Published in final edited form as:

Anesth Analg. 2017 September ; 125(3): 1032–1041. doi:10.1213/ANE.0000000000002128.

Nortriptyline Enhances Morphine-conditioned Place Preference in Neuropathic Rats: Role of the Central Noradrenergic System

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Abstract

BACKGROUND—Combination drug therapy is commonly used to treat chronic pain conditions such as neuropathic pain, and antidepressant is often used together with opioid analgesics. While rewarding is an intrinsic property of opioid analgesics, it is unknown whether the use of antidepressant would influence opioid reward, which may contribute to opioid addiction. In the present study, we examined whether nortriptyline (a tricyclic antidepressant and a first-line medication for neuropathic pain) would enhance the morphine rewarding property in both naïve and chronic constriction sciatic nerve injury (CCI) rats.

METHODS—The rewarding effect of these drugs was assessed using conditioned place preference (CPP). The real-time PCR, western blot and ELISA analysis were used to investigate the function of central noradrenergic system.

RESULTS—In naïve rats, co-administration of nortriptyline with morphine did not change the acquisition of morphine-induced CPP. However, nortriptyline enhanced the acquisition, delayed the extinction, and augmented the reinstatement of morphine-induced CPP in CCI rats. In CCI rats treated with both nortriptyline and morphine, the expression of $\alpha 2A$ adrenergic receptors, norepinephrine transporter, and tyrosine hydroxylase was markedly decreased in the locus coeruleus, whereas the norepinephrine concentration in the nucleus accumbens was remarkably increased.

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Conflicts of Interest: NONE

Authors contributions

WLM and JM were responsible for the study concept and design. WLM, ZRY, and SXW contributed to the acquisition of animal, WB and PCR data. GL, MFM, HK, LC assisted with data analysis and interpretation of findings. WLM drafted the manuscript. JM and LC provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

CONCLUSIONS—These results demonstrate that nortriptyline enhanced morphine reward when both drugs were used to treat neuropathic pain in rats and that this behavioral phenotype is likely to be mediated by upregulation of the central noradrenergic system. These findings may have implications in opioid therapy commonly used for chronic pain management.

Introduction

Neuropathic pain, pain resulting from injury or dysfunction of the nervous system, is a chronic pain condition that remains difficult to treat.¹ Commonly used drugs for neuropathic pain treatment include antidepressants, anticonvulsants, and opioids. However, when used alone, the effect of these drugs is rather limited due to dose-limiting adverse effects. Therefore, most patients with chronic neuropathic pain often receive more than one drug in order to achieve a better analgesic effect, hence the concept of combination drug therapy (CDT).² While reduction of common side effects is a benefit of CDT, it has been suggested that CDT may exacerbate certain side effects in both preclinical and clinical observations.³

Opioids such as morphine have an intrinsic rewarding property, which may contribute to opioid addiction associated with chronic pain management. Conditioned place preference (CPP) paradigms are widely used as experimental models examining behavioral phenotypes of drug addiction.⁴ Using such models, investigators have reported the enhanced expression of morphine-induced CPP in the presence of hypericin and/or synthetic antidepressants (e.g., fluoxetine),^{5, 6} whereas others have shown that administration of imipramine on the test day reversed morphine-induced CPP.⁷ To date, it remains unclear whether antidepressant such as nortriptyline (a tricyclic antidepressant and a first-line medication for neuropathic pain) would influence opioid reward. Therefore, the primary objective of the present study is to determine whether nortriptyline would enhance the morphine rewarding property using a CPP test in both naïve rats and rats with neuropathic pain induced by chronic constriction sciatic nerve injury (CCI).

The increased levels of endogenous dopamine in the mesolimbic dopaminergic system such as ventral tegmental area and nucleus accumbens (NAc) have linked to the rewarding properties of addictive drugs.⁸ However, norepinephrine (NE) could be an important mediator of drug reward as well.⁹ It has been shown that the endogenous NE system was essential for morphine-induced CPP and locomotion.¹⁰ In the central nervous system, 1) locus coeruleus (LC) is the principal site for NE synthesis, 2) NE is converted by dopamine beta-hydroxylase (DBH) to dopamine, 3) the biological effects of NE are mediated by pre- and post-synaptic adrenergic receptors, and 4) 80–90% of the released NE is re-uptaken through NE transporters (NET).¹¹ Moreover, α 2-Adrenergic receptors have been shown to affect morphine-induced CPP¹² and mice lack of DBH do not exhibit morphine-induced rewarding behavior.¹⁰ Since nortriptyline inhibits NE reuptake at the synaptic level, it is logical to ask whether nortriptyline may influence morphine-induced CPP when co-administered with morphine, a common drug combination for neuropathic pain treatment. Therefore, the second objective of this study is to examine whether co-administration of nortriptyline with morphine would alter the central NE system in rats with a phenotype of morphine reward.

