Supraspinal Neurotensin-Induced Antianalgesia in Mice
Is Mediated by Spinal Cholecystokinin

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ABSTRACT—Intracerebral injection of neurotensin into specific brain loci in rats produces hyperalgesia due to the release of cholecystokinin (CCK) in the spinal cord. The present purpose was to show in another species that neurotensin can antagonize the antinociceptive action of morphine through the spinal CCK mechanism in mice. Neurotensin given intracerebroventricularly (i.c.v.) at doses higher than 100 ng produced antinociception in the tail flick test. However, at lower doses between 1 pg to 25 ng, neurotensin antagonized the antinociceptive action of morphine given intrathecally (i.t.), thus demonstrating the antianalgesic activity of neurotensin. The rightward shift in the morphine dose-response curve produced by i.c.v. neurotensin was eliminated by an i.t. pretreatment with CCK₈ antibody (5 µl of antiserum solution diluted 1:1000). I.t. administration of lorglumide, a CCK₈-receptor antagonist (10−1000 ng), and PD135,158, a CCK₃-receptor antagonist (250−500 ng), also eliminated the antianalgesic action of neurotensin. Thus, the mechanism of the antianalgesic action of neurotensin given i.c.v. involved spinal CCK. This mode of action is similar to that for the antianalgesic action of supraspinal pentobarbital which also involves spinal CCK.

Keywords: Neurotensin (intracerebroventricular), Antianalgesia, Cholecystokinin spinal role, Morphine (intrathecal), Spinal cholecystokinin antagonist

Even though the predominant amount of work shows that neurotensin injected into the brain produces analgesia, hyperalgesia (measured as a decrease in the latency of the tail flick response to a nociceptive heat stimulus) occurs when small doses of neurotensin are administered intracerebrally into the brain of the rat (1−4). The locus of this action is in the medullary nucleus raphe magnus. Urban et al. (1) further demonstrated that the hyperalgesic action was mediated through the action of spinal cholecystokinin (CCK). This conclusion rests on the finding that intrathecal (i.t.) administration of the CCK₈-receptor antagonist L-365,260 and, to a lesser extent, the CCK₃ receptor antagonist devazepide inhibits the hyperalgesia. In addition to hyperalgesia, neurotensin administered into the nucleus raphe magnus has an antianalgesic action to eliminate the antinociception produced by morphine microinjected into the periaqueductal gray area (1−3). The antianalgesic action may also involve the nucleus reticularis gigantocellularis and nucleus reticularis paragigantocellularis lateralis because microinjection of (d-tryptophan¹³)-neurotensin or neurotensin antibody at these sites enhances morphine antinociception elicited from the periaqueductal gray area by antagonizing the action of endogenous neurotensin (2).

The purpose of the present study was to demonstrate the antianalgesic action of neurotensin in the mouse and presumptive involvement of spinal CCK in this antianalgesia. The advantage of using the mouse was that direct intracerebroventricular (i.c.v.) and i.t. injections can be made acutely, thus eliminating the need for chronic cannula placement. Positive results would extend the findings to another species, and the mouse model could then be used to find and compare other possible antianalgesic agents that may release spinal CCK. In the present study, the antianalgesic action of neurotensin given i.c.v. against the antinociceptive action of morphine administered i.t. was examined using the tail flick test (TFT). To evaluate the involvement of spinal CCK in this antianalgesic
action, CCK\textsubscript{8} antiserum and the CCK-receptor antagonists, lorglumide and PD135,158, were administered i.t.

