

Nocistatin, a peptide reversing acute and chronic morphine tolerance

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It has been reported that intracerebroventricular (i.c.v.) injection of nociceptin/orphanin FQ (OFQ) can antagonize morphine analgesia, whereas i.c.v. OFQ antibody can reverse morphine tolerance. Nocistatin (NST) is a recently characterized neuropeptide possessing an antagonizing effect on OFQ. Here we examine whether i.c.v. NST would result in a reversal of morphine tolerance. The results showed that: (1) i.c.v. NST at doses of 0.005, 0.05, 0.5, 5 or 50 ng per rat produced a bell-shaped dose-dependent reversal of chronic morphine tolerance,

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with maximum response at 0.5 ng. (2) Acute morphine tolerance could also be reversed, albeit partially, by i.c.v. NST at 0.5 ng. (3) The reversal of acute and chronic morphine tolerance by NST was completely abolished when NST (0.5 ng) was co-injected with (8 µg) OFQ. Since OFQ and NST are derived from the same preprohormone, the profile of its splicing in the CNS may play an important role in determining the effectiveness of morphine analgesia. *NeuroReport* 12:1789–1792 © 2001 Lippincott Williams & Wilkins.

INTRODUCTION

Nociceptin/orphanin FQ (OFQ) is an endogenous agonist for the orphan opioid-like (ORL₁) receptor [1]. Mogil and colleagues demonstrated that i.c.v. OFQ can antagonize systemic morphine analgesia, opioid-mediated stress-induced analgesia, as well as µ-, κ- and δ-receptor-mediated opioid analgesia in mice [2,3], and claimed OFQ to be an anti-opioid neuropeptide. We have demonstrated that i.c.v. OFQ can antagonize systemic morphine analgesia and opioid-mediated electroacupuncture (EA)-induced analgesia in rats [4,5], whereas i.c.v. OFQ antibody produced a partial reversal of morphine tolerance and electroacupuncture tolerance [6]. In addition, the biosynthesis and release of OFQ were found to be accelerated during morphine tolerance [7]. These results seem to substantiate the idea regarding OFQ as a supraspinal anti-opioid substance, and suggest its involvement in the development of morphine tolerance.

Recently, it was reported that the precursor of nociceptin comprised another bioactive peptide, nocistatin (NST), which was demonstrated to antagonize the effect of nociceptin in both *in vivo* and *in vitro* studies [8–12]. We have reported that i.c.v. NST indeed reverses the effect of OFQ in antagonizing morphine analgesia [9].

If OFQ plays an important role in the mediation of morphine tolerance and NST is indeed a functional antagonist of OFQ, then NST should be able to reverse morphine tolerance. This hypothesis was tested in the present study.

MATERIALS AND METHODS

Animals: Male Sprague–Dawley rats, bred by the Institute of Animal Research, Chinese Academy of Science, weighing 200–250 g at the start of the experiment, were used throughout. The rats were housed six/cage, with the room temperature maintained at 24 ± 1°C, relative humidity 50%, under a 12:12 h light:dark cycle. The experimental protocols were approved by the Animal Use Committee of Peking University Health Science Center. Cannulae for i.c.v. injection were performed stereotaxically under 10% chlorohydrate anaesthesia (0.3 ml/100 g body weight). Stainless steel tubing (0.8 mm o.d.) was fixed on the skull at coordinates A 5.4, L 1.5, H 3.0 mm, according to Pellegrino *et al.* [13]. Experiments involving i.c.v. injection started 5 days after the operation. The volume of each injection was 5 µl, administered over 10 s, followed by another 5 µl normal saline (NS) for flushing.

Drugs: Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang, China. NST and OFQ were products of Phoenix Pharmaceuticals, Inc. (USA). All chemicals were dissolved in sterile NS to desired final concentrations.

Nociceptive test: Experiments were performed at room temperature (20 ± 1°C). Nociception was assessed by the radiant heat tail-flick test [14]. Rats were kept in a plastic restrainer with hind limbs and tails exposed outside. Focused light from a 12.5 W projection bulb was applied to

the lower third of the tail, and the latency of tail flick reaction (TFL) was recorded to the nearest 0.1 s. Values from the first three measurements, with an inter-test interval of 5 min, were averaged as the basal nociceptive threshold, which was usually in the range 4–6 s. TFLs obtained in subsequent tests were expressed as percentage changes from basal levels, with a cutoff limit of 15 s to avoid unnecessary skin damage.

Statistical analysis: Data were processed by commercially available software GraphPad Prism 3.0. Results are presented as mean \pm s.e.m. Comparison between means of groups were analyzed with two-way or one-way ANOVA where appropriate, followed by Student–Newman–Keul's test. The accepted level of statistical significance was $p < 0.05$.

