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Attenuation of opioid analgesic tolerance in p75 neurotrophin receptor null mutant mice

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ABSTRACT

Repeated exposure to opioid drugs can lead to the development of tolerance, which manifests as a reduction in analgesic potency, and physical dependence, a response indicated by a withdrawal syndrome. Accumulating evidence suggests that the nerve growth factor (NGF) family of neurotrophins may have an important modulatory role in the induction of opioid analgesia and opioid addiction. Because neurotrophins universally bind the p75 neurotrophin receptor (p75NTR), we investigated whether the activity of this receptor is involved in the development of opioid analgesic tolerance and physical dependence. We found that in both the wild-type and p75NTR−/− mice an acute systemic (i.p.) injection of morphine produced a maximal analgesic response as measured by the thermal tail-immersion test. Repeated injection of morphine over 5 days in wild-type mice resulted in a progressive decline of the analgesic effect and a concomitant loss of the agonist potency, reflecting development of morphine tolerance. However, the loss of morphine analgesia was not observed in p75NTR−/− mice. In the second part of this study, mice were given escalating doses of systemic (i.p.) morphine over 5 days and subsequently challenged with the opioid receptor antagonist naloxone. This challenge precipitated a robust withdrawal syndrome that was comparable in wild-type mice and p75NTR−/− mice. The findings suggest that p75NTR activity plays a critical role in the development of opioid analgesic tolerance but not in the induction or the expression of opioid physical dependence.

The nerve growth factor (NGF) family of neurotrophins act on two types of receptors: the p75 neurotrophin receptor (p75NTR), which binds all neurotrophins and modulates specificity and affinity of their cognate tyrosine kinase receptors – TrkA, TrkB, and TrkC – which preferentially bind NGF, brain-derived neurotrophic factor (BDNF), and neurotrophin-3, respectively [7,22]. There is mounting evidence that activity of neurotrophins mediated by p75NTR and Trk receptors plays an important role in modulating the analgesic actions of opioid drugs [19]. A recent study reported that activation of p75NTR and TrkA receptors by NGF in sensory neurons causes an increase in mu-opioid receptor expression, a response which underlies the enhanced ability of opioid drugs to suppress inflammatory pain [19]. Intrathecal administration of NGF has been reported to restore the effectiveness of morphine in attenuating pain hypersensitivity following a peripheral nerve injury in rats [6]. Additionally, NGF has been directly implicated in the sorting and targeting of opioid receptors to the cell surface [15].

Because of the considerable interaction between the neurotrophin and opioid system, it is not surprising that neurotrophin activity has been linked to the development of opioid tolerance and opioid physical dependence. Chronic morphine treatment has been shown to upregulate expression of BDNF and neurotrophin-3 in the locus coeruleus and the ventral tegmental area (VTA), areas of the brain inextricably linked to drug dependence [13,21]. An increase in BDNF and neurotrophin-3 levels has also been demonstrated in the paragigantocellularis, which is one of two principle afferents innervating the locus coeruleus, following opioid withdrawal [13,21]. In addition, an infusion of BDNF or neurotrophin-4 into the VTA blocks both the neurochemical and the morphological changes that occur with chronic morphine treatment [4,5]. The neurochemical evidence implicating neurotrophins in the development of opioid tolerance and physical dependence is consistent with results showing a marked attenuation of opioid tolerance in neurotrophin-4 deficient transgenic mice [25] and a reduction in the intensity of the opioid withdrawal response in mice with a conditional deletion of BDNF [1]. Given the involvement of neurotrophins in opioid analgesia and opioid addiction, and the fact that neurotrophins

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universally bind the p75NTR, we sought to determine whether activity of p75NTR plays a role in the development of opioid analgesic tolerance and physical dependence.