MATERIALS AND METHODS

**Animals and measurement of antinociception**

Male CD-1 mice weighing 25 to 30 g were obtained from Charles Rivers (Wilmington, MA, USA). Each animal was used for only one experiment. Antinociception was measured by the radiant heat TFT using a cut-off time of 10 sec as the maximal antinociceptive response time as given in the method of D'Amour and Smith (5). This cut-off time prevented damage to the tail when the instrument was set to give control latencies (predrug times) of 2–4 sec. A timer and the radiant heat source were turned on simultaneously by pushing a button and turned off automatically by removal of the tail from the path of the light source or reaching the 10-sec cut-off time. The latency for removal of the tail was converted to percent maximum possible effect (% MPE) as calculated (6) by the following formula:

\[
% \text{MPE} = \frac{\text{Postdrug} - \text{Predrug}}{\text{10} - \text{Predrug}} \times 100
\]

**Drug administration**

Neurotensin was administered i.c.v. in a volume of 4 \(\mu l\) (7) under light halothane anesthesia, generally 10 min before the TFT. This time was chosen based on the duration of action studies (given in the results) where the time of administration of neurotensin was varied. Morphine, CCK\textsubscript{8} antiserum, lorglumide and PD135,158 were administered i.t. in a volume of 5 \(\mu l\) by the method of Hyldean and Wilcox (8). The morphine and CCK\textsubscript{8} antiserum were given 5 min and 1 hr before the TFT, respectively, as in a previous study (9). Duration of action studies were performed with the CCK antagonists, and the peak effect was observed to occur at 5 min. Doses and times of administration of drugs are given with each experiment. The times stated indicate the time between administration and the TFT. Appropriate vehicle solutions were given at each site when a drug was not given (e.g., for the control morphine dose-response curve where saline with 0.01% Triton X-100 was given i.c.v. in place of neurotensin). Drugs were dissolved in a 0.9% saline solution or for neurotensin, a 0.9% saline solution containing 0.01% Triton X-100. All studies were done in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

**Statistical analyses**

For the morphine dose-response data, the % MPE values were plotted versus the log doses for i.t. morphine (as altered by neurotensin and then CCK\textsubscript{8} antibody treatment), and ED\textsubscript{50} values were derived and compared by the method of Litchfield and Wilcoxon (10) with a computer version as described by Dewey et al. (6). This analysis involved transformation of the % MPE's to probits and included derivation of potency ratios for the ED\textsubscript{50} values of the various curves compared to one another. Evaluation of the slopes for parallelism and the potency ratios involved the Chi Squared test. Analysis of single dose experiments that involved a comparison between two groups was by Student's \(t\)-test. Those involving more than two groups were first analyzed by analysis of variance, and if a significant difference was found, a second step was performed. In the subsequent step, where several groups were compared to a single control group, Dunnnett's test was used and where several groups were compared to each other, Neuman-Keuls' test was used (11). In the latter situation, only the results for the main comparisons are given even though all possible comparisons were made. A \(P \leq 0.05\) was taken to indicate a significant difference between groups in all of these analyses.

**Source of drugs**

The drugs and commercial sources were as follows: morphine sulfate \(\cdot 5\text{H}_2\text{O}\) (Mallinckrodt Chemical Works, St. Louis, MO, USA); neurotensin (Peninsula Laboratories, Inc., Belmont, CA, USA); lorglumide sodium salt and PD135,158 \(N\)-methyl-DL-glucamine (Research Biochemicals, Natick, MA, USA) and CCK\textsubscript{8} antiserum (Chemicon International, Inc., Temecula, CA, USA). The doses of the drugs, given with the experiments, were for the forms stated above.

**RESULTS**

**Dose-response relationship for i.c.v. neurotensin to produce antinociception and to produce antianalgesia against i.t. morphine**

Figure 1 illustrates the dose-response relationship for the effect of neurotensin, given i.c.v., on the tail flick response. Doses above 100 ng produced an antinociceptive effect. A dose above 10 \(\mu g\) was not given; therefore, it is not known whether full efficacy for suppression of the tail flick response would have been obtained. The points of interest were at the lower doses where the tail flick response was not affected.

