Genetic deletion of pleiotrophin leads to disruption of spinal nociceptive transmission: Evidence for pleiotrophin modulation of morphine-induced analgesia.

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Abstract

Pleiotrophin (PTN) is a growth factor that exhibits neurotrophic actions and is upregulated at sites of nerve injury. Upregulation of PTN levels in injured dorsal root ganglion (DRG) correlates with decreased mechanical allodynia and faster recovery from Chronic Constriction Injury (CCI) of the rat sciatic nerve. Despite the evidence pointing to a role of PTN in the development of chronic pain, the role of this neurotrophic factor in pain transmission has not been assessed in acute pain models. We have now studied the behaviour of PTN genetically deficient (PTN-/-) and wild type (WT+/+) mice in the hot-plate and tail-immersion tests. We found that basal central pain responses do not differ between PTN-/- and WT+/+ mice in the hot-plate test. Very interestingly, basal latencies to a tail flick were significantly increased in PTN-/- mice as assessed in the tail-immersion test. It was also aimed to evaluate morphine-induced analgesia in PTN-/- and WT+/+ mice. We did not find differences among genotypes using a high dose of morphine (10 mg/kg) in the hot-plate test, reaching this dose the analgesia peak 25 minutes after injection (i.p.) and returning to almost basal values 125 minutes after injection. In contrast, we found that an intermediate dose of morphine (5 mg/kg) significantly delayed pain responses in PTN-/- mice compared to WT+/+ mice in both the hot-plate and tail-immersion tests. The data strongly suggest that PTN is of critical importance for pain processing at the spinal level and, furthermore, that endogenous PTN modulate morphine-induced analgesic effects in mice.

Keywords: Nerve injury, midkine, opioid, hot-plate, tail-flick.

1. Introduction

Pleiotrophin (PTN), also known as heparin binding growth-associated molecule (HB-GAM), is a 136 amino acid cytokine (Deuel et al., 2002; Li et al., 1990; Milner et al., 1989) that shares over 50% identity in amino acid sequence with midkine (MK), the only other member of the PTN/MK developmentally regulated gene family (Kadomatsu et al., 1988; Milner et al., 1989). Both PTN and MK have been found to play important roles in the development of the nervous system through their actions on neuronal differentiation (see for example review by Herradon and Ezquerra, 2009). In addition, it has been hypothesized that both PTN and MK may play important roles on survival of different cell types and wound repair since both cytokines are upregulated at sites of injury and repair in inflammatory macrophages, microglia, dermal fibroblasts, endothelial cells and other cells (Blondet et al., 2005; Kikuchi-Horie et al., 2004; Mi et al., 2007; Sakakima et al., 2004; Yeh et al., 1998; Herradon et al., 2009).

In the central nervous system, PTN has been found to be a key factor for survival of the injured dopaminergic neurons in vitro and in vivo (Hida et al. 2003; Jung et al. 2004; Hida et al. 2007; Gramage et al., 2010b), results that became promising when PTN was found to be significantly upregulated in the substantia nigra of patients with Parkinson´s disease (Marchionini et al., 2007). Interestingly, the role of PTN in wound repair in the periphery has been recently linked to its potential role in the guidance of neural axon regeneration in peripheral nervous system and muscle reinnervation (Jin et al., 2009). This is supported by the ability of PTN to promote regeneration of peripheral nerve axons after sciatic nerve transection (Mi et al., 2007). These actions of PTN in nerve repair could potentially be involved in the regulation of pain transmission in chronic states. For instance, higher levels of expression of PTN in

the dorsal root ganglion (DRG) of rats with chronic constriction injury (CCI) of the sciatic nerve correlate with decreased mechanical allodynia and faster recovery from CCI (Ezquerra et al., 2008). These evidences have led to the hypothesis that PTN may be important for the function of the spinal cord nociceptive neurons (Jin et al., 2009) and, thus, it may be an important factor in spinal pain processing. However, despite the existing evidences, the role of PTN in nociception has not been assessed in acute pain models directed to assess pain processing at either supraspinal or spinal level. To fill this gap in knowledge, we have now studied the behaviour of PTN genetically deficient (PTN-/-) and wild type (WT+/+) mice in the hot-plate and tail-immersion tests. In addition, we also aimed to evaluate morphine-induced analgesia in PTN-/- and WT+/+ mice in these tests.

2. Materials and Methods

Pleiotrophin knockout (PTN-/-) mice were kindly provided by Dr. Thomas F. Deuel (The Scripps Research Institute, La Jolla, CA). PTN-/- mice were generated on a 129/Ola x C57BL/6J background by deleting exons 2 to 4 as recently described (Del Olmo et al., 2009; Gramage et al., 2010a). In these studies we used 8 weeks old (20-25 g) female PTN-/- and WT+/+ mice. The genotypes of the PTN-/- mice were confirmed by polymerase chain reaction as previously described (Ezquerra et al., 2004). PTN-/- mice have been shown to be viable and fertile and lack gross anatomical abnormalities (Amet et al., 2001). All the animals used in this study were maintained according to European Union Laboratory Animal Care Rules (86/609/ECC directive).

