*Full Paper***Activation of Spinal Cholecystokinin and Neurokinin-1 Receptors Is Associated With the Attenuation of Intrathecal Morphine Analgesia Following Electroacupuncture Stimulation in Rats**

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Received February 27, 2007; Accepted April 17, 2007

Abstract. We previously demonstrated that electroacupuncture (EA) stimulation both produced antinociception and attenuated intrathecal (i.t.) morphine analgesia, suggesting that EA is capable of inducing two opposing systems, that is, opioid and anti-opioid mechanisms. This study examined the involvement of cholecystokinin (CCK) in the anti-opioid effects following EA in the spinal cord. EA was applied to commonly used acupoints for antinociception, ST-36 located 5-mm lateral to the anterior tubercle of the tibia, and analgesia was assessed by the hind-paw pressure test in male Sprague-Dawley rats. I.t. administration of CCK (0.01 – 10 μ g) attenuated i.t. morphine analgesia (10 μ g) dose-dependently. The attenuation of morphine analgesia following EA was reversed by i.t. proglumide, a CCK-receptor antagonist (0.01 μ g). CCK-like immunoreactivity was increased in lamina I and II in the dorsal horn, and expression of spinal CCK mRNA increased after EA. Moreover, i.t. pretreatment with the neurokinin-1 (NK1)-receptor antagonist L-703,606 (18 μ g) reversed both EA- and CCK-induced attenuation of morphine analgesia. These results suggest that CCK-mediated neural systems in the spinal cord may be involved in the attenuation of morphine analgesia following EA and that substance P-induced activation of NK1 receptors may be responsible for the downstream neuronal transmission of the CCK-mediated neuronal system.

Keywords: electroacupuncture, morphine, anti-opioid, cholecystokinin, neurokinin

Introduction

An anti-opioid system, which opposes the activity of the endogenous opioid system, is believed to be the one of the physiological feedback systems that maintain homeostatic control of nociceptive transmission (1). Previously we demonstrated that the analgesic effect of morphine was significantly attenuated by the activation of the endogenous opioid system via electroacupuncture (EA) stimulation (2), clearly showing involvement of endogenous opioid peptides in the activation of the anti-opioid system. In addition, analgesia produced by the intrathecal (i.t.), but not intracerebroventricular, admin-

istration of morphine was significantly attenuated following EA stimulation (2), indicating that the spinal cord is a major site for the activation of this anti-opioid system.

Several neuropeptides have been suggested to be responsible for this anti-opioid effect, including cholecystokinin (CCK) (3), nociceptin (4), neuropeptide FF (5), and others. These substances showed no effects on pain thresholds by themselves; however, coadministration of these compounds with opioid analgesics markedly attenuated the analgesic effect of the opioid. However, the physiological importance of the anti-opioid system and aspects of these anti-opioid substances are still obscure.

CCK is generally known as gastrointestinal hormone secreted from the mucosal membrane of the duodenum, but it is also a predominant component of the neuro-

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Published online in J-STAGE: June 8, 2007

doi: 10.1254/jphs.FP0070475

peptide located in the central nervous system (CNS). Accumulating evidence reveals that CCK in the CNS is involved in diverse functions, including feeding, satiety, cardiovascular regulation, anxiety, pain, analgesia, memory, neuroendocrine control, osmotic stress, dopamine-related behaviors, and neurodegenerative and neuropsychiatric disorders (6). In 1982, Itoh et al. (7) first reported that CCK suppressed β -endorphin-induced antinociceptive effects and other studies (8–10) also demonstrated that the administration of opioid analgesics elicited release of endogenous CCK from the spinal cord. In addition, Faris et al. (3) indicated that CCK antagonized opiate analgesia produced by front paw foot shock and morphine, but that opiate-independent analgesia induced by hind paw foot shock was not reduced by CCK. Taken together, these results suggest that CCK is closely related to the modulation of nociceptive transmission by endogenous opioid peptides and that the activation of the endogenous opioid system could induce the release of CCK in the spinal cord. Thus, CCK has been considered as one possible candidate related to the anti-opioid system. However, there is no evidence that determines whether activation of the endogenous opioid system induces release of CCK in the spinal cord.