In the study depicted in Fig. 2, the dose effect of neurotensin to antagonize i.t. morphine induced antinociception was evaluated. Neurotensin was given i.c.v. 10 min before the TFT at varying doses (1 pg to 100 ng) against a fixed dose (1 \(\mu g\)) of morphine given i.t. 5 min before the TFT. At doses of 1 pg up to 25 ng, neurotensin decreased the antinociceptive effect of i.t. morphine, an action designated as an antianalgesic effect of neurotensin. At the high dose of 100 ng (Fig. 2), the effect of neurotensin
was not significant. Response to doses above 100 ng of neurotensin were not tested because the presence of the overt analgesic action of neurotensin (as determined in Fig. 1) would make interpretation of the data difficult. In separate experiments, no antianalgesic action was found at a 0.1-ug dose (% MPE ± S.E.M. values were as follows: i.c.v. saline with 0.01% Triton X-100 + i.t. morphine = 78.1 ± 4.0%, i.c.v. neurotensin + i.t. morphine = 77.0 ± 5.4%) and a 0.5-ug dose (i.c.v. saline with 0.01% Triton X-100 + i.t. morphine = 76.6 ± 3.1%, i.c.v. neurotensin + i.t. morphine = 73.1 ± 6.9%). Neurotensin given intravenously in a dose of 1 pg or 1 ng 10 min before the TFT had no effect on the analgesic action of morphine (1 µg, i.t., 5 min) (data not given).

The time course of the antianalgesic action of the 1-ug and 10-ug doses of i.c.v. neurotensin against i.t. morphine was determined by increasing the time between neurotensin administration and the TFT while keeping the time and dose of i.t. morphine fixed (Fig. 3). At 10 min, both doses of neurotensin showed significant antagonism. At 20 min, even though the 1-ug dose of neurotensin showed significant antagonism (Fig. 3A) the 10-ug dose was no longer effective (Fig. 3B). This may be analogous to the shorter hyperalgesic response produced by a larger dose as reported by Urban et al. (1). In their studies, the duration of hyperalgesia produced by neurotensin in the nucleus raphe magnus was 20 min for the 0.1- and 0.3-nmol doses, whereas the duration was 30 min for the 0.03-nmol dose. This unusual pattern of action of neurotensin was not investigated further in the present study.
Effect of i.t. administration of CCK₈ antiserum on the antianalgesic action of i.c.v. neurotensin

Figure 4 shows the dose-response curve for i.t. morphine given 5 min before the TFT. The ED₅₀ (95% confidence interval) for i.t. morphine in the presence of i.c.v. saline was 0.36 (0.2-0.65) μg. When neurotensin (1 pg) was given i.c.v., the morphine dose-response curve was shifted significantly to the right in a parallel fashion. The new ED₅₀ was 1.8 (1.1-2.97) μg and represented a fivefold shift. The antianalgesic action of neurotensin was eliminated by giving i.t. CCK₈ antiserum (1:1000 dilution, 5 μl) 1 hr earlier. This curve was also parallel to the other two curves and had an ED₅₀ value of 0.11 (0.04-0.33) μg, which was not significantly different from the control morphine ED₅₀ value. When the CCK₈ antiserum prebound to CCK₈ was given 1 hr before the TFT, administration of neurotensin still antagonized the morphine effect (data not given). That is, prebinding eliminated the action of the antiserum.

Effect of i.t. administration of lorglumide and PD135,158 on the antianalgesic action of i.c.v. neurotensin

The antianalgesic action of i.c.v. neurotensin was replicated as the first and second bars in each set of experiments in Fig. 5A. Lorglumide, a CCK₆-receptor antagonist (12), at the dose of 0.1 ng coadministered with morphine i.t. 5 min before the TFT did not alter neurotensin antianalgesia. However, when given at doses of 10 and 100 ng, lorglumide eliminated the antianalgesic action of the 1-pg dose of i.c.v. neurotensin. In another experiment (data not given), 1 μg of lorglumide given i.t. eliminated the antianalgesic action of a 10-ng dose of neurotensin.

The duration of lorglumide action was determined by varying the time of lorglumide administration while keeping the time of i.t. morphine and i.c.v. neurotensin administration fixed. The results in Fig. 5B indicated that...
the action of the 100-ng dose of lorglumide diminished at 15 min and was gone by 30 min.