2.1. Hot-plate test.

To asses supraspinal nociceptive responses in both PTN-/- and WT+/+ mice, we used the hot-plate test. A metal hot-plate was maintained at either 53 ± 0.5°C or 55 ± 0.5°C. The time to when the mouse first exhibited nocifensive behaviour (licked its hind paw or jumping) was determined. The cutoff time for the first sign of nocifensive behaviour was 75 s in the case of the assays carried out at 53ºC and 30 s in the case of the assays carried out at 55ºC.

To study the effect of morphine on hot-plate response, saline (10 ml/kg) or morphine sulphate (Alcaliber, Madrid, Spain) were administered intraperitoneally (2.5, 5 and 10 mg/kg) after testing the baseline response for the hot-plate maintained at 53 ± 0.5°C. In additional studies, we also tested the baseline responses for the hot-plate maintained at 55 ± 0.5°C (n = 9/strain) in order to determine possible differences between genotypes depending on heat stimulus. To study the time course of the effect of

morphine, the hot-plate latency was recorded 25, 75 and 125 min after morphine (or saline, as a control) injection. Total number of animals used in every experimental group was as follows:

Saline (10 ml/kg): WT+/+, n = 24; PTN-/-, n = 18.

Morphine (2.5 mg/kg): WT+/+, n = 13; PTN-/-, n = 10.

Morphine (5 mg/kg): WT+/+, n = 14; PTN-/-, n = 8.

Morphine (10 mg/kg): WT+/+, n = 12; PTN-/-, n = 11.

2.2. Tail-immersion test.

To asses spinal nociceptive responses in both PTN-/- and WT+/+ mice, we used the tail-immersion test. First, the latency to a rapid tail-flick in a bath maintained at 50 ± 0.5ºC was registered in PTN-/- and WT +/+ mice (n = 5/strain) with a cutoff latency of 20 s to prevent tissue damage. Whereas WT+/+ mice showed normal baseline responses to the heat stimulus (~ 10 s), 4 of the 5 PTN-/- mice used in this study reached the cutoff latency of 20 s.

To study the effect of morphine on tail-immersion responses we used a bath maintained at a temperature of 55 ± 0.5ºC. We used the dose of morphine (5 mg/Kg) that provided pronounced differences concerning morphine analgesic effects between the mouse genotypes in the previously performed hot-plate test (see results section). Saline (10 ml/kg) or morphine sulphate (5 mg/kg) were administered i.p. after testing the baseline responses for the tail-immersion test using a cutoff latency of 15 s to prevent tissue damage. As shown in the results section, around 60% of the PTN-/- mice (n = 32) used in this study reached the cutoff value (15 s) in basal conditions. Importantly, none of the WT+/+ mice (n = 17) used reached the designated cutoff value. As a result, only the rest of PTN-/- mice that showed normal basal responses under the

designated cutoff value were used to assess morphine analgesic effects. To study the time course of the antinociceptive effect of morphine, the tail flick latency was recorded 25, 50, 75 and 125 min after morphine (or saline, as a control) injection. Total number of animals used in every experimental group was as follows:

Saline (10 ml/Kg): WT+/+, n = 7; PTN-/-, n = 5.

Morphine (5 mg/kg): WT+/+, n = 10; PTN-/-, n = 8.

2.3. Statistical Analysis.

The statistical significance of changes after every dose of morphine (or saline) injection was determined by 2-way ANOVA considering as factors the genotype (PTN-/- and WT+/+) and time point after injection. Bonferroni's post hoc tests were used to detect the sources of group differences revealed by the ANOVAs. Area under the curve values obtained from PTN-/- and WT+/+ mice, and basal values of both genotypes were analyzed using student's t test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Hot-plate test.

In the studies directed to assess morphine-induced antinociceptive effects in the hot-plate maintained at 53 ºC, we first analyzed together basal values from mice from both genotypes used in all experiments performed (WT+/+, n = 63; PTN-/-, n = 47). The latency to the first sign of nocifensive behaviour in PTN-/- mice was found to be essentially similar to that recorded in WT+/+ mice (Fig. 1A). We also assessed baseline responses of PTN-/- mice and WT+/+ mice (n = 9/strain) to the hot-plate maintained at 55 ºC, determining again similar latencies between both genotypes (Fig. 1B). Nociceptive responses after saline administrations were not altered compared to baseline values of either strain (Fig. 2A). Differences between PTN-/- and WT+/+ mice were clearly observed after the intermediate dosage of morphine used (5 mg/kg; Fig. 2C). At this dose, two-way ANOVA showed significant effects of the genotype ($F(1, 80) = 11.89$; $P = 0.0009$) and significant effects of time ($F(3, 80) = 12.58$; $P < 0.0001$)). Interestingly, except for the lowest dose of morphine used (2.5 mg/kg), which only caused modest antinociceptive effects (Fig. 2B), the analgesic effect of morphine was maximal at 25 min after morphine injection and was dose-dependent (Fig. 2). Considering alone the 25 min time point, we found that morphine (5 mg/kg) exerts significantly enhanced analgesic effects in PTN-/- mice compared to WT+/+ mice (Fig. 2C) whereas a higher dose of morphine (10 mg/kg) almost achieved maximum analgesic effects in both strains in a similar manner (Fig. 2D).