Methods

Animals

Experiments were performed on adult male Sprague-Dawley rats (Charles River Laboratories) weighing 250–300g at the beginning of each experiment. Rats were housed 3 to 4 per cage and maintained under a controlled condition ($21 \pm 2^{\circ}\text{C}$, 7 a.m. to 7 p.m. alternate light-dark cycles) with access to food and water *ad libitum*. The animal protocol was approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee and the experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals¹³. Experimental study groups were randomly assigned, and the researchers were blinded for the behavior tests.

Drugs administration

Morphine and nortriptyline (Sigma-Aldrich, USA) were dissolved in saline. Nortriptyline (5 mg/kg) was administered intraperitoneally (i.p.) 30 min before each morphine (i.p., 10 mg/kg) injection.

CCI model

CCI was induced by loosely ligating one side of common sciatic nerve, as previously described¹⁴. Briefly, under isoflurane anesthesia, the right common sciatic nerve was exposed by blunt dissection through biceps femoris and four 4-0 chronic gut sutures were tied loosely around the sciatic nerve. For sham surgery, rats underwent the same procedure without nerve ligation.

CPP test

Acquisition—An unbiased, fully counterbalanced CPP procedure was used, including apparatus and pre-conditioning session as described previously.¹⁵ Rats exhibited no preference for either of the place cues in the apparatus during the pre-conditioning test. During conditioning, drug treatment (intraperitoneal saline or morphine) and placement into a black or white side compartment were randomly assigned and counter-balanced. In the morning, rats were injected with saline or morphine and placed into one compartment for 30 min. Four hours later, after morphine or saline administration (opposite to the morning treatment), they were placed into the other compartment for 30 min. This daily process was repeated for six consecutive days. Twenty-four hours after the last conditioning session, post-conditioning test (10 min) was performed in the same conditions as in the pre-conditioning test. Changes in CPP behaviors were presented as CPP scores determined by the increase in the time spent in the compartment previously paired with morphine than in the compartment previously paired with saline as previously reported.¹⁶ To examine extinction and reinstatement, rats were kept for the following experiments (see below). In separate groups, rats were immediately sacrificed after the post-conditioning test and their brains samples were rapidly extracted and dissected according to regions of interest and frozen at -80°C till use.

Extinction and reinstatement procedure—After post-conditioning test, rats were tested weekly until no significant differences were observed between the time spent in the

morphine-paired and saline-paired compartment. For morphine reinstatement, morphine (5 mg/kg) was injected intraperitoneally one day after the confirmation of CPP extinction. Twenty minutes after injection, each group of rats underwent the same CPP test used for the initial post-conditioning test.

Nociceptive behavioral tests

The hindpaw withdrawal latency (PWL) to radiant heat was examined as previously described.¹⁷ Briefly, after accommodation of 30 minutes, a radiant heat source using a 390 Analgesia Meter (IITC Inc., Woodland Hills, CA) was aimed at the plantar surface until the rat lifted its hindpaw. The time from the onset of radiant heat stimulation to hindpaw withdrawal was defined as PWL. All experiments were carried out between 8 a.m. and 12 p.m. to avoid diurnal variation in behavioral tests. Each rat underwent two trials and the results were averaged to yield mean withdrawal latencies.