The effectiveness of PD135,158, a CCKₐ-receptor antagonist (13, 14), was evaluated in a similar manner. The results in Fig. 6A show that the 250-ng and 500-ng doses of PD135,158 were effective in eliminating the antianalgesic action of i.c.v. neurotensin, while 100 ng was ineffective. The duration of action of i.t. PD135,158, like

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**Fig. 5.** Inhibition of the antianalgesic action of i.c.v. neurotensin against i.t. morphine by i.t. lorglumide, a CCKₐ-receptor antagonist. A: Lorglumide dose-response effect. Three separate experiments are shown: in each set, the open bars represent i.t. morphine (1 μg, 5 min before the TFT); the striped bars represent i.t. morphine + i.c.v. neurotensin (1 pg, 10 min before the TFT) combination, and the stippled bars are the same morphine neurotensin combination plus i.t. lorglumide. Lorglumide, at the doses indicated, was coadministered with i.t. morphine. Asterisk indicates significantly different from other groups not similarly marked within the same experiment (P ≤ 0.05) as evaluated by Neuman-Keul's test. B: Lorglumide time course. The doses and times of administration of morphine, i.t., and neurotensin, i.c.v., were fixed as indicated, while lorglumide was given i.t. at a 100-ng dose at the different times indicated (the time indicated is that between lorglumide administration and the TFT). Asterisk has the same meaning as in panel A.
lorglumide, was short (Fig. 6B); the effect of the 500-ng dose of PD135,158 was no longer significant at 15 min.

**DISCUSSION**

Administration of high doses (>100 ng) of neurotensin i.c.v. produced antinociception. This finding agrees with the literature which suggests that analgesia is the predominant action of supraspinal neurotensin in mice (15, 16). Work in rats suggests that the antinociceptive action of neurotensin occurs through sites in the rostro-ventral medulla, periaqueductal gray area and perhaps other sites (1–4, 17–20).

In the present study, the primary interest was in the

![Graph](image)

**Fig. 6.** Inhibition of the antinociceptive action of i.c.v. neurotensin against i.t. morphine by i.t. PD135,158. A: PD135,158 dose-response effect. All groups were given i.t. morphine, 1 μg, 5 min before the TFT. Neurotensin was given i.c.v., 1 pg, 10 min before the TFT. PD135,158 was given i.t. at the doses indicated along with the morphine. Other designations are as in previous figures. B: PD135,158 time course. The doses and times of administration of morphine, i.t., and neurotensin, i.c.v., were fixed as indicated. A 500-ng dose of PD135,158 was given i.t. at the different times indicated (time is that between PD135,158 administration and the TFT). Asterisk has same meaning as in Fig. 5A.
antianalgesic action of neurotensin given i.c.v. in mice. This action was demonstrated by the ability of i.c.v. neurotensin to produce a rightward shift of the dose-response curve for i.t. morphine-induced analgesia. The doses of neurotensin (1 pg and 10 ng) that produced the antianalgesic effect are well below the dose needed to produce analgesia. The dose-dependent selectivity for neurotensin action may explain the longer duration of antianalgesic action of the 1-pg as compared to the 10-ng dose of neurotensin.

Urban et al. (1, 2) discuss several alternatives for the biphasic action of neurotensin in the rat. A plausible argument would be that the antianalgesic site of action is more sensitive than the analgesic site; however, recruitment of analgesic sites predominates when larger doses are given. Because previous studies in mice gave no indication of the antianalgesic action of neurotensin, it was not studied before. For instance, the non-analgesic doses of neurotensin did not produce hyperalgesia that would have indicated the possibility of an antianalgesic action. In the rat, the presence of both hyperalgesic and antianalgesic responses to neurotensin (2, 3) ultimately led Urban et al. (1) to discover that intracerebral administration of neurotensin releases spinal CCK. It is well established in the literature and confirmed by work in this laboratory that CCK released endogenously in the spinal cord and administered intrathecally antagonizes morphine analgesia (9, 12, 21–25).

