*Full Paper***Activation of Spinal Anti-analgesic System Following Electroacupuncture Stimulation in Rats**

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Abstract. We evaluated the interaction between electroacupuncture (EA)-induced antinociception and an endogenous anti-analgesic system. EA was applied to the ST-36 acupoint for 45 min in male Sprague-Dawley rats, and pain thresholds were assessed by the hind-paw pressure test. EA produced a marked increase in pain thresholds and its antinociceptive action was completely reversed by naloxone (5 mg/kg). The analgesic effects of subcutaneous morphine (7 mg/kg) following EA stimulation were significantly attenuated. The attenuation of morphine analgesia was inversely proportional to the time intervals between EA termination and morphine injection, and the effect was not observed 120 min after EA stimulation. The analgesic effects of i.t. morphine (10 μ g), but not i.c.v. morphine (25 μ g), following EA were also attenuated. On the other hand, systemic morphine (7 mg/kg)-induced hyperthermia was not affected by EA. Moreover, i.c.v. morphine, but not i.t. morphine, produced hyperthermia. The i.c.v. morphine-induced hyperthermia was not affected by EA, similar to i.c.v. morphine analgesia. These results suggest that the attenuation of morphine analgesia following EA, that is, the activation of an endogenous anti-analgesic system, is closely related to the activation of an analgesic system by EA and that the spinal cord plays a critical role in the activation of the endogenous anti-analgesic systems.

Keywords: morphine, anti-analgesic system, electroacupuncture, spinal

Introduction

Activation of antinociceptive systems by the release of endogenous opioid peptides is one of the intrinsic protections against a stressful environment. However, prolonged antinociception has the potential to cause a maladaptive delay in initiation of fight-or-flight reactions. It is speculated that there are intrinsic anti-analgesic systems that normalize pain thresholds to permit adaptive response to a sense of danger. Although the mechanisms of these systems for pain modulation have been intensively studied, endogenous anti-analgesic mechanisms are still not fully understood.

Recently some endogenous substances have been identified that have anti-opioid actions, attenuating the

antinociceptive effects of opioids without themselves producing any effect on pain thresholds (1–3). An anti-opioid theory was postulated to explain some aspects of the endogenous anti-analgesic systems. This theory asserts that neuropeptides released in the central nervous system are involved in the homeostatic mechanisms that attenuate the analgesic effects of morphine (4).

Electroacupuncture (EA) is the one of the therapeutic techniques used in Oriental Medicine for the treatment of nausea, vomiting, asthma, headache, and myofascial pain (5). EA stimulation involves the application of certain frequencies of electrical current through acupuncture needles to specific locations termed acupoints. It has been established that the therapeutic effect of EA is associated with the release of various neurotransmitters and/or neuropeptides in the central nervous system (6, 7). Extensive studies demonstrate

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that the antinociceptive effects produced by EA are antagonized by the opioid receptor antagonist naloxone (8–10). Furthermore, low frequency (2 Hz) EA stimulation released enkephalins in the spinal cord (8, 11), whereas high frequency (100 Hz) stimulation induced the release of dynorphins (11), indicating that antinociception induced by EA stimulation is mediated by the activation of endogenous opioid systems (12).

If the pain modulation is one aspect of the physiological feedback system, activation of endogenous opioid systems by EA stimulation might also elicit an endogenous anti-analgesic effect. The current experiment was designed to investigate the possible existence of an endogenous anti-analgesic system induced by the activation of endogenous opioid systems by EA. Thus, we examined the effects of EA-induced activation of opioid receptor on the analgesic effects of morphine administered after EA stimulation. Besides analgesia, morphine has other pharmacological functions, including body temperature change, constipation, respiratory depression, and endocrine effects. The administration of naloxone completely blocks hyperthermia and analgesia induced by morphine (13, 14), suggesting that opioid receptor activation is the primary mechanism shared by the production of hyperthermia and analgesia. To determine the effects of EA-induced activation of opioid receptor on another function of morphine, we also examined the effects of EA on morphine-induced body temperature change.