To examine the role of p75NTR in opioid analgesic tolerance and physical dependence, we used adult male wild-type SV129 mice and p75NTR−/− at exon III SV129 mice [8,10,14]. As described previously by Hannila and Kawaja [11], the genotype of each progeny was determined by obtaining tail DNA samples from postnatal day 35 mice. Samples were digested with EcoRI and amplified with primers for either p75NTR (5′WT: 5′-GTGTTACGTTCTCTGACGTTGTG; 3′/WT: 5′-TCTCATTCGGCGTCA GCCCAGGG; and 3′/Neo: 5′-GATTCGCAG-CGCATCGCCTT). The cDNA products were separated on a 0.8% agarose gel, viewed under ultraviolet light, and photographed.

All experiments were performed in strict accordance with the guidelines of the Canadian Council on Animal Care using protocols approved by the University Animal Care Committee. Animals weighing 25–30 g were maintained on a 12 h light/12 h dark cycle with access to food and water ad libitum. Morphine sulfate (BDH Pharmaceuticals, Canada) and naloxone HCl (DuPont NEN, USA) were dissolved in physiological saline (0.9%).

In the opioid tolerance paradigm, animals were treated with systemic (i.p.) injection of morphine (15 mg/kg) once daily for 5 days between 11 a.m. and 1 p.m. Control groups were given systemic (i.p.) injection of saline once daily for 5 days. Nociception was assessed using the thermal tail-immersion test [24] which entailed gentle restraint of the animal and immersion of the lower 3 cm portion of its tail in a water bath maintained at a constant temperature of 51 °C. The time latency for removal of the tail (indicated by a distinct tail-flick) from the stimulus was recorded. A maximum immersion time of 10 s was used to prevent tissue damage. On day 1, the thermal tail-immersion test was performed prior to morphine injection (baseline latency) and at 30, 60, 90, 120, 150, and 180 min time-points after the drug injection. On subsequent days (days 2–5), the thermal tail-immersion test was performed immediately before and 30 min after morphine injection. On day 6, a cumulative dose–response curve for the acute effect of morphine was obtained as described previously [23] to determine the agonist ED_{50} value, an indicator of the drug potency. Briefly, the animals were given ascending doses of morphine every 30 min and the response to the drug determined in thermal tail-immersion test until a maximal level of the analgesic response was reached. In all experiments, behavioral assessments were performed with the experimenter being unaware of the genetic profile of the mice. The latency values for tail-flick response were converted to a maximal possible effect (MPE): MPE (%) = 100 × [postdrug response − baseline response]/[cut-off value − baseline response]. Morphine ED_{50} values were determined from the cumulative dose response using a non-linear regressional analysis (Prism 2, Graph-Pad Software Inc., San Diego, CA, USA).

In the second part of this study, opioid physical dependence was established by administering ascending doses of systemic (i.p.) morphine at 8 h intervals: day 1: 20 and 30 mg/kg; day 2: 40 and 50 mg/kg; day 3: 60 and 70 mg/kg; and day 4: 80 and 90 mg/kg. On day 5, animals received a morning injection of 100 mg/kg and 2 h later a single systemic injection of naloxone (2 mg/kg; i.p.) to precipitate opioid withdrawal. Control animals received comparable injections of saline and challenged with naloxone on the final day of treatment. On the day of the test, animals were acclimatized to a clear Plexiglass testing chamber (25 cm × 25 cm × 30 cm) 1 h prior to the naloxone challenge and the elicited withdrawal symptoms evaluated at 10 min intervals for a total testing period of 30 min. The number of episodes of headshakes, jumping, and