To better analyze these differences between WT+/+ and PTN-/- mice treated with morphine, we calculated the areas under the curves (AUC). We found a

significantly higher AUC value only in PTN-/- compared to WT+/+ mice treated with 5 mg/kg morphine (Fig. 3).

3.2. Tail-immersion test.

First, we assessed baseline responses of PTN-/- and WT+/+ mice (n = 5/strain) to the tail-immersion test using a bath maintained at 50 ± 0.5 ºC. PTN-/- mice showed a significantly delayed latency to a tail-flick compared to WT+/+ mice (Fig. 4A). More importantly, it has to be noted that 4 of the 5 PTN-/- mice used reached the experimental cutoff time (20 s) whereas none of the 5 WT+/+ mice used reached cutoff value. In the studies designed to test morphine antinociceptive effects, we aimed to use a higher bath temperature (55 ± 0.5 ºC). Taking together the basal values of all mice from both strains used in the studies assessing morphine effects, we found that PTN-/- mice still exhibited significantly delayed pain responses (Fig. 4B) compared to WT+/+ mice and, interestingly again, 19 of the 32 PTN-/- mice used reached the experimental cutoff time (15 s). Again, none of the 17 WT+/+ mice used reached cutoff values.

To evaluate morphine (and saline, as control)-induced analgesia in this test in PTN-/- mice, we only used the 13 PTN-/- mice that showed latencies under the cutoff value in the bath maintained at 55 ± 0.5 ºC. Interestingly, the basal latencies of these PTN-/- mice were only slightly, not significantly, higher than those of the WT+/+ mice (Fig. 5). Nociceptive responses after saline administrations were not altered compared to baseline values of either genotype (Fig. 5A). Differences between PTN-/- and WT+/+ mice were clearly observed after morphine (5 mg/kg) administration (Fig. 5B). In this case, two-way ANOVA showed significant effects of the genotype ($F(1, 80) = 39.99$; $P < 0.0001$) and significant effects of time ($F(4, 80) = 11.14$; $P < 0.0001$)). Twenty five and 50 minutes after morphine administration, PTN -/- mice exhibited very significant

increases in their latencies compared to WT+/+ mice (Fig. 5B). As expected, we also found a significantly higher AUC value in PTN-/- compared to WT+/+ mice treated with 5 mg/kg morphine (Fig. 5C).

4. Discussion

The present study reveals for the first time that PTN deficiency does not influence pain processing at the supraspinal level but critically alters spinal pain processing. In the hot-plate test, the nocifensive behaviour involves licking, flinching and head, trunk and limb coordination. Compared to the spinal reflexive behaviours measured by other acute pain models such as the tail withdrawal test, these behaviours are more complex, organized and unlearned behaviours and involve purposeful actions requiring supraspinal sensory processing, being all these qualities apparently unaffected in PTN-/- mice. In contrast, when the less complex spinal reflexive behaviour is evaluated using the tail-immersion test, striking differences are observed in mice lacking endogenous PTN. Around 60 % of the PTN-/- mice used in these experiments were unresponsive to the highest heat stimulus used (55ºC) in our experimental conditions. Since, in some cases, hypoalgesia observed in genetically altered mice using this same test has been found to be dependent on the intensity of the stimulus used (see for example Ferré et al., 2007), we performed studies at different temperatures. Our data suggest that hypoalgesia observed in mice lacking endogenous PTN is not dependent on the intensity of the stimulus used.

Interestingly, it was previously found that higher levels of expression of PTN in DRG of rats with chronic neuropathic pain correlate with a faster recovery and reduced allodynic and hyperalgesic symptoms (Ezquerra et al., 2008), suggesting an important role of PTN in the control of chronic pain states. In contrast, the data presented here suggest that PTN is not involved in pain transmission at the supraspinal level in acute pain states. However, absence of endogenous PTN seems to lead to a very impressive hypoalgesia when spinal nociceptive transmission is tested, suggesting PTN is critical

for normal pain processing at the spinal level. Although these evidences suggest opposite roles of PTN in chronic and acute pain states, it seems reasonable to think that attenuation of chronic neuropathic pain responses could result from the neurotrophic actions of PTN after injury (Mi et al., 2007) and its capacity to form functional neovasculature in the injured area (Christman et al., 2005), functions of PTN that should not influence acute pain responses.