Materials and Methods

Subjects

Male Sprague-Dawley rats (SLC, Shizuoka), weighing 250–350 g, were used, and they were housed in groups of two in plastic cages with food and water available ad libitum. Rats were maintained on a 12-h light-dark cycle controlled (lights on at 8:00 h) and air conditioned (23°C–24°C, 60% humidity) room. Pharmacological tests and care of the animals were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of the Wakayama Medical University.

Experiment procedure

Surgical procedure: For intracerebroventricular (i.c.v.) administration, stainless steel guide cannulae (20 gauge, hypodermic injection needle 1/1; Sato, Tokyo) were stereotactically implanted in the skull of rats unilaterally, according to the method of de Balbian Vester et al. (15), under pentobarbital anesthesia (50 mg/kg, i.p.). Stereotaxic coordinates were 2-mm left lateral to the sagittal suture and 1-mm caudal to the coronal

suture. A dummy stylet was inserted into the guide cannula to prevent its occlusion. For intrathecal (i.t.) administration, rats were implanted with spinal catheters as previously described (16) under anesthesia. In brief, 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 short length of stainless steel wire. After surgery, the animals were housed individually to prevent them from destroying the guide cannulae or catheters and allowed 10 days of postoperative recovery before experiments. Rats showing any neurological defects resulting from the surgical procedure were excluded from the experiments.

Microinjection procedure: For i.c.v. administration, a 29-gauge injection needle connected by polyethylene tubing to a 25- μ l Hamilton syringe was inserted 5.0 mm from the surface of the skull into the left lateral ventricle, and either sterile saline or drug solution was microinjected in a volume of 10 μ l over 60 s. I.t. injection was delivered at a volume of 5 μ l of drug solution followed with 15 μ l of sterile saline flush delivered slowly over 30 s. I.c.v. and i.t. injection sites were verified at the end of experiments by observing the distribution of 1% methylene blue after i.c.v. or i.t. injection. For systemic administration, all compounds were administered subcutaneously at a volume of 0.2 ml/kg.

Electroacupuncture stimulation: The Zusanli (ST-36) acupoint, located 5-mm lateral to the anterior tubercle of the tibia, is the most frequently used acupoint to produce antinociception in humans (17) and animals (18, 19), and it was selected for EA stimulation site in this study. Two stainless steel acupuncture needles (Seilin, Shizuoka) were bilaterally inserted to a depth of 5 mm into the ST-36 acupoint. Electrical stimulation (3 Hz, rectangular pulse, 0.1-ms duration) was applied to the needles for 45 min, using a Tokki-Model II stimulator (Igarashi Ika Kogyo, Tokyo). Electrical stimulation was applied bilaterally to the middle of the gluteus maximus muscle as a control, non-acupoint stimulation. The intensity of the stimulation was maintained to induce twitching of the hind legs, but not strong enough for rats to exhibit the escape response or squeaking. Rats were softly held in both hands during EA application to avoid any restraint stress. No significant behavioral changes were observed during EA stimulation.

Nociceptive test: The hind-paw pressure test was performed 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), where a constantly increasing pressure was applied to the hind paw until the rat squeaked 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 120 min. EA-induced antinociception and morphine analgesia were evaluated as the time course of nociceptive thresholds and the area under the nociceptive curve (AUC: $g \times \min$).

Body temperature measurements: Body temperatures were measured using a Thermo-Finer Type N-1 thermometer (Terumo, Tokyo) and thermistor probe (No. 5). To minimize the possible stress, the insertion of the probe was performed by holding the rat's tail softly by the index finger and thumb. The probe was lubricated with olive oil to prevent tissue damage and was inserted 4 cm into the rectum and allowed to equilibrate for 30 s before the temperature reading. Body temperatures measured twice before the experiment were averaged to establish a baseline body temperature. All body temperatures were measured at 30-min intervals during the experiments and were expressed as changes (ΔT) from each of the baseline body temperatures. Experiments were carried out at an environmental temperature of 23°C.