The present results show that i.c.v. administration of neurotensin inhibited i.t. morphine-induced analgesia. Because the tail flick response is a spinal reflex that remains intact and can still be inhibited by morphine after spinal transection in mice (26), the effect of i.c.v. neurotensin indicates that neurotensin has a descending modulatory influence on spinal morphine action. That this antianalgesic action of i.c.v. neurotensin is mediated by spinal CCK was supported by elimination of the antianalgesia by i.t. pretreatment with CCK8 antisera. Administration of CCK-receptor antagonists, lorglumide and PD135,158, i.t. also eliminated the antianalgesic action of i.c.v. neurotensin. Even though both antagonists were previously shown to inhibit the antianalgesic action of CCK8 given i.t. or released spinally by pentobarbital given i.c.v. in mice (9), the relative receptor selectivities were not established pharmacologically by evaluating differential effectiveness. Therefore, no conclusion can be given as to which CCK-receptor subtype played the major role in neurotensin antianalgesia. For the hyperalgesic effect of neurotensin in the rat, Urban et al. (1) conclude that the spinal CCK8 receptor is involved due to the greater potency of the CCK8 receptor antagonist L-365,260 compared to the CCK4 receptor antagonist devazepide. Evidence exists, at least in the rat, for the necessary connection between the brain and the spinal cord of a descending neuronal pathway mediated by spinal CCK (27, 28).

The CCK8 antiserum treatment not only attenuated the antianalgesic action of i.c.v. neurotensin against i.t. morphine-induced analgesia but also brought the dose-response curve for morphine analgesia a little to the left beyond the control dose-response curve. Even though this was not a significant effect, it may indicate that morphine itself releases spinal CCK. Spinal release of CCK due to morphine administration has been reported in the rat (29). Also, others have reported that CCK antagonists enhance morphine analgesia, which further suggests the release of CCK by morphine (12, 30–34). This aspect was not studied further because it was beyond the immediate intent of the present work.

The hyperalgesic and antianalgesic actions of neurotensin appear to occur at similar doses in the rat (1–4), and the question arises as to whether hyperalgesia causes the antianalgesic action. It is possible for agents to have separate hyperalgesic and antianalgesic actions (35). In the present study where i.c.v. neurotensin produced antianalgesia, hyperalgesia could not be detected in the TFT with groups given the various doses of neurotensin (Fig. 1). Similarly, in an earlier study, the antianalgesic actions of CCK occurred in a dose range where a hyperalgesic response in the TFT was not observed (9). Thus, the present concept that i.c.v. neurotensin antianalgesia was mediated by spinal CCK in mice is consistent in that neither i.c.v. neurotensin nor its spinal intermediary, CCK, produced hyperalgesia. In our hands, the mouse TFT can detect the hyperalgesic action of i.c.v. pentobarbital, but the lack of regression of the response with dose puts a severe limitation on attempting to relate hyperalgesia to antianalgesic action (36). I.t. CCK8 also produces antianalgesia in the rat TFT against PL017, a µ-receptor-selective peptide, without producing hyperalgesia (36). Thus, hyperalgesia may not play an integral part in antianalgesia. In the physiological functioning of the CCK system, the antiopioid action appears to maintain homeostasis through its antianalgesic action rather than produce a hyperalgesic state (35).

Even though the present study demonstrated that i.c.v. neurotensin produces antagonism of i.t. morphine analgesia by releasing CCK in the spinal cord, CCK is only one of a number of endogenous peptides that have antiopioid activities. As reviewed by Rothman (37), other endogenous peptides with antiopioid actions include MIF-1, FMRFamide, thyrotropin releasing hormone, corticotropin releasing factor and dynorphin A (1–17). More recently, a peptide referred to as nociceptin, has been identified as the product of an opioid-receptor-like (ORL-1) gene (38) that has antiopioid actions. Because
spinal dynorphin A (1–17) has an antianalgesic action (26), we initially tested whether neurotensin-induced antianalgesia was mediated by spinal dynorphin A (1–17). Dynorphin antisera had no effect on the antianalgesic action of i.c.v. neurotensin; thus spinal dynorphin A (1–17) did not mediate the antianalgesic action of neurotensin (B.B. Holmes et al., unpublished data). The concept of endogenous antiopioid systems has heuristic value (22, 35, 37). Just as there are diverse systems for producing analgesia, existence of diverse antianalgesic or antiopioid systems for modulating analgesia and hyperalgesic systems provide for fine gain control over the physiological responses to pain. The present mouse model offers a facile approach to evaluating various descending antianalgesic systems. In our own case, the points of commonality between the antianalgesic actions of spinal dynorphin A (1–17) (26), i.c.v. pentobarbital (9) and i.c.v. neurotensin can be studied further.

