentrations, opioids have an inhibitory effect to shorten the action potential duration. This view would reconcile the antianalgesic action obtained in the present study at a low dose of LE with the analgesic action obtained at high concentrations of LE (Ossipov et al., 1996; Vanderah et al., 1996). Furthermore, the antianalgesic action of i.t. Dyn is inhibited by i.t. cholera toxin, suggesting that an excitatory action occurs through activation of opioid receptors coupled to Gs regulatory proteins (Arts et al., 1993). Even though we would like to think that the antianalgesic action of LE is an excitatory action, evidence is lacking for an excitatory action in vivo with its implication for opioid receptor Gs protein coupling.

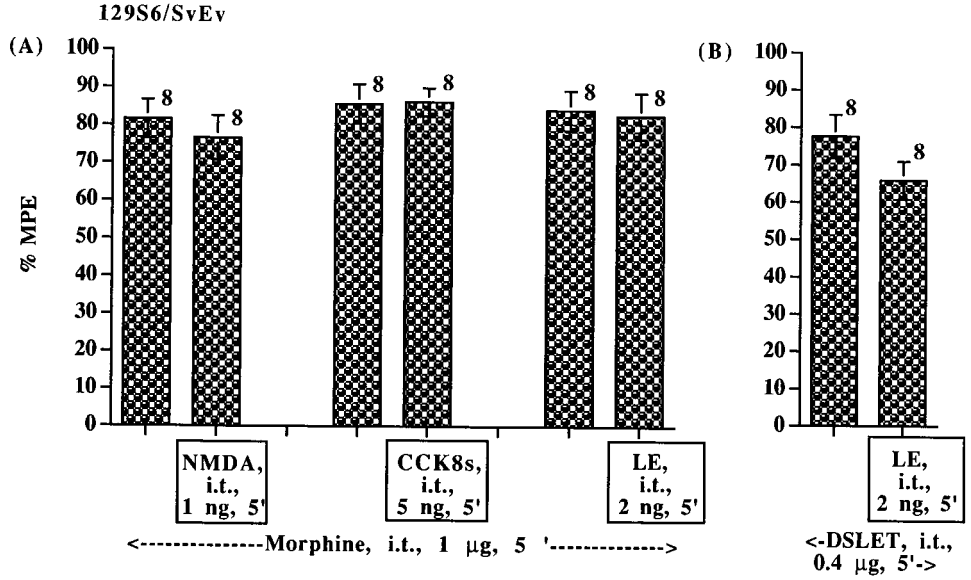

Fig. 9. Absence of antianalgesic response in 129S6/SvEv mice. A, analgesic action of i.t. morphine was not attenuated by i.t. NMDA, CCK8s, and LE. B, analgesic action of DSLET present in 129S6/SvEv mice was also not attenuated by LE.

Thus, at present, it appears that the term inverse agonist action is appropriate for the situation.

The linear sequence of steps proposed here offers the heuristic possibility of bringing together several separate concepts. The antiopioid action of CCK and Dyn may have implications in relation to morphine action and morphine tolerance (Rothman, 1992; Goodman et al., 1995; Wiesenfeld-Hallin and Xu, 1996). Repeated intrathecal treatment of rats with μ- and δ-agonists not only produces tolerance but also allodynia and hyperalgesia; resistance to the analgesic action of morphine found in the nerve ligation model of neuropathic pain in rats has similarities to morphine tolerance; increased lumbar concentrations of Dyn are found in these situations and the pharmacological responses are reversed by Dyn antiserum (Gardell et al., 2000). Concomitant administration of δ_2-opioid receptor antagonists with morphine inhibits the development of tolerance to morphine (Abdelhamid et al., 1991; Miyamoto et al., 1993a,b, 1994) and could be consistent with the sensitization to an excitatory action (Chakrabarti et al., 1998; Crain and Shen, 2000a) and perhaps to an endogenous opioid, LE. Furthermore, implications may exist regarding physiological function. Rats conditioned to give a stress-induced analgesic response can be further conditioned to a safety cue so that when the latter cue is activated, the rat responds by terminating the stress-induced analgesia (Wiertelak et al., 1992). This termination depends on the release of spinal CCK. The safety cue is also sufficient to inhibit the antinociceptive action of morphine. It is tempting to suggest that the inverse agonist action of LE (released by spinal CCK) might be involved.

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