The data presented here clearly support that PTN plays a critical role in activating pain processing at the spinal level and, thus, suggest the different molecules involved in the signaling pathways initiated by PTN as potential drug targets for pain treatment. Pleiotrophin binds the Receptor Protein Tyrosine Phosphatase (RPTP)β/ζ (Meng et al., 2000; Fukada et al., 2006), causing the inactivation of its phosphatase activity. As a result, PTN induces significant increases in the tyrosine phosphorylation levels of the different substrates of RPTPβ/ζ identified so far, β-catenin (Meng et al., 2000), β-adducin (Pariser et al., 2005a; Pariser et al., 2005b), Fyn (Pariser et al., 2005c), p190 RhoGAP and membrane-associated guanylate kinase, WW, and PDZ domain containing 1 (Fukada et al., 2005) and GIT1/Cat-1 (Kawachi et al., 2001). Anaplastic lymphoma kinase (ALK), another proposed receptor for PTN (Stoica et al., 2001), has been shown as a substrate of RPTPβ/ζ (Perez-Pinera et al., 2007). Studies directed to clarify the possible contribution of each of these substrates and the receptor RPTPβ/ζ to the PTN mediation of pain processing at the spinal level could result in very significant contributions to the field in the near future.

We also found that the antinociceptive effect of morphine was dose-dependent in both PTN-/- and WT+/+ mice in the hot-plate test. Differences concerning morphine-induced analgesia were found at an intermediate dose (5 mg/kg) that caused greater analgesia in PTN-/- mice compared to WT+/+ mice. In contrast, morphine at a dose of

10 mg/kg almost reached experimental cutoff time in both PTN-/- and WT+/+ mice 25 minutes after the administration of the drug, suggesting this dose is too high to reflect strain differences. Interestingly, when morphine (5 mg/kg) analgesic effects were tested on the tail-immersion test, we found greater analgesic effects of morphine in PTN-/- mice compared to WT+/+ mice. These data may be important since they identify, for the first time, PTN as an endogenous modulator of morphine-induced analgesic effects at both spinal and supraspinal levels.

It is interesting to note that the endogenous expression of another neurotrophic factor, brain-derived neurotrophic factor (BDNF), is key for morphine-induced plasticity of noradrenergic neurons (Akbarian et al., 2002) which could be important for morphine to trigger descending inhibitory pathways in the control of pain transmission (Millan, 2002). Our results strongly support the novel neurotrophic factor PTN as another important regulator of morphine-induced analgesic effects. Our data also suggest that individual differences in the expression levels of PTN could potentially underlie some of the well-documented differences concerning the efficacy of morphine to induce satisfactory analgesic effects in humans (for a review see Smith, 2008).

In summary, the data presented here suggest a critical role of PTN in acute pain transmission at the spinal level. Furthermore, the data identify previously unexpected roles of endogenous PTN in the modulation of morphine-induced analgesia at the spinal and supraspinal levels.

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5. **REFERENCES**

Akbarian, S., Rios, M., Liu, R.J., Gold, S.J., Fong, H.F., Zeiler, S., Coppola, V., Tessarollo, L., Jones, K.R., Nestler, E.J., Aghajanian, G.K., Jaenisch, R., 2002. Brain-derived neurotrophic factor is essential for opiate-induced plasticity of noradrenergic neurons. J. Neurosci 22, 4153-62.

Amet, L.E., Lauri, S.E., Hienola, A., Croll, S.D., Lu, Y., Levorse, B.J., Prabhakaran, M., Taira, T., Rauvala, H., Vogt, T.F., 2001. Enhanced hippocampal long-term potentiation in mice lacking heparin-binding growth-associated molecule. Mol. Cell. Neurosci. 17, 1014-24.

Blondet, B., Carpentier, G., Ferry, A., Courty, J., 2005. Pleiotrophin cellular localization in nerve regeneration after peripheral nerve injury. J. Histochem. Cytochem 53, 971-7.

Christman, K.L., Fang, Q., Kim, A.J., Sievers, R.E., Fok, H.H., Candia, A.F., Colley, K.J., Herradon, G., Ezquerra, L., Deuel, T.F., Lee, R.J., 2005. Pleiotrophin induces formation of functional neovasculature in vivo. Biochem. Biophys. Res. Commun 332, 1146-52.

Del Olmo, N., Gramage, E., Alguacil, L.F., Pérez-Pinera, P., Deuel, T.F., Herradon, G., 2009. Pleiotrophin inhibits hippocampal long-term potentiation: a role of pleiotrophin in learning and memory. Growth Factors 27, 189-94.

Deuel, T. F., Zhang, N., Yeh, H.J., Silos-Santiago, I., Wang, Z.Y., 2002. Pleiotrophin: a cytokine with diverse functions and a novel signaling pathway. Arch. Biochem. Biophys 397, 162-71.