Western blot analysis

Rats were sacrificed by decapitation under pentobarbital anesthesia. Brain regions of interest were separately removed and homogenized in an SDS buffer containing a mixture of proteinase inhibitors (Sigma-Aldrich). After 12,000 r.p.m. centrifugation for 10 min, the supernatant was used for analysis. Samples (30 µg of total protein) were dissolved with an equal volume of loading buffer, separated via 10 % SDS-PAGE and then electro-transferred at 300 mA to Immuno-Blot PVDF membranes for 1 h. Membranes were blocked in TBST containing 5 % non-fat milk overnight at 4 °C before incubation for 1 hr at room temperature with rabbit anti-SLC6A2 (NET) antibody (1:1000, SAB2102224-50UG, Sigma-Aldrich, USA), rabbit anti-TH (1:1000, ab41528, abcam, USA), rabbit anti-α2-Adrenergic receptors (1:500, A271, Sigma-Aldrich, USA), or mouse anti-β-actin antibody (1:10000, ab6276, abcam, USA), diluted in TBST containing 5 % BSA. These blots were washed extensively in TBST and incubated with goat-anti-rabbit IgG (1:10000, PI-1000, Vector, USA) or Horse-anti-mouse IgG conjugated to horseradish peroxidase (1:10000, PI-9401, Vector, USA) in TBST/1.25 % BSA for 1 hr at room temperature. The blots were visualized in ECL solution (Millipore) for 5 min and exposed to hyperfilms (Kodak) for 1–10 min. The density of the specific bands from Western blot gels was measured with a computer-assisted imaging analysis system (Image J, NIH).

Real-time RT-PCR

Total RNA was isolated from the locus coeruleus samples using Trizol reagent (Invitrogen). Two micrograms of total RNA was used to synthesize the first strand of cDNA using SuperScript III kit (Invitrogen) according to the manufacturer's protocol. Four microliters of cDNA was put into 20 µl vessel with 1 µl of 20× TaqMan Gene Expression Assay (Applied Biosystems). The genes examined were Act b (Rn00667869_M1), Adra2a (Rn00567876_M1), and Dbh (Rn00565819_M1). The reaction was performed in duplicates with the following conditions: denaturing at 95°C for 30 s, annealing at 60°C for 2 min, extension at 68°C for 2 min for 35 cycles using an ABI Prism 7500 Sequence Detector System maintained by the MGH Neuroscience Center nucleic acid quantitation core facilities. Beta-actin RNA gene expression was measured as control.

ELISA

A standard curve was generated for each assay using the reference set supplied in the Norepinephrine ELISA kit (IB89537, IBL-America, Minneapolis, MN, USA). Samples were read using a microplate reader (Bio-Tek Instruments) at wavelengths of 450 nm and a reference wavelength between 620 nm and 650 nm. The NE content in NAc was assayed based on the manufacturer's instructions and the sample concentration was calculated based on a standard curve.

Statistical analysis

Data were presented as the mean $\pm$ the standard error of the mean (SEM), and all statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL). For behavior tests in Fig. 1 and Fig. 2, we used 4–12 animals per group, and mostly more than 6 per group. For Western blot, 3 animals per group were used; For ELISA, 5 animals per group were used; and PCR, 3–4 animals per group were used. We calculated the observed power of these tests using the SPSS statistical software, and the observed powers are higher than 0.70 in all of these tests (from 0.74 – 0.99). One-way analysis of variance (ANOVA) followed by the Bonferroni test was used to detect the CPP in the naïve rats. Two-way ANOVA was used and followed by the Bonferroni test to detect CPP in sham and CCI rats as well as the thermal hyperalgesia in all rats. PCR, Western blot and ELISA data were analyzed using ANOVA followed by a Bonferroni posttest. In all statistical analyses, $P < 0.05$ was considered the criteria for significance.

Results

No effect of nortriptyline on morphine-induced CPP in naïve rats

We first observed the effect of nortriptyline on morphine-induced CPP in naïve rats. Intraperitoneal injection of morphine (10 mg/kg) for six days significantly increased the CPP score, as compared with the saline vehicle control group (One-way ANOVA, $F_{3,17} = 11.295$, $P < 0.05$; Fig 1A), establishing a CPP model under our experimental condition. However, combination of morphine with nortriptyline (5 mg/kg) did not further enhance the CPP score, as compared with the morphine alone group ($P = 1.000$), nor did nortriptyline alone produce any place preference ($P = 0.616$), indicating that nortriptyline has no effect on morphine-induced CPP in naïve rats.

Nociceptive threshold in naïve rats before and after the six-day administration of nortriptyline and/or morphine was also observed. After repeated morphine injection, the withdrawal latency on both hindpaws was significantly decreased, as compared with the saline group, indicating that repeated morphine administration also induced hyperalgesia (Two-way ANOVA, $F_{6,31} = 3.842$, $P < 0.01$, for Fig 1B, and $F_{6,31} = 4.319$, $P < 0.05$, for Fig 1C). However, the morphine plus nortriptyline group ($P = 0.396$) or the nortriptyline alone group ($P = 0.158$) showed no significant changes in the hindpaw withdrawal latency after repeated administration.