Substance P is considered to be one of the neurotransmitters released from primary afferent neurons during nociceptive transmissions (11, 12). One mechanism of opioid analgesia is suppression of substance P release from the primary afferent neurons by presynaptic inhibition. Indeed, i.t. administration of morphine inhibited the release of substance P in the spinal dorsal horn (13). In studies using substance P knockout mice, the response to moderate to intense pain was significantly reduced, although the behavioral response to mildly painful stimuli was unchanged (14, 15). These results suggest that substance P may have an important modulatory role on nociceptive transmission. We therefore hypothesized that the enhanced activity of spinal substance P may be involved in attenuation of morphine analgesia following EA stimulation through the activation of an anti-opioid property of CCK.

This study was designed to elucidate the involvement of CCK in the attenuation of the analgesic effect of morphine following EA stimulation and to determine the involvement of substance P in the mechanism of the anti-opioid property of CCK.

Materials and Methods

Animals

Male Sprague-Dawley rats (250–350 g; SLC, Shizuoka) were used. The animals were kept on a 12-h light-

dark cycle condition (lights on at 8:00 h) in an air conditioned environment (23°C–24°C, 60% humidity) and were housed in groups of two in plastic cages with food and water available ad libitum. Behavioral tests and care of the animals were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of Wakayama Medical University.

Experimental procedures

Surgical procedure: Rats were implanted with spinal catheters for i.t. administration as previously described (16). In brief, under pentobarbital anesthesia (50 mg/kg, i.p.), a midline dorsal incision was made and the lumbar vertebrae from L₂ to L₃ were exposed unilaterally. An intervertebral puncture between L₂ and L₃ was made with a 21-gauge needle and a PE-10 polyethylene tube (14 cm in length) filled with sterile saline was inserted 2-cm rostrally into the subarachnoid space to locate its tip at T₁₂. The outer end of the catheter was passed subcutaneously, exteriorized between the scapulae, and plugged with a short length of stainless steel wire. After surgery, the animals were housed individually and allowed 10 days of postoperative recovery. Rats showing any neurological deficits resulting from the surgical procedure were excluded from the experiments.

Microinjection procedure: A 25- μ l Hamilton syringe was connected by polyethylene tubing to the implanted catheter, and either sterile saline or drug solution was microinjected in a volume of 5 μ l followed with 15 μ l of sterile saline flush delivered slowly over 30 s. I.t. injection sites were verified at the end of experiments by observing the distribution of 1% methylene blue after i.t. injection.

EA stimulation: The Zusanli (ST-36) acupoint, located 5-mm lateral to the anterior tubercle of the tibia, is frequently used to produce antinociception in humans (17) and animals (18, 19). Electrical stimulation (3 Hz, rectangular pulse, 0.1-ms duration for 45 min) was supplied to two acupuncture needles (Seirin, Shizuoka) bilaterally inserted to a depth of 5 mm into ST-36 acupoint using a Tokki-Model II stimulator (Igarashi Ika Kogyo, Tokyo). The intensity of the stimulation was sufficient to produce twitching of the hind legs, but not strong enough for rats to exhibit an escape response or squeaking. Rats were softly held in both hands during EA stimulation to avoid any restraint stress. No significant behavioral changes were observed during EA stimulation.

Nociceptive test: Rats were habituated to the experimental handling and condition for 3–5 days before experiments. On the day of the experiment, rats were acclimated for 2 h in the behavioral test room before the behavioral experiments. The hind-paw pressure test

was conducted to evaluate the nociceptive thresholds to mechanical stimulation in hand-held rats. Nociceptive thresholds were estimated by the Randall-Selitto method (Basile analgesimeter; Ugo Basile, Milan, Italy), in which a constantly increasing pressure was applied to the hind paw until the rat vocalized or withdrew the hind paw. A 1500-g cut-off value was employed to prevent tissue damage. Paw-pressure nociceptive thresholds (g) were measured every 15 min for 180 min. EA-induced antinociception and morphine analgesia were graphed as the time course of nociceptive thresholds and the area under the nociceptive thresholds curve (AUC: g $\times$ min).