Ezquerra, L., Alguacil, L.F., Nguyen, T., Deuel, T.F., Silos-Santiago, I., Herradon, G., 2008. Different pattern of pleiotrophin and midkine expression in neuropathic pain: correlation between changes in pleiotrophin gene expression and rat strain differences in neuropathic pain. Growth Factors 26, 44-8.

Ezquerra, L., Herradon, G., Nguyen, T., Vogt, T.F., Bronson, R., Silos-Santiago, I., Deuel, T.F., 2004. Pleiotrophin is a major regulator of the catecholamine biosynthesis pathway in mouse aorta. Biochem. Biophys. Res. Commun 323, 512-7.

Ferré, S., Diamond, I., Goldberg, S.R., Yao, L., Hourani, S.M., Huang, Z.L., Urade, Y., Kitchen, I., 2007. Adenosine A2A receptors in ventral striatum, hypothalamus and nociceptive circuitry implications for drug addiction, sleep and pain. Prog. Neurobiol 83, 332-47.

Fukada, M., Kawachi, H., Fujikawa, A., Noda, M., 2005., Yeast substrate-trapping system for isolating substrates of protein tyrosine phosphatases: Isolation of substrates for protein tyrosine phosphatase receptor type z. Methods 35, 54-63

Gramage, E., Putelli, A., Polanco, M.J., González-Martín, C., Ezquerra, L., Alguacil, L.F., Pérez-Pinera, P., Deuel, T.F., Herradon, G., 2010a. The neurotrophic factor pleiotrophin modulates amphetamine-seeking behaviour and amphetamine-induced neurotoxic effects: evidence from pleiotrophin knockout mice. Addict. Biol, in press. DOI: 10.1111/j.1369-1600.2009.00202.x

Gramage, E., Rossi, L., Granado, N., Moratalla, R., Herradon, G., 2010b. Genetic inactivation of Pleiotrophin triggers amphetamine-induced cell loss in the substantia nigra and enhances amphetamine neurotoxicity in the striatum. Neuroscience, in press. DOI: 10.1016/j.neuroscience.2010.06.078.

Herradon, G., Ezquerra, L., 2009. Blocking receptor protein tyrosine phosphatase beta/zeta: a potential therapeutic strategy for Parkinson's disease. Curr. Med. Chem 16, 3322-9. Review.

Herradon, G., Ezquerra, L., Gramage, E., Alguacil, L.F., 2009. Targeting the pleiotrophin/receptor protein tyrosine phosphatase beta/zeta signaling pathway to limit neurotoxicity induced by drug abuse. Mini Rev. Med. Chem 9, 440-7. Review.

Herradon, G., Ezquerra, L., Nguyen, T., Silos-Santiago, I., Deuel, T.F., 2005. Midkine regulates pleiotrophin organ-specific gene expression: evidence for transcriptional regulation and functional redundancy within the pleiotrophin/midkine developmental gene family. Biochem. Biophys. Res. Commun 333, 714-721.

Hida, H., Jung, C.G., Wu, C.Z., Kim, H.J., Kodama, Y., Masuda, T., Nishino, H., 2003. Pleiotrophin exhibits a trophic effect on survival of dopaminergic neurons in vitro. Eur. J. Neurosci 17, 2127-2134.

Hida, H., Masuda, T., Sato, T., Kim, T.S., Misumi, S., Nishino, H., 2007. Pleiotrophin promotes functional recovery after neural transplantation in rats. Neuroreport 18, 179-183.

Jin, L., Jianghai, C., Juan, L., Hao, K., 2009. Pleiotrophin and peripheral nerve injury. Neurosurg. Rev 32, 387-93.

Jung, C.G., Hida, H., Nakahira, K., Ikenaka, K., Kim, H.J., Nishino, H., 2004. Pleiotrophin mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon and the product promotes production of dopaminergic neurons from embryonic stem cell-derived nestin-positive cells. FASEB J 18, 1237-1239.

Kadomatsu, K., Tomomura, M., Muramatsu, T., 1988. cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis, Biochem. Biophys. Res. Commun 151, 1312-1318.

Kawachi, H., Fujikawa, A., Maeda, N., Noda, M., 2001. Identification of GIT1/Cat-1 as a substrate molecule of protein tyrosine phosphatase zeta /beta by the yeast substrate-trapping system. Proc. Natl. Acad. Sci. U S A 98, 6593-6598

Kikuchi-Horie, K., Kawakami, K., Kamata, M., Wada, M., Hu, J.G., Nakagawa, H., Ohara, K., Watabe, K., Oyanagi, K., 2004. Distinctive expression of midkine in the repair period of rat brain during neurogenesis: immunohistochemical and immunoelectron microscopic observations. J. Neurosci. Res 75, 678-87.

Li, Y.S., Milner, P.G., Chauhan, A.K., Watson, M.A., Hoffman, R.M., Kodner, C.M., Milbrandt, J., Deuel, T.F., 1990. Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. Science 250, 1690-4.