Enhancement of nortriptyline on the acquisition, extinction and reinstatement of morphine-induced CPP in CCI rats

We then observed the effect of nortriptyline on the acquisition, extinction and reinstatement of morphine-induced CPP in CCI rats with neuropathic pain. After the preconditioning test, rats were subjected to the CCI surgery. Seven days after surgery when neuropathic pain was fully demonstrable, CCI rats were subjected to morphine and/or nortriptyline conditioning for six consecutive days, using the same protocol for naïve rats. Twenty-four hours after the last conditioning session, the post-conditioning test was performed. As expected, morphine conditioning significantly increased the CPP score in both sham and CCI rats ($P<0.001$ for sham rats and $P<0.01$ for CCI rats). However, combination of morphine with nortriptyline further enhanced the CPP score, as compared with the morphine alone group, in CCI rats (Two-way ANOVA, $F_{6,50} = 26.367$, $P<0.05$) but not in sham rats ($P<0.001$) (Fig 2A). And nortriptyline alone did not change the CPP score in both sham and CCI rats ($P=1.000$).

We also observed changes in baseline nociceptive threshold at 7 days after CCI surgery and after post-conditioning (Fig 2B, C). Seven days after CCI surgery, the ipsilateral (Two-way ANOVA, $F_{15,93}=9.383$, $P<0.001$), but not contralateral ($P=1.000$), hindpaw withdrawal latency was significantly decreased as compared with the sham CCI group. After six-days of morphine and/or nortriptyline conditioning, the hindpaw withdrawal latency was remarkably increased in CCI rats, suggesting the analgesic effect of the morphine and/or nortriptyline treatment ($P<0.05$). However, nortriptyline did not further increase the morphine analgesic effect in CCI rats (Fig 2B, C).

To examine whether nortriptyline would alter the CPP extinction, the above groups of rats were again subjected to the CPP test at 7 days after post-conditioning. The CPP score in the morphine plus nortriptyline group remained higher as compared with that of the morphine alone group in CCI (Two-way ANOVA, $F_{4,29} = 29.959$, $P<0.01$) (Fig 2D), indicating that nortriptyline delayed the CPP extinction in CCI rats.

Relapse is a characteristic of drug addiction.¹⁸ The reinstatement model, based on a CPP paradigm, has been widely used to study relapse to drug seeking.^{19, 20} We next observed the effect of nortriptyline on the reinstatement of morphine-induced CPP. To examine reinstatement, 5 mg/kg morphine was given for priming 20 min before the CPP test to each of the above groups. The CPP score was significantly higher in both sham and CCI rats with prior morphine conditioning as compared with the saline group (Two-way ANOVA, $F_{4,19} = 20.058$, ($P<0.01$ for sham rats and CCI rats), indicating the effectiveness of the reinstatement model under the experimental condition. Of interest, CCI rats with the prior morphine plus nortriptyline conditioning showed a higher CPP score than that of the morphine alone group (Two-way ANOVA, $F_{4,19} = 20.058$, $P<0.05$; Fig 2E), whereas the priming morphine dose did not change the CPP score in the prior nortriptyline conditioning group.

Taken together with the data from all sets of experiments, the results showed that nortriptyline enhanced the acquisition, delayed the extinction, and augmented the reinstatement of morphine-induced CPP in CCI rats.

Effect of nortriptyline and/or morphine conditioning on the NE system in the LC of CCI rats