RESULTS

Effect of i.c.v. NST on chronic morphine tolerance: Male Sprague–Dawley rats were given repeated s.c. injections of increasing doses of morphine (5, 10, 20, 40, 50, 60 mg/kg, t.i.d.) for 6 days. In order to observe the development of morphine tolerance, a testing dose of morphine (5 mg/kg) was injected s.c. at 07.00 h every day, and TFLs were determined prior to and 30 min after the injection. A progressive decrease of morphine analgesia indicated the development of morphine tolerance (Fig. 1).

On the seventh day, the rats were randomly divided into six groups, which were given i.c.v. NS or NST (0.005, 0.05, 0.5, 5 or 50 ng), and followed immediately by a testing dose of morphine (5 mg/kg, s.c.). TFL was measured at 10 min intervals for a total of 60 min after the i.c.v. injection (Fig. 2a). The analgesic effect induced by morphine (5 mg/kg, s.c.) was expressed as the mean area under the curve (MAUC) after the injection of morphine, i.e. over the 10–60 min period of the testing session in the presence and absence of NST (Fig. 2b). Morphine (5 mg/kg) showed no analgesic effect in the saline control group. In contrast, NST (0.005, 0.05, 0.5, 5 or 50 ng) reinstated morphine analgesia in a dose-dependent manner (Fig. 2b). The dose–

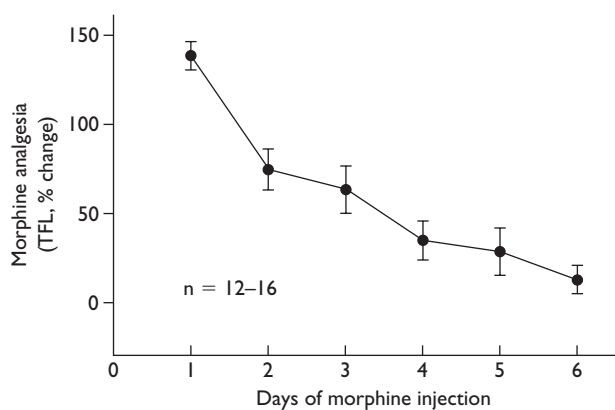


Fig. 1. The development of chronic tolerance to morphine-induced analgesia in rats. Male Sprague–Dawley rats were given repeated subcutaneous injection of morphine (5, 10, 20, 40, 50, 60 mg/kg, t.i.d.) for 6 days. The development of tolerance was monitored at 07.00 h every day by measuring the percentage increase of tail flick latency (TFL) 30 min after the injection of a testing dose of morphine (5 mg/kg).

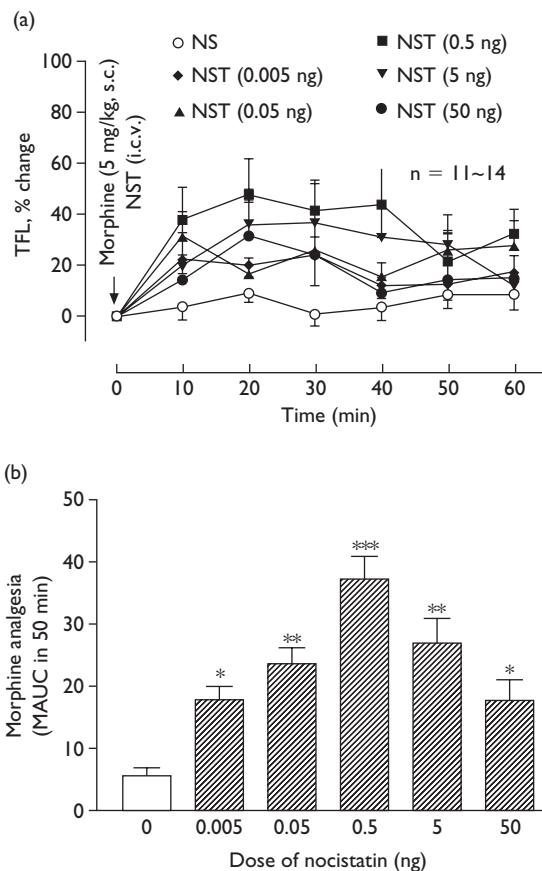


Fig. 2. NST reversed the chronic tolerance to morphine-induced analgesia. (a) The time course of the analgesic effect induced by a testing dose of morphine (5 mg/kg, s.c.). (b) The dose–response relationship using the mean area under the curve (MAUC) after the injection of morphine as the index of nociception. Columns and vertical lines represent the means and s.e.m., respectively. Statistical analysis was carried out by one way ANOVA followed by Student–Newman–Keul's test. $p < 0.05$, ** $p < 0.01$, compared with NS group.

response curve is bell-shaped, with the maximum response at 0.5 ng.