Marchionini, D.M., Lehrmann, E., Chu, Y., He, B., Sortwell, C.E., Becker, K.G., Freed, W.J., Kordower, J.H., Collier, T.J., 2007. Role of heparin binding growth factors in nigrostriatal dopamine system development and Parkinson's disease. Brain Res 1147, 77-88.

Meng, K., Rodriguez-Pena, A., Dimitrov, T., Chen, W., Yamin, M., Noda, M., Deuel, T.F., 2000. Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta. Proc. Natl. Acad. Sci. U S A 97, 2603-2608.

Mi, R., Chen, W., Hoke, A., 2007. Pleiotrophin is a neurotrophic factor for spinal motor neurons. Proc. Natl. Acad. Sci. U S A 104, 4664-9.

Millan, M.J., 2002. Descending control of pain. Prog. Neurobiol 66, 355-474.

Milner, P.G., Li, Y.S., Hoffman, R.M., Kodner, C.M.,Siegel, N.R., Deuel, T.F., 1989. A novel 17 kD heparin-binding growth factor (HBGF-8) in bovine uterus: purification and N-terminal amino acid sequence. Biochem. Biophys. Res. Commun 165, 1096-103.

Pariser, H., Herradon, G., Ezquerra, L., Perez-Pinera, P., Deuel, T.F., 2005a. Pleiotrophin regulates serine phosphorylation and the cellular distribution of beta-adducin through activation of protein kinase C. Proc. Natl. Acad. Sci. U S A 102, 12407-12412.

Pariser, H., Perez-Pinera, P., Ezquerra, L., Herradon, G., Deuel, T.F., 2005b. Pleiotrophin stimulates tyrosine phosphorylation of beta-adducin through inactivation of the transmembrane receptor protein tyrosine phosphatase beta/zeta. Biochem. Biophys. Res. Commun 335, 232-239

Pariser, H., Ezquerra, L., Herradon, G., Perez-Pinera, P., Deuel, T.F., 2005c. Fyn is a downstream target of the pleiotrophin/receptor protein tyrosine phosphatase beta/zeta-signaling pathway: regulation of tyrosine phosphorylation of Fyn by pleiotrophin. Biochem. Biophys. Res. Commun 332, 664-669

Perez-Pinera, P., Zhang, W., Chang, Y., Vega, J.A., Deuel, T.F., 2007. Anaplastic lymphoma kinase is activated through the pleiotrophin/receptor protein-tyrosine phosphatase beta/zeta signaling pathway: an alternative mechanism of receptor tyrosine kinase activation. J. Biol. Chem 282, 28683-28690

Sakakima, H., Yoshida, Y., Kadomatsu, K., Yuzawa, Y., Matsuo, S., Muramatsu, T., 2004. Midkine expression in rat spinal motor neurons following sciatic nerve injury. Brain Res. Dev. Brain Res 153, 251-60.

Smith, H.S., 2008. Variations in opioid responsiveness. Pain Physician 11, 237-48. Review.

Stoica, G.E., Kuo, A., Aigner, A., Sunitha, I., Souttou, B., Malerczyk, C., Caughey, D.J., Wen, D., Karavanov, A., Riegel, A.T., Wellstein, A., 2001. Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. J. Biol. Chem 276, 16772-16779

Yeh, H. J., He, Y.Y., Xu, J., Hsu, C.Y., Deuel, T.F., 1998. Upregulation of pleiotrophin gene expression in developing microvasculature, macrophages, and astrocytes after acute ischemic brain injury. J. Neurosci 18, 3699-707.

6. FIGURES

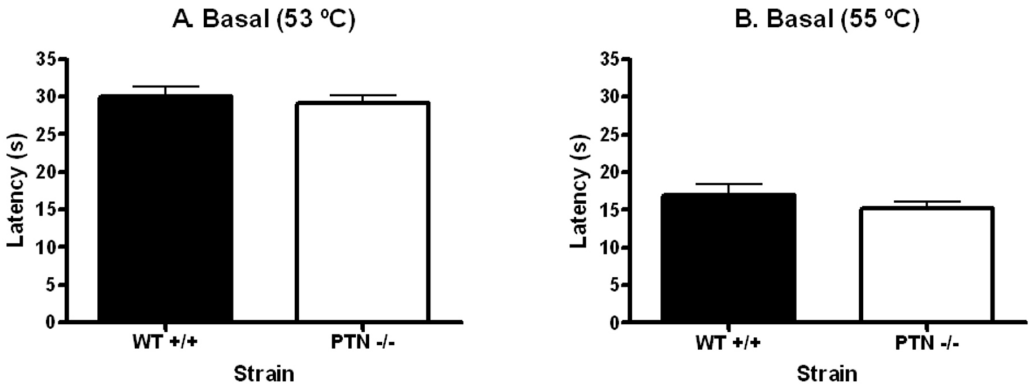

Fig. 1. Basal nociceptive behavioural responses of WT+/+ and PTN-/- mice in the hot-plate test. Analysis using student's t test did not show statistical differences between genotypes (P (53ºC) = 0.61; P (55ºC) = 0.35). Data show mean ± SEM.