Immunohistochemistry: If CCK is involved in the attenuation of morphine analgesia, it is speculated that EA stimulation triggers the induction of CCK production. CCK-like immunoreactivity (LI) in the spinal dorsal horn 1 h after EA stimulation was examined. Rats were deeply anesthetized with intraperitoneal administration of sodium pentobarbital (60 mg/kg, i.p.) and perfused transcardially with cold periodate-lysine-paraformaldehyde solution (pH 7.4) (20). The lumbar spinal cord and the dorsal root ganglia (DRG) were removed and placed in the same fixative for 12 h at 4°C. Then, samples were stored in 10% sucrose in 0.01 M phosphate-buffered saline (PBS) for 24 h and snap-frozen at -85°C. Transverse section of L₄ to L₅ cords and DRG (20- μ m-thick) were prepared in a cryostat (Leica, Wetzlar, Germany) and then thaw-mounted onto glass slides. After drying, tissue sections were washed three times for 10 min in PBS and incubated with 3% normal goat serum for 2 h to block non-specific binding. The sections were washed three times for 10 min in PBS and incubated with primary rabbit anti-rat CCK IgG (diluted 1:700; Affinity, Exeter, UK), diluted in PBS (pH 7.4) containing 0.3% Triton X-100 (Sigma, St. Louis, MO, USA), at 4°C for 40 h. After rinsing three times for 10 min in PBS, the sections were incubated with biotinylated goat anti-rabbit IgG (diluted 1:200; Jackson ImmunoResearch, West Grove, PA, USA) at 4°C for overnight. After three washes in PBS, the chromogenic detection of the deposited biotin was carried out by treatment with fluorescein isothiocyanate-conjugated streptavidin (diluted 1:200; Amersham Bioscience, Buckinghamshire, UK) at 4°C for 2 h. The stained sections were examined under a fluorescent microscope.

RNA isolation and cDNA synthesis: To examine the EA stimulation-induced CCK production, the lumbar enlargement of the spinal cord 1 h after EA stimulation was removed from rats that were deeply anesthetized with sodium pentobarbital (60 mg/kg). The tissue samples were immediately frozen in liquid nitrogen and stored at -85°C. PCR experiments were performed on total RNA isolated from harvested tissues by the

acid guanidine thiocyanate-phenol/chloroform (AGPC) method. In brief, the frozen tissues were homogenized in a denaturing solution (4 M guanidine thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sodium *N*-lauroyl sarcosine, 0.1 M 2-mercaptoethanol) with a Polytron homogenizer (PT-1200; Kinematica AG, Luttach, Switzerland). Subsequently, the following components were added to the homogenate to isolate total RNA: 2 M sodium acetate (pH 4.0), water saturated phenol, and chloroform/isoamyl alcohol. The mixed solutions were vortexed well and settled in an ice bath for 15 min. After centrifugation (5,000 $\times$ g for 20 min at 4°C), the aqueous layer was transferred and total RNA was precipitated with isopropyl alcohol for 1 h at -20°C. After centrifugation, RNA pellets were washed with 80% ethanol.

RT-PCR: Total RNA was reverse-transcribed to cDNA using a random primer (Takara Bio, Shiga) and ReverTra Ace reverse transcriptase (Toyobo, Osaka). Reactions were conducted at 37°C for 1 h, followed by a 10-min step at 68°C to inactivate enzymatic activity. PCR was performed in a final volume of 20 μ l containing 25 μ M each primer and 1 U of AmpliTaq DNA polymerase (Perkin Elmer, Branchburg, NJ, USA). Primers were designed using Primer3 software, with a specified amplicon length between 100 and 300 bp. The following primers were used: the control gene rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer 5'-ACCACAGTCCATGCCATCAC, reverse primer 5'-TCCACCACCCTGTTGCTGTA; CCK forward primer 5'-TACATGGGCTGGATGGATT, reverse primer 5'-AGCCACCAGAGGGAAACATT.

The amplification conditions were denaturation at 94°C for 40 s (5 min for the first cycle), annealing at 62°C for 30 s, and extension at 72°C for 40 s (5 min for the last cycle) for 32 cycles. GAPDH, which served as a control for RNA quality, was amplified for 27 cycles. PCR products were electrophoresed on ethidium bromide-stained agarose gels (1.5%) and visualized under ultraviolet light. The band intensity of PCR products was semi-quantified by densitometric analysis and normalized against the expression of GAPDH using Image J software.