Drugs

Morphine hydrochloride (Takeda, Osaka) and naloxone hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in sterile saline. Naloxone at the dose of 5 mg/kg was administered 15 min before EA stimulation. This large dose of naloxone acts on not only μ -opioid receptors, but also the other subtypes of opioid receptors as an antagonist. The probe dose of morphine was determined to be 7 mg/kg for systemic, 25 μ g for intracerebroventricular, and 10 μ g for intrathecal administration to produce submaximal analgesia. Morphine or saline was administered 15 min after the termination of EA stimulation in the experiment measuring pain thresholds. In the experiment on body temperature, morphine or saline was injected 60 min after the termination of EA, because EA-induced hyperthermia returned to the baseline temperature by 60 min.

Data analyses

The trapezoidal rule was used to calculate area under the pain thresholds *versus* time curves (AUCs). Data were expressed as the mean $\pm$ S.E.M. Statistical analysis was carried out using 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

Effect of EA stimulation on subcutaneous morphine analgesia

EA stimulation produced a gradual increase in nociceptive thresholds, which diminished completely within 15 min after the termination of EA (Fig. 1A). Non-acupoint stimulation had no effect on the nociceptive thresholds. The antinociception induced by EA stimulation was completely blocked by systemic injection

Fig. 1. Effects of naloxone (NLX) on the antinociception induced by electroacupuncture (EA). A: time course of EA-induced antinociception estimated by the hind-paw pressure test. B: the area under the pain thresholds curve (AUC) of EA-induced antinociception. Naloxone (5 mg/kg, s.c.) was administered 15 min before EA stimulation. EA was applied to ST-36 acupoints (0.1-ms duration at 3 Hz for 45 min). Electrical stimulation was applied to the middle of the gluteus maximus muscle as a non-acupoint stimulation (non-EA). Each point and column represents the mean and vertical bars indicated by S.E.M. The number in parentheses is the number of rats. ***P*<0.01 vs EA. ##*P*<0.01 vs non-EA.

tion of naloxone at the dose of 5 mg/kg (Fig. 1). In naive rats, systemic administration of morphine (7 mg/kg) produced analgesia that peaked at 45 min after the injection and declined gradually over the next 75 min. The analgesic effect of morphine was significantly attenuated in rats exposed to EA stimulation, whereas no attenuation of morphine analgesia was observed in rats receiving non-acupoint stimulation (Fig. 2).

The suppressive effects of morphine analgesia at variable time intervals

Various time intervals between EA termination and

morphine administration were selected to evaluate the time course of the suppressive effect of EA stimulation on morphine analgesia. The magnitude of morphine analgesia was evaluated by AUC. The peak attenuation of morphine analgesia appeared when morphine was administered immediately after the termination of EA (Fig. 3). The attenuation of morphine analgesia was inversely proportional to the time intervals between EA termination and morphine injection. Attenuation of morphine-induced analgesia was not observed when morphine was administered 120 min after EA termination (Fig. 3).

Effects of EA stimulation on i.t. or i.c.v. morphine analgesia

To determine the responsible site for attenuation of systemic morphine-induced analgesia, we evaluated the effects of EA stimulation on i.t. or i.c.v. morphine analgesia. The analgesic action of i.t. morphine was also significantly attenuated following EA stimulation, whereas EA stimulation had no effect on i.c.v. morphine analgesia (Fig. 4). There was no statistical significance of the magnitude of EA-induced attenuation of morphine analgesia between systemic and intrathecal administration.