The effect of NST on acute morphine tolerance: Twenty-five rats were randomly divided into two groups (NS group and NST group). All rats were given eight consecutive injections of morphine (5 mg/kg, s.c.) with an inter-injection interval of 2 h to induce an acute tolerance to morphine analgesia. Challenging doses of morphine (5 mg/kg, s.c.) were given 2, 6, 10 and 14 h after the 8th injection. NS or NST were injected i.c.v. prior to the first challenging dose of morphine, and TFL was measured 30 min after each morphine injection. Compared to the NS control group, the NST (0.5 ng) group produced a gradual recovery of morphine-induced analgesia, peaked at 8 h after the i.c.v. injection ($p < 0.01$ compared with NS control group; Fig. 3).

OFQ abolished the reversing effect of NST on acute morphine tolerance: The acute tolerance to morphine was prepared as stated above. Two hours after the 8th mor-

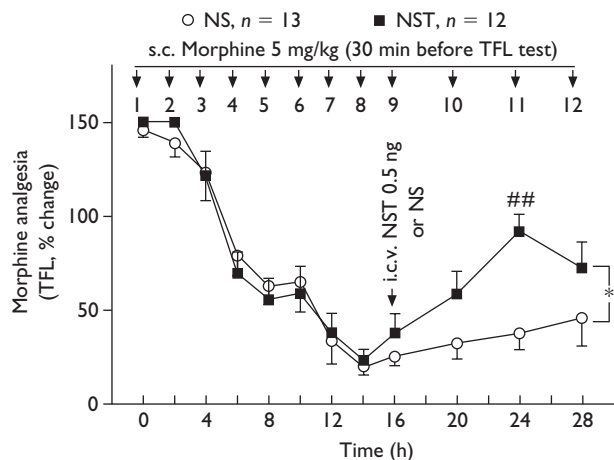


Fig. 3. Reversal of the acute tolerance to morphine-induced analgesia by i.c.v. injection of nocistatin. Arrows show the injection of a testing dose of morphine (5 mg/kg, s.c.), and the analgesic effect was assessed 30 min after morphine injection. The solid arrow head indicates i.c.v. injection of nocistatin 0.5 ng or normal saline (NS) 30 min prior to the TFL test. Vertical bars represent s.e.m. Significance of difference of the two lines between 16h and 28h was tested by two-way ANOVA followed by Student–Newman–Keul’s test. * $p < 0.05$, compared between the two groups; ## $p < 0.01$ compared with NS group at the corresponding point by Student–Newman–Keul’s test.

phine injection, the rats were randomly divided into two groups, i.c.v. injection with NS, OFQ (8 μ g), NST (0.5 ng), and NST (0.5 ng) plus OFQ (8 μ g), respectively. After the i.c.v. injection, all four groups were given a challenging dose of morphine (5 mg/kg, s.c.) immediately. The same doses of morphine were injected again 4, 8, and 12 h after the i.c.v. injection, respectively. TFL measured before the first morphine injection was employed as a baseline. It was repeated in 30 min after each injection of morphine to monitor the possible recovery of morphine analgesia. The results are shown in Fig. 4. NST (0.5 ng) produced a marked reversal of morphine tolerance. This effect of NST was totally abolished by OFQ (8 μ g) co-administered with NST.

OFQ abolished the reversing effect of NST on chronic morphine tolerance: Chronic tolerance to morphine analgesia was induced as stated above. At the seventh day of morphine injection, the rats were randomly divided into four groups, administered i.c.v. injection with NS, OFQ (8 μ g), NST (0.5 ng), and NST (0.5 ng) plus OFQ (8 μ g), respectively, then followed immediately by a testing dose of morphine (5 mg/kg, s.c.). TFL was measured every 10 min for a total of 60 min after the i.c.v. injection. As shown in Fig. 5, i.c.v. injection of NST produced a partial reinstatement of morphine analgesia. This effect of NST was totally abolished by OFQ co-administered with NST.

DISCUSSION

NST and OFQ are the expression products from the same gene with opposing effects on several central nervous system functions. Okuda-Ashitaka *et al.* [8] demonstrated that the allodynia and hyperalgesia induced by OFQ can be reversed by NST, and the pain responses evoked by