Drugs

The drugs used in the present experiment were morphine hydrochloride (Takeda Pharmaceutical Company, Osaka); cholecystokinin octapeptide (CCK-8; Peptide Institute, Osaka); proglumide (CCK antagonist) and L-703,606 [neurokinin-1 (NK1) tachykinin receptor antagonist] (Sigma). Sterile saline solution was used as the solvent. Proglumide was coadministered with morphine or saline intrathecally 15 min after the termination of EA stimulation. L-7073,606 was injected

immediately after the termination of EA stimulation or 5 min before CCK administration.

Data analysis

Data were expressed as the mean $\pm$ S.E.M. Statistical analysis was carried out by Student's *t*-test or analysis of variance followed by the Tukey test for multiple comparisons. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Attenuation of the analgesic effect of morphine administered intrathecally following EA stimulation

Behavioral nociceptive thresholds determined by the hind-paw pressure test indicated that EA stimulation produced a significant antinociceptive effect, which peaked at 45 min and diminished completely within 15 min after the termination of EA stimulation; and the analgesic effects of i.t. morphine administered 15 min after EA stimulation was attenuated from 15 to 75 min after morphine administration (Fig. 1A). Morphine-induced analgesia following EA was significantly attenuated in comparison with i.t. morphine alone (Fig. 1B).

Involvement of spinal CCK in EA-induced attenuation of morphine analgesia

To elucidate the involvement of CCK receptors in the attenuation of i.t. morphine analgesia following EA stimulation, the selective CCK-receptor antagonist proglumide (0.01 μ g, i.t.) was coadministered with morphine (10 μ g, i.t.) 15 min after EA stimulation. Coadministration of proglumide completely reversed the EA-induced suppression of morphine analgesia (Fig. 2). Neither basal nociceptive thresholds nor i.t. morphine analgesia was affected by proglumide.

I.t. morphine analgesia was dose-dependently attenuated by pretreatment with i.t. administration of CCK (0.01–10 μ g) 15 min before morphine injection (Fig. 3).

Increase in CCK-LI in the spinal dorsal horn after EA stimulation

Weak intensity of CCK-LI was observed in lamina I of naïve rats (Fig. 4). One hour after the termination of EA stimulation, a marked increase in CCK-LI was observed in lamina I and II of the spinal dorsal horn. The filamentous appearance of CCK-LI inferred that intracellular CCK was mainly located in the nerve fiber. In the DRG, no CCK-LI was detected in either the non-EA control or EA stimulated rats (data not shown).

Fig. 1. Electroacupuncture (EA)-induced antinociception and effects of EA on the analgesic effects of intrathecal administered morphine (Mor). A: time course of the EA-induced antinociception and intrathecal morphine analgesia estimated by the hind-paw pressure test. B: the area under the analgesic curve (AUC) of morphine. EA was applied to ST-36 acupoints (0.1-ms duration at 3 Hz for 45 min). An arrow corresponds to the injection of morphine (10 μ g, i.t.). Each point and column represents the mean and vertical bars indicate the S.E.M. The number in parentheses is the number of rats. ***P*<0.01 vs open circle (A) or open column (B).

Expression of CCK mRNA in the spinal cord in response to EA stimulation

RT-PCR was performed to determine EA-induced CCK mRNA expression in the lumbar enlargement of the spinal cord. The expression of CCK mRNA was observed in both non-EA control and EA-stimulated rats (Fig. 5A). Semi-quantitative analysis of CCK mRNA indicated that EA stimulation significantly enhanced CCK mRNA expression in the spinal cord (Fig. 5B).

Effects of a NK1-receptor antagonist on the EA-induced attenuation of morphine analgesia and anti-opioid effects of CCK

To elucidate the involvement of substance P in the attenuation of morphine analgesia, we examined the

Fig. 2. Effects of proglumide on attenuation of the analgesic effect of intrathecal morphine (Mor) following electroacupuncture (EA) stimulation. Proglumide (0.01 µg, i.t.) was coadministered with Mor 15 min after EA stimulation. Analgesic effects were expressed as the area under the pain thresholds curve (AUC). EA was applied to ST-36 acupoints (0.1-ms duration at 3 Hz for 45 min). Each column represents the mean $\pm$ S.E.M. of 5 rats. ** P <0.01 vs open column, ## P <0.01 vs closed column.