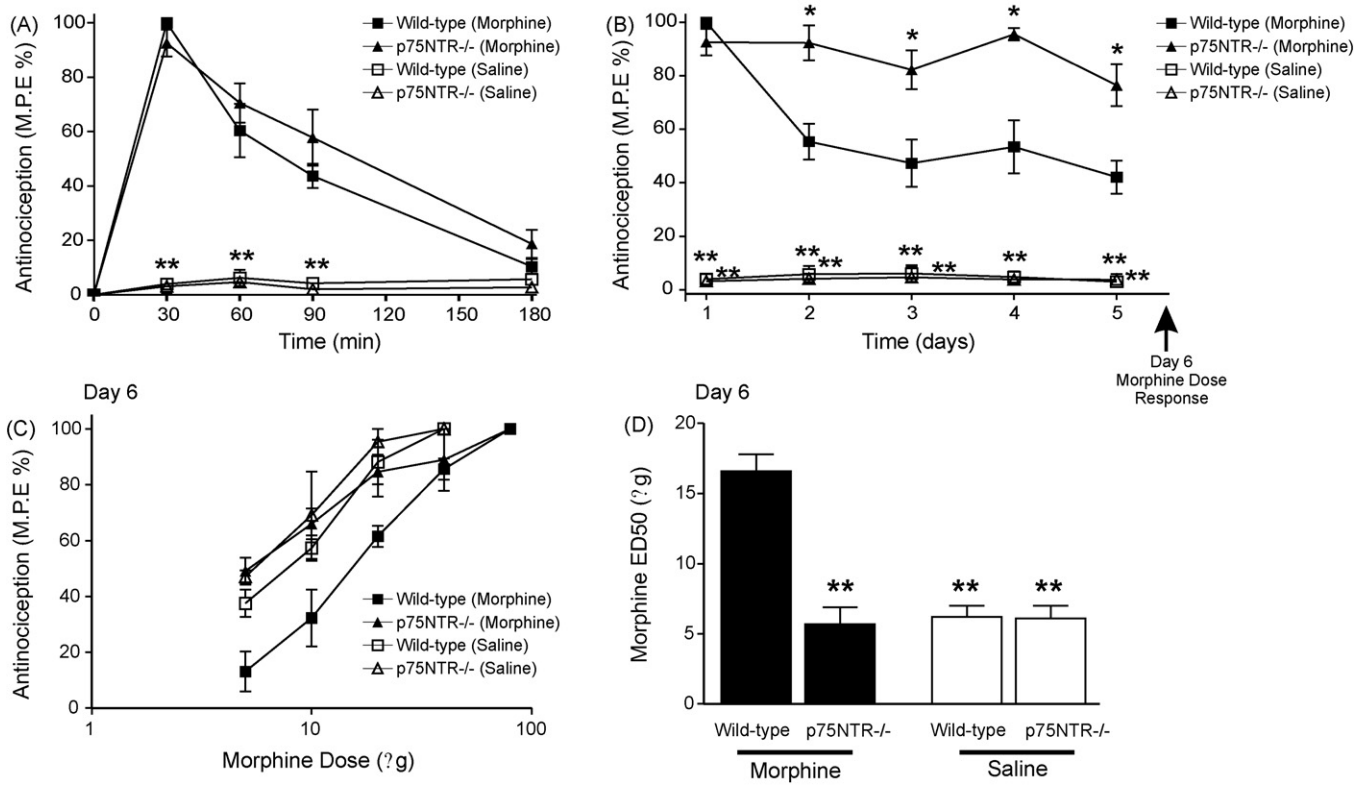

Fig. 1. The development of tolerance to the antinociceptive action of morphine is attenuated in p75NTR−/− mice. Wild-type and p75NTR−/− mice were given systemic (i.p.) injection of morphine or saline and the antinociceptive response was assessed using the thermal tail-immersion test. (A) The time-course of acute antinociceptive response to morphine on day 1 following a single injection of the opioid agonist (15 mg/kg). (B) Peak antinociceptive response to a systemic injection of morphine or saline delivered once daily from day 1 to 5. (C) Cumulative morphine dose–response curves obtained on day 6 following the 5-day treatment with morphine or saline. (D) The ED_{50} values for the antinociceptive effect of morphine (a measure of drug potency) derived from the cumulative dose–response curves obtained on day 6. The data are presented as mean ± S.E.M. (n = 5–10 animals per group). Significant difference from value in the morphine-treated wild-type mice: $^{*}p < 0.01$, $^{**}p < 0.001$.