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REFERENCES

1 Urban MO, Smith DJ and Gebhart GF: Involvement of spinal cholecystokinin receptors in mediating neurotensin hyperalgesia from the medullary nucleus raphe magnus in the rat. J Pharmacol Exp Ther 278, 90–98 (1996)


25 Magnusson DSK, Sullivan AF, Simonnet G, Roques BP and Dickenson AH: Differential interactions of cholecystokinin and FLFQPQRF-NH2 with mu and delta opioid antinoception in
the rat spinal cord. Neuropeptides 16, 216–218 (1990)
26 Wang FS, Rady JJ and Fujimoto JM: Elimination of the anti-
  analgesic action of dynorphin A (1–17) by spinal transection in
  barbital anesthetized mice. J Pharmacol Exp Ther 268, 873–
  880 (1994)
27 Mantyh PW and Hunt SP: Evidence for cholecystokinin-like
  immunoreactive neurons in the rat medulla oblongata which
28 Skirboll L, Hokfelt T, Dockray G, Rehfeld J, Brownstein M
  and Cuello A.: Evidence for periaqueductal cholecystokinin-
  substance P neurons projecting to the spinal cord. J Neurosci 3,
  1151–1157 (1983)
29 Zhou Y, Sun YH, Zhang ZW and Han JS: Increased release of
  immunoreactive cholecystokinin octapeptide by morphine and
  potentiation of mu-opioid analgesia by CCK\textsubscript{\textdelta} receptor antag-
  onist L-365,260 in rat spinal cord. Eur J Pharmacol 234,
  147–154 (1993)
30 Watkins LR, Kinscheck IB and Mayer DJ: Potentiation of
  morphine analgesia by the cholecystokinin antagonist
31 Wiesenfeld-Hallin Z, Xu XI, Hughes J, Horwell DC and
  Hokfelt T: PD134308, a selective antagonist of cholecystokinin
  type B receptor, enhances the analgesic effect of morphine and
  synergistically interacts with intrathecal galanin to depress
  spinal nociceptive reflexes. Proc Natl Acad Sci USA 87,
  7105–7109 (1990)
32 Dourish CT, O'Neill MF, Schaffer LW, Siegl PK and Iversen
  SD: The cholecystokinin receptor antagonist devazepide en-
  hances morphine-induced analgesia but not morphine-induced
  respiratory depression in the squirrel monkey. J Pharmacol Exp
  Ther 255, 1158–1165 (1990)
33 Ossipov MH, Kovelowski CJ, Vanderah T and Porreca F:
  Naltrindole, an opioid \textdelta antagonist, blocks the enhancement of
  morphine-antinociception induced by a CCK\textsubscript{\textdelta} antagonist in the
34 Lavigne GL, Millington WR and Meuller GP: The CCK\textsubscript{\textalpha} and
  CCK\textsubscript{\textdelta} receptor antagonists, devazepide and L-365,260, enhance
  morphine antinociception only in non-acclimated rats exposed
35 Maier SF, Wiertelak EP and Watkins LR: Endogenous pain
  facilitatory systems. Analgesia and hyperalgesia. Am Pain Soc J
  1, 191–201 (1992)
36 Wang FS and Fujimoto JM: Pentobarbital administered
  intracerebroventricularly antagonizes morphine-induced anti-
  nociception in mice. J Pharmacol Exp Ther 265, 1361–1368
  (1993)
37 Rothman RB: A review of the role of anti-opioid peptides in
  morphine tolerance and dependence. Synapse 12, 129–138
  (1992)
38 Meunier JC, Mollerau C, Toll L, Suaudeau C, Moisand C,
  Alvinerie P, Batour JL, Guillemont JC, Ferrara P, Monsarrat
  B, Mazarguil H, Vassart G, Parmentier M and Costentin J: Isola-
  tion and structure of the endogenous agonist of opioid recep-