Fig. 3. Anti-opioid property of intrathecal administered cholecystokinin (CCK) on the analgesic effect of intrathecal morphine (Mor). CCK (dose range from 0.01 – 10 µg) was pretreated 15 min before Mor (10 µg) administration. Analgesic effects were expressed as the area under the pain thresholds curve (AUC). Each column represents the mean and vertical bars indicate the S.E.M. of 5 rats. ** P <0.01 vs open column.

Fig. 4. Representative immunofluorescence micrographs of cholecystokinin-like immunoreactivity (CCK-LI) in the spinal dorsal horn. A marked increase in CCK-LI was observed in lamina I and II in the spinal dorsal horn 1 h after electroacupuncture (EA) stimulation. A schematic representation of the micrographs is indicated on the left. Calibration bar = 250 µm.

effect of the NK1-receptor antagonist L-703,606 on EA- and CCK-induced attenuation of morphine analgesia. L-703,606 (18 µg, i.t.) was administered immediately after the termination of EA stimulation or 5 min before CCK (10 µg, i.t.) injection. Pretreatment with L-703,606 significantly reversed the attenuation of i.t. morphine analgesia produced by previous EA stimulation. Moreover, pretreatment with L-703,606 also completely inhibited the attenuation of i.t. morphine analgesia induced by i.t. administration of CCK. On the other hand, neither the basal nociceptive thresholds nor i.t. morphine analgesia was affected by L-703,606 (Fig. 6).

Discussion

Antinociception induced by EA stimulation has been shown to be completely antagonized by the opioid receptor antagonist naloxone (2), indicating that the antinociceptive effect of EA is due to the release of endogenous opioid peptides. On the other hand, the present study demonstrated that i.t. morphine analgesia was paradoxically attenuated following EA stimulation. The exogenous opioid-induced nociceptive response or the reduction of nociceptive thresholds has been reported in clinical observations (21, 22) and is experimentally validated (23 – 26). Thus, it has been postulated that potent opioid analgesics activate both a facilitatory system of nociceptive transmission and an

Fig. 5. Expression of cholecystokinin mRNA in the spinal cord in response to electroacupuncture (EA) stimulation. A: Representative images of RT-PCR products 1 h after EA stimulation. B: Densitometric analysis of the band intensity of PCR products. The band intensity was normalized against the expression of GAPDH mRNA. Each column represents the mean and vertical bars indicate the S.E.M. of 8 rats. ** $P < 0.01$ vs open column.

Fig. 6. Effects of L-703,606 on electroacupuncture (EA)- or cholecystokinin (CCK)-induced attenuation of intrathecal administered morphine (Mor) analgesia. L-703,606 (18 µg, i.t.) was administered immediately after EA stimulation or 5 min before CCK (10 µg, i.t.) injection. EA was applied to the ST-36 acupoint (0.1-ms duration at 3 Hz for 45 min). Analgesic effects were expressed as the area under the pain thresholds curve (AUC). Each column represents the mean and vertical bars indicate the S.E.M. of 5–7 rats. ** $P < 0.01$ vs open column, ## $P < 0.01$ vs closed column, §§ $P < 0.01$ vs heavily hatched column.

inhibitory system responsible for the analgesia. However, the mechanism (including opioid receptor subtypes) underlying these two opposing systems activated by endogenous opioids (e.g., in EA) or exogenous opioid analgesics is still obscure.

Our result demonstrated that the i.t. administration of CCK attenuated morphine analgesia dose-dependently. Moreover, the attenuation of morphine analgesia following EA stimulation was completely reversed by the coadministration of the CCK-receptor antagonist proglumide, suggesting that CCK participated in the attenuation of morphine analgesia and that EA stimulation-induced mechanisms are essential for the increments in CCK-LI and CCK mRNAs in the spinal cord. Indeed, increases in both CCK-LI and CCK mRNA in the spinal cord were observed after EA stimulation in the present study. Moreover, it has been reported that i.t. administration of opioids induces CCK release in the spinal cord (8, 10). Taken together, it is strongly speculated that the activation of opioid receptors involves both the release of CCK and CCK-related anti-opioid effects in the spinal cord.

In agreement with previous studies, CCK-LI was observed in lamina I and II of the spinal dorsal horn. Although the type of neurons responsible for the release of CCK was not determined in this experiment, the interneurons in the lamina I and II would be the main source of CCK since DRG neurons showed no CCK-LI either in the present observation or in the previous reports (27). The μ -opioid receptor is distributed in spinal neurons in lamina I and II of the dorsal horn (28, 29). This morphological overlap infers that CCK and μ -opioid receptors are closely related to the modulation of nociceptive signal transmission (6, 29).