Effect of EA stimulation on body temperature change induced by morphine

EA stimulation produced hyperthermia, which had recovered 60 min following termination. Administration of i.c.v. morphine (25 μ g) to control animals produced

Fig. 2. Electroacupuncture (EA)-induced antinociception and effects of EA on the analgesic effects of morphine (Mor). A: time course of the EA-induced antinociception and systemic 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). Electrical stimulation was applied to the middle of the gluteus maximus muscle as a non-acupoint stimulation (non-EA). The arrow corresponds to the injection of morphine (7 mg/kg, s.c.). Each point and column represents the mean and vertical bars indicated by the S.E.M. The number in parentheses is the number of rats. ** $P < 0.01$, * $P < 0.05$ vs control.

Fig. 3. Effects of different time intervals between electroacupuncture (EA) stimulation and morphine administration. Morphine (7 mg/kg, s.c.) was administered at 0 to 120 min after the termination of 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 6 rats. ** $P < 0.01$ vs open column.

significant increases in body temperature, lasting over 3 h (Fig. 5). In contrast, injection of i.t. morphine (10 μ g) into control animals had no effect on body temperature (data not shown). EA stimulation showed no effects on the hyperthermic action of i.c.v. morphine (Fig. 5). Moreover, systemic administration of morphine (7 and 14 mg/kg)-induced hyperthermia was not affected by the prior EA stimulation (data not shown).

Fig. 4. The area under the pain thresholds curve (AUC) of intrathecal (i.t.) or intracerebroventricular (i.c.v.)-induced morphine (Mor) analgesia following electroacupuncture (EA). Mor was administrated 15 min after the termination of EA stimulation. EA was applied to ST-36 acupoints (0.1-ms duration at 3 Hz for 45 min). Each column represents the mean and vertical bars indicate the S.E.M. of 6 or 8 rats. ** $P < 0.01$ vs open column.

Fig. 5. The change in rectal temperature after intracerebroventricular (i.c.v., 25 μ g) administration of morphine (Mor) following electroacupuncture (EA). The rectal temperatures were measured at 30-min intervals for 4 h. EA was applied to the ST-36 acupoint (0.1-ms duration at 3 Hz for 45 min). An arrow corresponds to the injection of Mor. Each point represents the mean and vertical bars indicate the S.E.M. The number in parentheses is the number of rats.

Discussion

The present study revealed the possible existence of an anti-analgesic system following activation of the opioid system using EA stimulation.

Non-acupoint stimulation, which elicited no antinociceptive effect, had no influence on the analgesic action of systemically administered morphine, whereas stimulation of the ST-36 acupoint, which produced antinociceptive effects, significantly attenuated the analgesic effect of systemic morphine. Attenuation of morphine analgesia was inversely proportional to the time intervals between EA termination and morphine injection. These results indicate that there are possible interactions between EA-induced antinociception and attenuation of morphine analgesia following EA stimulation. Accumulating evidence indicates that EA stimulation produces antinociceptive effects as a result of the release of endogenous opioid peptides in the central nervous system (12, 20, 21). Consistent with these reports, the antinociception induced by EA stimulation was completely antagonized by naloxone, indicating that EA-induced antinociception under our experimental condition was apparently produced by the activation of the endogenous opioid system. Therefore, it is presumed that the attenuation of morphine analgesia is also likely to be the result of release of endogenous opioid peptides. Indeed, a number of studies have indicated that the administration of exogenous opioids unexpectedly produced a nociceptive response or reduction of baseline nociceptive thresholds in rodents and humans. For example, Mao et al. (22) demonstrated that rats receiving repeated intrathecal morphine administration over a 7-day period clearly showed a progressive reduction of baseline nociceptive thresholds; and in a human study, intraoperative remifentanyl increased postoperative pain and morphine requirement (23). These paradoxical effects of opioids imply that exogenous opioids can activate both the antinociceptive and the nociceptive system (24) and antinociceptive or nociceptive behavior will appear as the result of the balance of the two systems (25, 26). EA-induced opioid peptide release could have complex downstream effects that would attenuate morphine-induced analgesia. Such downstream effects would include opioid receptor-mediated effects such as desensitization/internalization or non-opioid effects such as involvement of anti-opioid peptides. In addition, we found that the analgesic effect of i.t. morphine, but not i.c.v. morphine, was significantly attenuated following EA stimulation, suggesting that the spinal cord, perhaps not the brain, plays an important role in the activation of such downstream effects.