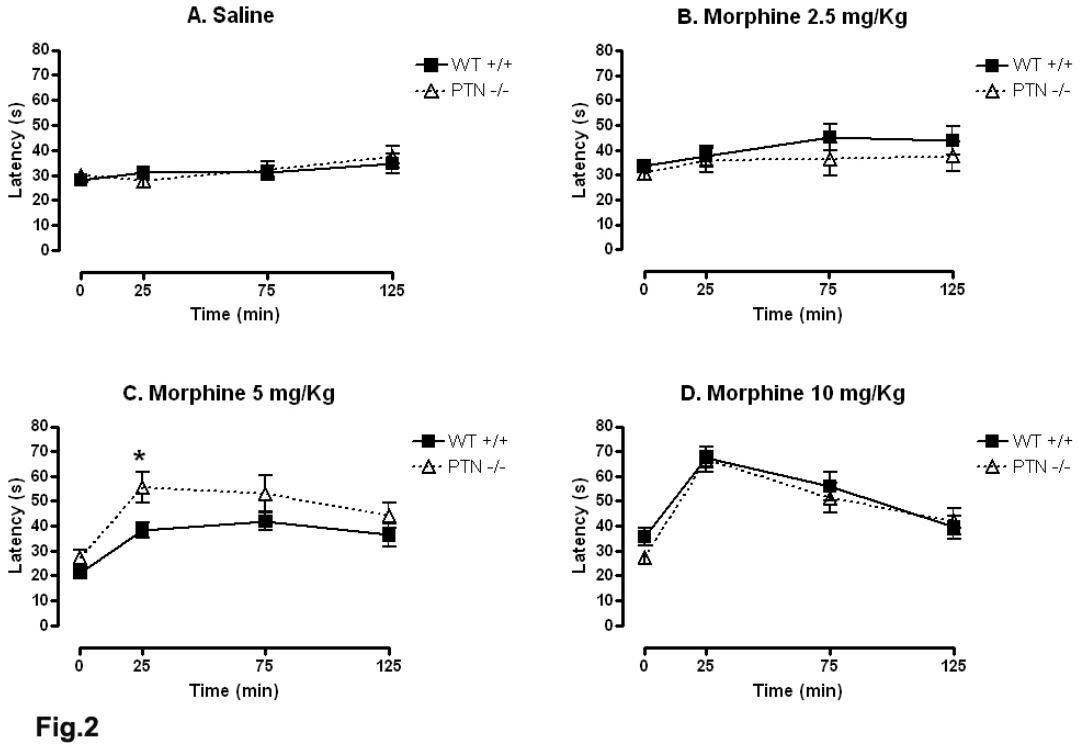

Fig. 2. Comparison of nociceptive behavioural responses and the antinociceptive effect of morphine in WT+/+ and PTN-/-mice. Results from hot-plate maintained at

53 ºC are shown. The effects of saline administration used as a control (A) and the analgesic effects of morphine at a dose of 2.5 mg/kg (B), 5 mg/kg (C) and 10 mg/kg (D) on the hot-plate test are shown as a function of time. Data show mean ± SEM. * P < 0.05 vs. WT+/+.