To investigate whether the development of CPP phenotypes in CCI rats would be associated with changes in the central NE system, we observed the expression of DBH (dopamine beta-hydroxylase) and TH (tyrosine hydroxylase) related to NE synthesis, NET (NE transporter) responsible for the reuptake of NE released into the synaptic cleft, and also α 2A adrenergic autoreceptors in the LC of CCI rats subject to nortriptyline and/or morphine conditioning as described above. The real-time PCR results showed that the expression of DBH was significantly decreased in LC in both morphine alone and morphine plus nortriptyline groups (One-way ANOVA, $F_{2,8} = 6.783$, $P < 0.05$, Fig 3A), and the mRNA level of α 2A is not changed in the morphine alone group but greatly reduced in the nortriptyline and morphine group (One-way ANOVA, $F_{2,9} = 7.767$, $P < 0.05$, Fig 3B). In addition, the Western blot results demonstrated that the protein level of α 2A (One-way ANOVA, $F_{3,8} = 1.964$, $P < 0.05$, Fig 3C, D) and NET (One-way ANOVA, $F_{3,8} = 7.810$, $P < 0.01$, Fig 3C, E) is not changed in the morphine alone group but markedly decreased in the nortriptyline and morphine group. Also, the expression of TH was significantly increased in the morphine group as compared with the morphine plus nortriptyline group and saline group (One-way ANOVA, $F_{3,8} = 8.132$, $P < 0.05$, Fig 3C, F). Thus, the enhanced CPP phenotype due to co-administration of morphine with nortriptyline in CCI rats was accompanied by changes of the NE synthesis and regulation of NE release and removal at the synaptic site in LC.

Effect of nortriptyline and/or morphine on the NE system in the NAc of CCI rats

We further explored the role of NE in the NAc in the aggravation of nortriptyline on the morphine induced CPP in CCI rats. Western blot results showed that the expression of α 2A (One-way ANOVA, $F_{3,8} = 5.231$, $P < 0.05$, Fig 4A, B) and NET (One-way ANOVA, $F_{3,8} = 6.657$, $P < 0.05$, Fig 4A, C) in NAc in CCI rats in the morphine plus nortriptyline group is significantly decreased compared with the morphine alone group. ELISA results demonstrated that the NE concentration in the NAc is remarkably increased after morphine combined with nortriptyline treatment, when compared with the morphine alone (One-way ANOVA, $F_{3,16} = 14.762$, $P < 0.001$, Fig 4D). The results suggested that the aggravation of nortriptyline on the morphine induced CPP in CCI rats is possibly through decreasing the α 2A receptor and NET expression and thus increase the NE concentration in the NAc.

Discussion

Neuropathic pain is a debilitating chronic condition that remains very difficult to treat. Although combined drug therapy has been shown to be beneficial for certain chronic pain conditions²¹, some studies have shown that CDT may exacerbate certain side effects in both preclinical and clinical observations.²² A guideline sponsored by the International Association for the Study of Pain recommends nortriptyline as a first-line medication for neuropathic pain,²³ but its influence on morphine reward and opioid addiction remains unknown.

Our results showed that, in naïve rats, co-administration of nortriptyline with morphine did not change the acquisition of morphine-induced CPP. However, nortriptyline enhanced the acquisition, delayed the extinction, and augmented the reinstatement of morphine-induced

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CPP in rats with neuropathic pain induced by CCI. And this effect is not due to on the further pain relief as shown in the pain behavior test. In CCI rats treated with both nortriptyline and morphine, the expression of $\alpha 2A$ adrenergic receptors, norepinephrine transporter, and tyrosine hydroxylase (real-time PCR; western blot) was markedly decreased in the locus coeruleus, whereas the norepinephrine concentration (ELISA) in the nucleus accumbens was remarkably increased under the same condition. These results demonstrate that nortriptyline enhanced morphine reward when both drugs were used to treat neuropathic pain in rats and that this behavioral phenotype is likely to be mediated by upregulation of the central noradrenergic system.

Changes in morphine reward in a preclinical model of neuropathic pain

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Clinical and experimental studies have suggested that opioid addictive behaviors may be attenuated under a neuropathic pain condition.^{24–26} Clinical survey studies suggest that a rather small number of chronic pain patients prescribed on opioid therapy may end up with opioid addiction.²⁷ Pre-clinical studies have shown that the rewarding effect of morphine was “suppressed” under inflammatory (carrageenan- and formalin-induced pain) and neuropathic pain (CCI) in rodents using a similar CPP paradigm.^{24, 28, 29} Studies also have shown that morphine-induced CPP in rats with neuropathic pain would depend on the morphine dose administered during conditioning sessions.³⁰ In rats with neuropathic pain, the dose-dependent effect of morphine on CPP appeared to show a bell-shaped dose-response relationship such that a low dose of morphine (2 mg/kg) produced a greater CPP than a higher dose of morphine (8 mg/kg).²⁴ Similarly, it has been reported that a morphine dose at 3.5 mg/kg induced CPP only in sham rats but not in rats with spared nerve injury (another preclinical neuropathic pain model). CPP was demonstrated in nerve-injured rats only when the morphine dose was increased to 5 mg/kg, and there were no differences in CPP scores between nerve-injured and sham rats when morphine dose was at 7.5 mg/kg.³⁰ Consistently, in the present study, we found that morphine (10 mg/kg)-induced CPP in CCI rats was comparable to that of naïve or sham rats, suggesting that nerve injury did not deter the development of morphine reward under our experimental condition.