To determine whether the attenuation of morphine

action following EA stimulation is specific for analgesia, effects of EA stimulation on systemic morphine-induced hyperthermia, which is antagonized by naloxone, were evaluated. It has been demonstrated that the thermoregulatory responses of opioids are greatly affected by the species and strain, ambient temperature, level of restraint, dose, and type of opioid analgesics (14). In agreement with the previous observation reported by Ushijima et al. (13), subcutaneous administration of morphine produced dose-dependent hyperthermia over a range of 1.25 to 10 mg/kg (data not shown). Although the analgesic effects of s.c. morphine administered 60 min after EA was significantly attenuated (Fig. 3), EA stimulation had no effect on the hyperthermic action of morphine administered at the same time interval (60 min).

To clarify the different effects of EA on antinociception and hyperthermia, morphine was administered by two different routes (i.c.v. and i.t.). I.c.v. administration of analgesic doses of morphine (25 μ g), but not that of i.t. morphine (10 μ g), produced significant increases in body temperature, suggesting that the center of hyperthermic action induced by opioid is located in the brain. Indeed, microinjection of μ -opioid receptor agonist into the preoptic anterior hypothalamus (POAH), generally considered to be the primary site of the central control of body temperature, produces hyperthermia (27). Thus, it appears that i.c.v. morphine-induced hyperthermia is likely to be mediated by opioid receptors located in the POAH. The hyperthermic action of i.c.v. morphine was not influenced by EA stimulation. In agreement with the observation of the anti-analgesic effects induced by EA stimulation, these results strongly suggest that supraspinal sites are not likely to be responsible to the activation of anti-opioid system.

Tolerance is defined as a decrease in the effect of a drug after repeated exposure to the same or a similar drug (28). From this standpoint, the attenuation of morphine analgesia following EA observed in the present study may result from acute cross-tolerance to the endogenous opioid peptides released by EA stimulation. Although the precise details of tolerance were not fully elucidated, many studies conducted to explore the mechanisms of acute tolerance have typically observed a phenomenon that appears 3 to 8 h after opioid administration and persists for at least 20 h (29–32). It has also been reported that the protein synthesis inhibitor cycloheximide suppresses the development of tolerance (33, 34), indicating that the time lag necessary for the development of acute tolerance to opioid analgesics may be related to the synthesis of new proteins. Based on the observation in this study that the attenuation of morphine analgesia occurred immediately after the

termination of EA stimulation, rapid onset mechanisms might be involved in the development of EA-induced attenuation of morphine analgesia. One possibility to account for the rapid onset mechanisms is the opioid receptor-mediated downstream effects such as desensitization/internalization. In fact, the observation of opioid receptor internalization suggests that internalization of the opioid receptor peaks at 15 min and returns to the control level by 60 min (35), and there are several lines of evidence for the involvement of desensitization/internalization in the development of acute tolerance (36). However, further investigations are required to elucidate whether EA-induced attenuation of morphine analgesia is a phenomenon categorized as acute tolerance.

Alternatively, it is possible that anti-opioid peptides may be involved. Recently, it was observed that several endogenous substances, including CCK (1), nociceptin (2) and neuropeptide FF (3), have anti-opioid properties that result in attenuation of the analgesic action of opioids without inducing nociception by themselves. Indeed, we demonstrated that systemic coadministration of proglumide, cholecystokinin-receptor antagonist, with morphine completely reversed the attenuation of morphine analgesia following EA stimulation (unpublished data). It is possible that anti-opioid peptides may be involved in the underlying mechanisms of the EA-induced anti-analgesic effect.