It is worthwhile to note that some descending projections from the rostral ventromedial medulla (RVM) to the spinal dorsal horn contains CCK-positive fibers (30). The RVM, which includes the nucleus raphe magnus and the medullary reticular formation, has been identified as a critical region with respect to nociceptive processing and control (31, 32). Recently Xie et al. (33) reported that CCK in the RVM engages in descending pain facilitatory pathways to enhance spinal nociceptive transmission and attenuate opioid antinociception. Thus, we cannot rule out the possibility that the descending projections from RVM to the spinal cord as well as the interneurons in the spinal dorsal horn might be the origin of CCK in the dorsal horn.

There are two major possible explanations to account for the mechanisms of attenuation of morphine analgesia induced by i.t. administration of CCK and/or the release of endogenous CCK in the spinal cord. One is a receptor-receptor interaction between CCK and opioids. It has

been reported that activation of CCK receptors by CCK-octapeptide reduces the binding affinity of opioids to their receptor (34). Another possible explanation is physiological interaction of second messenger systems between CCK receptors and opioid receptors. CCK receptors belong to the superfamily of G-protein coupled receptors (6). The type of G-protein associated with CCK receptors is G_q , which is known to be related to neurotransmitter release by the activation of inositol triphosphate and diacylglycerol through increases in the calcium conductance. CCK receptors distribute in the central terminals of the primary afferent nociceptive neurons (35). In addition, opioid receptors also belong to the superfamily of G-protein coupled receptors, but the type of G-protein is G_i , which has inhibitory effects on neurotransmitter release. The opioid receptors are expressed on the central terminal of primary afferent neurons and the projection neurons (36). The overlap in expression between CCK receptors and opioid receptors on the terminals of primary afferent small fibers (6, 29) suggests that these two receptors have physiological counter modulatory interaction on one another.

It is also postulated that CCK may be an opioid receptor ligand and prevent the activation of opioid receptors directly. There is one study, showing that a CCK-receptor antagonist has a modest affinity for opioid receptors (37). However, no direct evidence for the binding affinity of CCK for opioid receptors has been obtained, and this hypothesis remains to be proven.

The present study revealed that attenuation of i.t. morphine analgesia following both EA stimulation and i.t. administration of CCK was completely antagonized by the coadministration of the NK1-receptor antagonist L-703,606. This is the first report describing the involvement of substance P in the mechanism of CCK-induced anti-opioid effects. These results indicate that activation of the NK1 receptor is markedly involved in the CCK-induced attenuation of morphine analgesia, suggesting that the release of substance P and subsequent activation of the secondary afferent neuron by substance P might be the key mechanism of the attenuation of morphine analgesia following EA stimulation and i.t. administration of CCK. Indeed, CCK receptors are located on the central terminal of small fiber neurons (35), which is associated with the nociceptive transmission, and substance P is colocalized with glutamate in primary afferent terminals (38, 39). Furthermore, activation of G_q -linked CCK receptor is likely to be involved in the release of neurotransmitter from the small fiber primary afferent neuron. Taken together, EA-induced activation of spinal CCK may increase the release of substance P from primary afferent terminal, indicating that facilitation of excitatory neurotransmitter release in the spinal

cord becomes an opposing force to morphine-induced analgesia. Therefore, EA-induced paradoxical attenuation of morphine analgesia was associated with facilitation of excitatory neurotransmitter from the primary afferent neuron induced by spinal CCK that is consequently released by activation of the opioid system via EA stimulation.

In summary, this study demonstrated that the activation of the CCK system in the spinal cord by an endogenous opioid peptide is involved in the attenuation of i.t. morphine analgesia following EA stimulation, and the activation of NK1 receptors might be a key mechanism of the anti-opioid effect of CCK in the spinal cord. Ultimately, knowledge of the present finding as relevant to the presence of both anti-analgesic and pro-analgesic actions as a result of endogenous opioid peptide release may allow the development of a new clinical approach that can reduce the adverse effects induced by opioids.

Acknowledgments

The authors thank Dr. James H. Woods and Dr. Gail Winger (Department of Pharmacology, the University of Michigan) for their review of the manuscript.

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