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Of interest to note is that previous studies, including ours, have suggested an interaction between opioid analgesia and opioid rewarding.^{15, 31} In our previous study, morphine-induced CPP was enhanced in rats with CFA-induced ankle monoarthritis.¹⁵ A previous study has shown that local anesthetic blockade of the afferent input alone can establish CPP in a model of experimental postsurgical pain.³¹ These data indicate that morphine analgesia itself may contribute to the opioid rewarding effect in addition to the intrinsic rewarding effect of opioids. Nonetheless, further studied may examine the impact of various pain conditions on opioid reward, including inflammatory, neuropathic and cancer-related pain.

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Furthermore, in the present study, opioid-induced hyperalgesia was observed in naïve rats but in sham rats, which may due to influence by the opioid treatment regimen, and also the confounding effect of the surgical procedure could not be ruled out. Also, we also did not observe potentiation of the analgesic effect when morphine was combined with nortriptyline in CCI rats, suggested that the enhancement of morphine rewarding is not due to the effect of the pain relief. This finding is consistent with a previous report that no additive effect of

nortriptyline on morphine analgesia,^{32, 33} although a recent study reports a better analgesia effect with a nortriptyline and morphine combination.²¹ Therefore, more preclinical, clinical, and translational studies are needed to improve the efficacy, and avoid side-effects, of combination drug therapy.

Effect of antidepressants on morphine-induced CPP

In this study, nortriptyline alone did not produce CPP, a finding that is in agreement with a previous report that tricyclic antidepressants such as desipramine or imipramine failed to produce a rewarding effect.³⁴ Previous studies have also shown that intraperitoneal or intracerebroventricular injection of hypericin, synthetic antidepressants (fluoxetine, imipramine and tranylcypromine), amantadine or fluvoxamine can induce CPP and potentiate morphine-induced CPP.^{5, 6} However, another study showed that the expression of morphine-induced CPP was attenuated by pretreatment of mirtazapine (an atypical antidepressant).³⁵ The data showed that nortriptyline did not affect the acquisition of morphine-induced CPP in naïve rats, similar to the finding in a previous report using a combination of imipramine and morphine.⁷ All of these studies used naïve rats and the differences regarding the effect of antidepressant on morphine-induced CPP may be related to different antidepressants (e.g., selective serotonin reuptake inhibitor versus selective norepinephrine reuptake inhibitor).

In the present study, we further examined the effect of nortriptyline on morphine-induced CPP in rats with CCI-induced neuropathic pain in order to mimic the clinical situation of opioid therapy. In addition to examining the acquisition of morphine-induced CPP, as in many previous studies, we also observed the effect of nortriptyline on the extinction and reinstatement of morphine-induced CPP in neuropathic pain rats. Our results showed that nortriptyline enhanced the development, delayed the extinction, and augmented the reinstatement of morphine-induced CPP in CCI rats. The data suggest that future studies on this topic may need to consider the influence of antidepressant on various states of opioid reward.

Role of the central NE system in the enhancement by nortriptyline of morphine reward

Nortriptyline is a NE reuptake inhibitor, which effectively increases synaptic NE concentration. NE is the primary neurotransmitter for the endogenous noradrenergic system that serves multiple functions including arousal, attention, mood, learning, memory and stress response, and also pain control. To date, the role of the endogenous NE system in opioid reward remains controversial, ranging from the lack of influence³⁶ to serving an essential role in morphine-induced CPP and/or locomotion¹⁰ in DBH knockout mice as well as in the acquisition and reinstatement of CPP by selective depletion of the medial prefrontal cortical noradrenergic afferents.³⁷

LC is the principal site for NE synthesis and NAc is a main area in rewarding. It has been demonstrated that LC and NAc have both anatomical connections and functional interactions.⁹ These two brain regions are also involved in opioid addiction and relapses. Consistently, in the present study, the expression of $\alpha 2A$ autoreceptors and NET in LC and NAc, as well as TH in LC, of CCI rats exposed to morphine plus nortriptyline conditioning

was significantly decreased as compared to the morphine conditioning alone group. Moreover, the NE concentration in the NAc was remarkably increased in the morphine and nortriptyline conditioning group. These results suggest that the effect of nortriptyline on morphine-induced CPP in CCI rats could be mediated by an increased NE concentration in the NAc due to a decrease in the α 2A autoreceptor and NET expression. This possibility is supported by the increased NE concentration in the NAc in these same rats.