In summary, this study demonstrated that the activation of endogenous opioid systems paradoxically produced an anti-analgesic effect in the spinal cord observable after the acute antinociceptive effects of EA subsided. This anti-analgesic system may play an important role in the adaptive regulation of responses to potentially damaging situations.

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References

- 1 Faris PL, Komisaruk BR, Watkins LR, Mayer DJ. Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. *Science*. 1983;219:310–312.
- 2 Heinricher MM, McGaraughty S, Grandy DK. Circuitry underlying antinociceptive actions of orphanin FQ in the rostral ventromedial medulla. *J Neurophysiol*. 1997;78:3351–3358.
- 3 Yang HY, Fratta W, Majane EA, Costa E. Isolation, sequencing, synthesis, and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. *Proc Natl Acad Sci U S A*. 1985;82:7757–7761.
- 4 Rothman RB. A review of the role of anti-opioid peptides in

- morphine tolerance and dependence. *Synapse*. 1992;12:129–138.
- 5 NIH. Acupuncture. NIH Consensus Statement. 1997;15:1–34.
 - 6 Han JS, Terenius L. Neurochemical basis of acupuncture analgesia. *Annu Rev Pharmacol Toxicol*. 1982;22:193–220.
 - 7 He LF. Involvement of endogenous opioid peptides in acupuncture analgesia. *Pain*. 1987;31:99–121.
 - 8 Yaksh TL, Huang SP, Rudy TA. The direct and specific opiate-like effect of met5-enkephalin and analogues on the spinal cord. *Neuroscience*. 1977;2:593–596.
 - 9 Bing Z, Villanueva L, Le Bars D. Acupuncture and diffuse noxious inhibitory controls: naloxone-reversible depression of activities of trigeminal convergent neurons. *Neuroscience*. 1990;37:809–818.
 - 10 He LF, Dong WQ, Wang MZ. Effects of iontophoretic etorphine and naloxone, and electroacupuncture on nociceptive responses from thalamic neurones in rabbits. *Pain*. 1991;44:89–95.
 - 11 Ulett GA, Han S, Han JS. Electroacupuncture: mechanisms and clinical application. *Biol Psychiatry*. 1998;44:129–138.
 - 12 Chen XH, Han JS. All three types of opioid receptors in the spinal cord are important for 2/15 Hz electroacupuncture analgesia. *Eur J Pharmacol*. 1992;211:203–210.
 - 13 Ushijima I, Tanaka M, Tsuda A, Koga S, Nagasaki N. Differential effects of morphine on core temperature in stressed and non-stressed rats. *Eur J Pharmacol*. 1985;112:331–337.
 - 14 Baker AK, Meert TF. Functional effects of systemically administered agonists and antagonists of mu, delta, and kappa opioid receptor subtypes on body temperature in mice. *J Pharmacol Exp Ther*. 2002;302:1253–1264.
 - 15 De Balbian Verster F, Robinson CA, Hengeveld CA, Bush ES. Freehand cerebroventricular injection technique for unanesthetized rats. *Life Sci*. 1971;10:1395–1402.
 - 16 Miyamoto Y, Morita N, Kitabata Y, Yamanishi T, Kishioka S, Ozaki M, et al. Antinociceptive synergism between supraspinal and spinal sites after subcutaneous morphine evidenced by CNS morphine content. *Brain Res*. 1991;552:136–140.
 - 17 Lin JG, Lo MW, Wen YR, Hsieh CL, Tsai SK, Sun WZ. The effect of high and low frequency electroacupuncture in pain after lower abdominal surgery. *Pain*. 2002;99:509–514.
 - 18 Lee JH, Beitz AJ. The distribution of brain-stem and spinal cord nuclei associated with different frequencies of electroacupuncture analgesia. *Pain*. 1993;52:11–28.