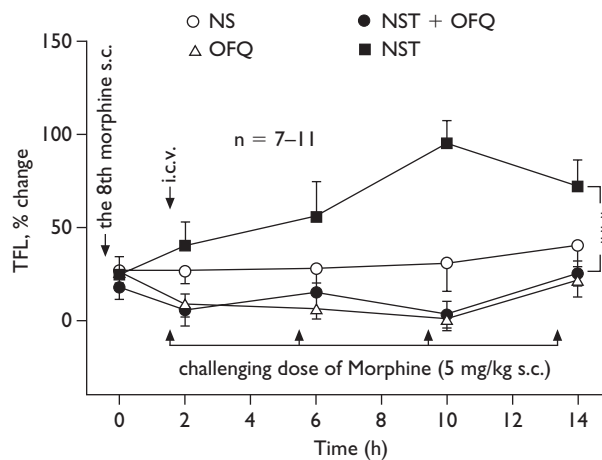


Fig. 4. Orphanin FQ abolished the effect of nocistatin against acute morphine tolerance. All rats were given challenging doses of morphine (5 mg/kg, s.c.) 2, 6, 10 and 14 h after the 8th morphine injection. NS, OFQ, NST, or NST+OFQ were given immediately prior to the 1st challenging dose of morphine. TFL was assessed 30 min after each injection of morphine. Other annotations are the same as in Fig. 3.

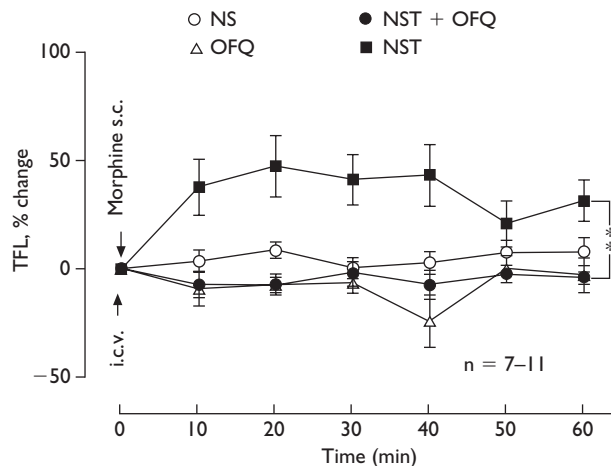


Fig. 5. Orphanin FQ abolished the effect of nocistatin against chronic morphine tolerance. All rats were given morphine injection for 6 days (5, 10, 20, 40, 50, 60 mg/kg, s.c. t.i.d.) followed by a test dose (5 mg/kg, s.c.) of morphine on day 7. NS, OFQ, NST, or NST + OFQ was administered immediately prior to the test injection of morphine. TFL was measured every 10 min for 60 min. Other annotations are the same as in Fig. 2.

prostaglandin E₂ can also be attenuated by NST. We have reported that i.c.v. injection of OFQ at 8 μ g showed significant antagonistic effect on morphine analgesia, and that i.c.v. injection of NST (0.005, 0.05, 0.5, 5 or 50 ng) reversed the effect of OFQ (8 μ g), with a bell-shaped dose–response curve, peaking at 0.5 ng [9]. Recently, Zeilhofer *et al.* [15] have shown that NST selectively inhibits transmitter release from spinal inhibitory interneurons, whereas OFQ inhibits excitatory transmission in the superficial layers of the rat spinal cord horn. All the above experiments demonstrated that NST and OFQ may play opposing roles in modulating pain transmission. In line with the study of Tian *et al.*, which stated that i.c.v. injection of OFQ anti-

body reversed morphine tolerance [6], our present study demonstrated that i.c.v. injection of NST could also reverse morphine tolerance. Nevertheless, this effect of NST could be reversed by OFQ. These results demonstrated that NST and OFQ might play opposing roles in the modulation of morphine tolerance.

An antagonistic effect between two neuropeptides derived from the same precursor is likely to be one kind of intramolecular regulation that helps an organism maintain homeostasis [16]. However, the mechanism of the antagonistic interaction is not clear at the present time. It has been shown that NST binds to the membrane of mouse brain and spinal cord with a high affinity in a saturable manner [8], but not to the OFQ receptor, thus suggesting that NST is not an endogenous antagonist of OFQ receptor.

Concerning the mechanism of the nocistatin-induced reversal of morphine tolerance, it is interesting to note that i.c.v. injection of NST did not affect the baseline nociception, nor did it affect the analgesic effect produced by a single injection of morphine [9]. It was only after repeated injection of morphine (8 injections in 14h or 18 injections in 6 days) that NST produced a potentiation of reduced morphine analgesia or a reversal of morphine tolerance (Fig. 2, Fig. 3). However, with the existence of exogenous OFQ, NST seemed to have no power at all (Fig. 4, Fig. 5). It remains to be studied whether NST is a product produced simultaneously with OFQ [7], or that its expression can be regulated by an independent mechanism. Furthermore, it remains to be studied whether NST specifically antagonizes the effect of OFQ, or is a common inhibitor of anti-

morphine substance, such as cholecystokinin octapeptide [17].

CONCLUSION

The current study demonstrated that i.c.v. injection of nocistatin could reverse both acute and chronic tolerance to morphine analgesia.

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