While the exact mechanism by which an altered endogenous NE system may regulate opioid reward is still in investigation, it has been shown that chronic neuropathic pain alone resulted in noradrenergic dysfunction shown as the increased bursting activity in LC accompanied by an increase in the expression of TH, NET transporter and α 2-adrenoceptors in the LC.³⁸ Future studies will explore the cellular mechanisms of these changes and the brain network involved in the development of various phenotypes of opioid reward with or without antidepressant.

The present data may have significant clinical implications for chronic pain management. If co-administration of an opioid (morphine) and a TCA (nortriptyline) could enhance morphine reward, it becomes possible that such a drug combination may increase the demand of opioid dose escalation for pain management. Future studies need to explore whether the other opioids or TCA have the similar potentiating effect and further examine the underlying mechanisms, in order to search for effective drug combinations that could provide effective analgesia but fewer side effects in pain clinical.

Acknowledgments

This work was supported by the NIH grant R01 DE022901 and R01 DE018214 (J.M.).

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Figure 1. Effect of nortriptyline (Nor) on morphine-induced CPP (A) and effect of nortriptyline and/or morphine (Mor) administration on nociceptive threshold in naive rats (B, C)

Nortriptyline (5 mg/kg) was given alone or at 30 min before morphine (10 mg/kg) injection followed by a 30-min conditioning session. Data are presented as the mean $\pm$ S.E.M. * $P < 0.05$. n.s. = no significant difference. $n = 4-6$ rats for each group.

Figure 2. Enhancement of nortriptyline on the acquisition (A), extinction (D) and reinstatement (E) of morphine-induced CPP in CCI rats

Nortriptyline (5 mg/kg) was given alone or 30 min before morphine (10 mg/kg) during the initial conditioning sessions. The analgesic effect of nortriptyline and/or morphine in the CCI rats were evaluated using a thermal hyperalgesia test (B, C). The hindpaw withdrawal latency was performed before, days 7 after CCI or sham operation, and after the post-conditioning test. The CPP score was again obtained at 7 days after the last post-conditioning test to examine the time course of CPP extinction (D). Reinstatement of morphine-induced CPP was examined by morphine priming (5 mg/kg), followed 20 min later by another CPP test (E). Data are presented as the mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$. $n = 4-12$ rats for each group.

Figure 3. Effect of nortriptyline and/or morphine on the NE system in the locus coeruleus (LC) of CCI rats

Nortriptyline (5 mg/kg) was administered intraperitoneally (i.p.) 30 min before morphine (i.p., 10 mg/kg) injection for 6 days since the day 7 after CCI surgery. Real-time PCR results showed the mRNA expression of DBH (A) and $\alpha 2A$ (B) in the LC of CCI rats. Western blot analysis showed changes in the expressions of $\alpha 2A$ (C, D), NET (C, E) and TH (C, F) after nortriptyline and/or morphine administration in CCI rats. Representative bands are shown in C, and the data summary is shown in D, E, F. * $P < 0.05$; $n=3-4$ for PCR and $n=3$ for Western blot analysis.

Figure 4. Effect of nortriptyline and/or morphine on the NE system in the nucleus accumbens (NAc) of CCI rats

Nortriptyline (5 mg/kg) was administered intraperitoneally (i.p.) 30 min before morphine (i.p., 10 mg/kg) injection for 6 days since the day 7 after CCI surgery. Western blot analysis showed changes in the expressions of α2A (A, B), NET (A, C) after nortriptyline and/or morphine administration in CCI rats. Representative bands are shown in A, and the data summary is shown in B, C. The NE concentration in NAc was measured by ELISA (D). *P < 0.05, **P < 0.01; n = 3 rats for WB and n = 5 for ELISA analysis.