 - 19 Zhang YQ, Ji GC, Wu GC, Zhao ZQ. Excitatory amino acid receptor antagonists and electroacupuncture synergistically inhibit carrageenan-induced behavioral hyperalgesia and spinal fos expression in rats. *Pain*. 2002;99:525–535.
 - 20 Mayer DJ, Price DD, Rafii A. Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. *Brain Res*. 1977;121:368–372.
 - 21 Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci*. 2003;26:17–22.
 - 22 Mao J, Price DD, Mayer DJ. Thermal hyperalgesia in association with the development of morphine tolerance in rats: roles of excitatory amino acid receptors and protein kinase C. *J Neurosci*. 1994;14:2301–2312.
 - 23 Guignard B, Bossard AE, Coste C, Sessler DI, Lebrault C, Alfonsi P, et al. Acute opioid tolerance: intraoperative remifentanyl increases postoperative pain and morphine requirement. *Anesthesiology*. 2000;93:409–417.
 - 24 Celerier E, Laulin JP, Corcuff JB, Le Moal M, Simonnet G. Progressive enhancement of delayed hyperalgesia induced by repeated heroin administration: a sensitization process. *J Neurosci*. 2001;21:4074–4080.
 - 25 Han JS. Cholecystokinin octapeptide (CCK-8): a negative feedback control mechanism for opioid analgesia. *Prog Brain Res*. 1995;105:263–271.
 - 26 Dickenson AH. Plasticity: implications for opioid and other pharmacological interventions in specific pain states. *Behav Brain Sci*. 1997;20:392–403; discussion 435–513.
 - 27 Xin L, Geller EB, Adler MW. Body temperature and analgesic effects of selective mu and kappa opioid receptor agonists microdialyzed into rat brain. *J Pharmacol Exp Ther*. 1997;281:499–507.
 - 28 Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science*. 1991;251:85–87.
 - 29 Kishioka S, Morita N, Kitabata Y, Yamanishi T, Miyamoto Y, Ozaki M, et al. Dynorphin-(1-13): antinociceptive action and its effects on morphine analgesia and acute tolerance. *Jpn J Pharmacol*. 1992;60:197–207.
 - 30 Rosenfeld GC, Burks TF. Single-dose tolerance to morphine hypothermia in the rat: differentiation of acute from long-term tolerance. *J Pharmacol Exp Ther*. 1977;202:654–659.
 - 31 Narita M, Mizoguchi H, Tseng LF. Inhibition of protein kinase C, but not of protein kinase A, blocks the development of acute antinociceptive tolerance to an intrathecally administered mu-opioid receptor agonist in the mouse. *Eur J Pharmacol*. 1995;280:R1–R3.
 - 32 Fairbanks CA, Wilcox GL. Acute tolerance to spinally administered morphine compares mechanistically with chronically induced morphine tolerance. *J Pharmacol Exp Ther*. 1997;282:1408–1417.
 - 33 Way EL, Loh HH, Shen F. Morphine tolerance, physical dependence, and synthesis of brain 5-hydroxytryptamine. *Science*. 1968;162:1290–1292.
 - 34 Loh HH, Shen FH, Way EL. Inhibition of morphine tolerance and physical dependence development and brain serotonin synthesis by cycloheximide. *Biochem Pharmacol*. 1969;18:2711–2721.
 - 35 Trafton JA, Abbadie C, Marek K, Basbaum AI. Postsynaptic signaling via the [mu]-opioid receptor: responses of dorsal horn neurons to exogenous opioids and noxious stimulation. *J Neurosci*. 2000;20:8578–8584.
 - 36 von Zastrow M, Svingos A, Haberstock-Debic H, Evans C. Regulated endocytosis of opioid receptors: cellular mechanisms and proposed roles in physiological adaptation to opiate drugs. *Curr Opin Neurobiol*. 2003;13:348–353.