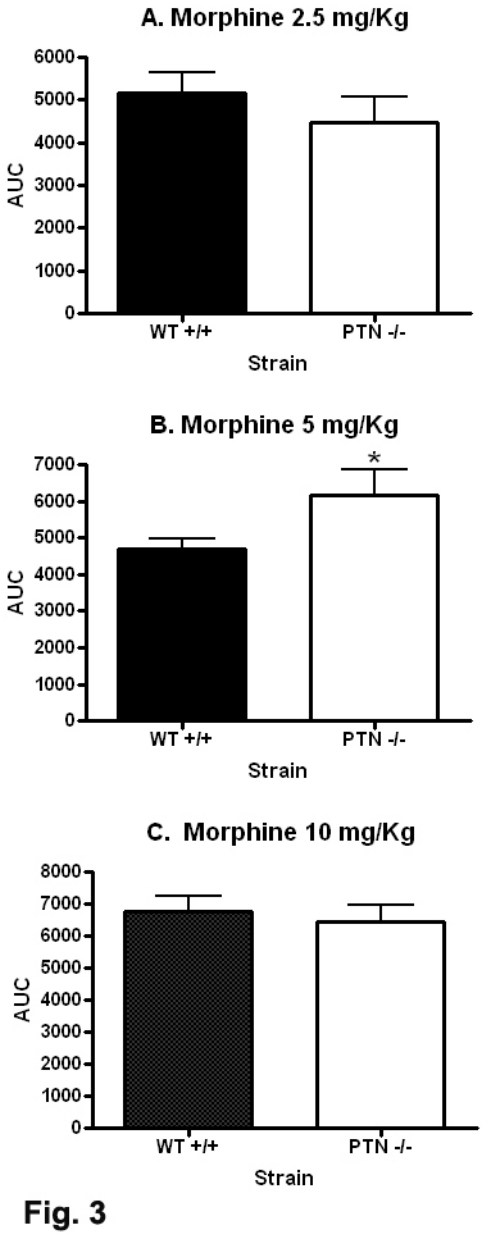

Fig. 3

Fig. 3. Comparison of nociceptive behavioural responses and the antinociceptive effect of morphine in WT+/+ and PTN-/- mice. Results from hot-plate maintained at 53 ºC are shown. Area under the curve (AUC) values for WT+/+ and PTN-/- mice

treated with morphine (2.5, 5 and 10 mg/kg) are compared. Data show mean ± SEM. * P < 0.05 vs. WT+/+.

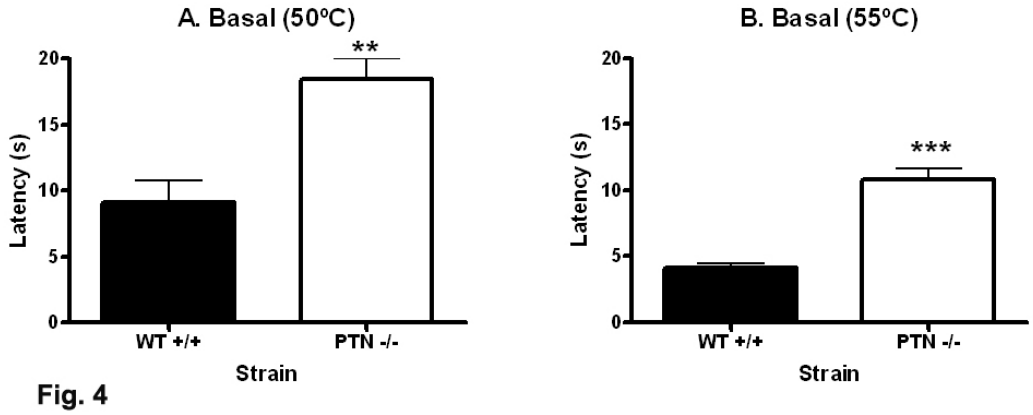

Fig. 4. Basal nociceptive behavioural responses of WT+/+ and PTN-/- mice in the tail-immersion test. Analysis using student's t test revealed statistical differences between genotypes (P (50ºC) = 0.003; P (55ºC) < 0.0001). Data show mean ± SEM.

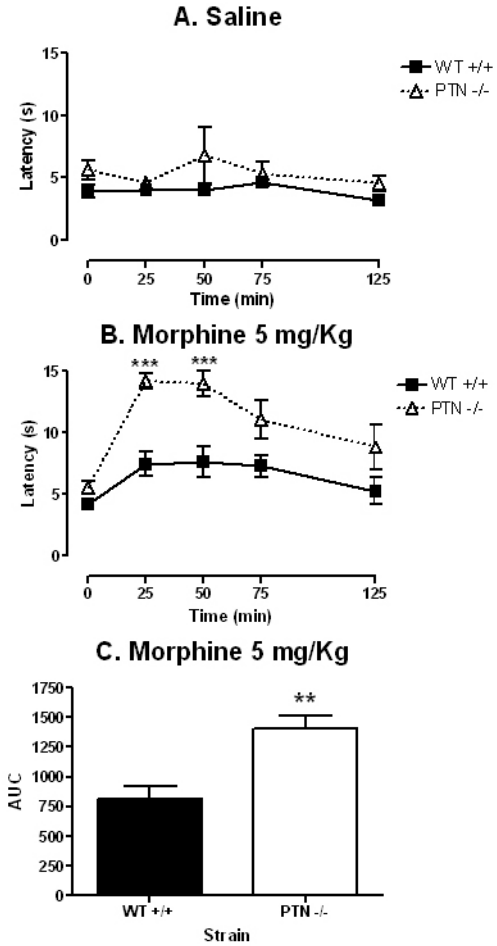

Fig. 5

Fig. 5. Comparison of nociceptive behavioural responses and the antinociceptive effect of morphine in WT+/+ and PTN-/-mice. Results from tail-immersion test using a bath maintained at 55 ºC are shown. The effects of saline administration used as a control (A) and the analgesic effects of morphine at a dose of 5 mg/kg (B) on the tail-immersion test are shown as a function of time. Area under the curve (AUC) values for WT+/+ and PTN-/- mice treated with morphine (5 mg/kg) are also compared (C). Data show mean ± SEM. ** $P < 0.01$ vs. WT+/+, *** $P < 0.001$ vs. WT+/+.