Original research Article

Pleiotrophin modulates morphine withdrawal but has no effects on morphine-conditioned place preference

Esther Gramage*, Marta Vicente-Rodríguez, Gonzalo Herradón

Pharmacology lab, Department of Pharmaceutical and Health Sciences. Facultad de Farmacia. Universidad CEU San Pablo, Madrid, Spain.

*Corresponding author:

Esther Gramage, PhD
Lab. Pharmacology
Faculty of Pharmacy
Universidad CEU San Pablo
Urb. Montepríncipe
28668 Boadilla del Monte, Madrid, Spain.
Tel: 34-91-3724700 (Ext. 4843)
E-mail: esther.gramagecaro@ceu.es

Abstract

Pleiotrophin (PTN) is a neurotrophic factor with important functions in addiction and neurodegenerative disorders. Morphine administration induces an increase in the expression of PTN and Midkine (MK), the only other member of this family of cytokines, in brain areas related with the addictive effects of drug of abuse, like the Ventral Tegmental Area or the hippocampus. In spite of previous studies showing that PTN modulates amphetamine and ethanol rewarding effects, and that PTN is involved in morphine-induced analgesia, it was still unknown if the rewarding effects of morphine may be regulated by endogenous PTN. Thus, we aim to study the role of PTN in the reward and physical dependence induced by morphine.

We used the Conditioned Place Preference (CPP) paradigm in PTN genetically deficient (PTN-/-) and wild type (WT) mice to assess the rewarding effects of morphine in absence of endogenous PTN. Secondly, to study if PTN may be involved in morphine physical dependence, naloxone-precipitated withdrawal syndrome was induced in PTN-/- and WT morphine dependent mice. Although the increase in the time spent in the morphine-paired compartment after conditioning tended to be more pronounced in PTN-/- mice, statistical significance was not achieved. The data suggest that PTN does not exert an important role in morphine reward. However, our results clearly indicate that PTN-/- mice develop a more severe withdrawal syndrome than WT mice, characterized as a significant increase in the time standing and in the total incidences of forepaw licking, forepaw tremors, wet dog shake and writhing. The data presented here suggest that PTN is a novel genetic factor that plays a role in morphine withdrawal syndrome.

Keywords: Midkine; morphine; dependence; withdrawal; naloxone; addiction

Introduction

Pleiotrophin (PTN), also known as heparin binding growth associated molecule (HB-GAM), is a 136 amino acid cytokine [6] that shares over 50% identity in amino acid sequence with midkine (MK), the only other member of the PTN/MK developmentally regulated gene family [19, 29]. PTN is widely expressed in the Central Nervous System (CNS) during early development [24], while in adult stages it appears to be restricted to some cell types in brain cortex, hippocampus, and cerebellum and in some striatal interneurons ([3, 41]; reviewed in [12]). In spite of this restricted expression, both PTN and MK are known to be upregulated in several brain areas after the administration of different drugs of abuse [8, 9, 22]. In particular, morphine administration is known to upregulate MK in the hippocampus [8] and PTN in the Ventral Tegmental Area (VTA) [11], brain areas involved in the reinforcing effects of addictive drugs [20]. Accordingly, these cytokines have been found to play important roles in addiction (see for example review [18]).

Previous studies from our group have demonstrated that PTN regulates the rewarding effects of amphetamine and ethanol [16, 26, 40]. On the other hand, the deficiency of endogenous PTN has been demonstrated to be a key factor that increases the vulnerability against the neurotoxic effects of the amphetamine. Interestingly, amphetamine induces a significant decrease in the number of dopaminergic neurons in the substantia nigra only in mice constitutively lacking PTN [17], supporting the role of PTN in the neuroprotection of the nigrostriatal pathway after the amphetamine insult. Furthermore, periadolescent amphetamine treatment causes transient cognitive deficits and long-term alterations of hippocampal long-term potentiation (LTP) depending on the endogenous expression of PTN [13], what indicates that PTN is involved in diverse effects of drug of abuse.

With regard to opioid drugs, it was found that PTN is of critical importance for pain processing at the spinal level and, importantly, that endogenous PTN modulates morphine-induced analgesic effects in acute pain models in mice [14, 15]. However, it remained unknown if PTN is modulating the main side effects associated with the analgesic uses of morphine, the psychological and physical dependence. To fill this gap in knowledge, we have now studied the behavior of PTN genetically deficient (PTN-/-) mice and wild type (WT) mice in the Conditioned Place Preference (CPP) induced by morphine, a traditional paradigm used to assess the rewarding effects of drugs in rodents. In addition, naloxone-precipitated withdrawal was induced in morphine dependent PTN-/- and WT mice to study if the absence of endogenous PTN modulates morphine-induced physical dependence.

Material and methods

Animals

PTN-/- mice on a C57BL/6J background, generated by methods previously described [2, 15] were kindly donated by Dr. Thomas F. Deuel (The Scripps Research Institute, La Jolla, CA). All the animals used in this study were maintained in accordance with the European Union Laboratory Animal Care Rules (86/609/ECC directive) and the protocols were approved by the Animal Research Committee of USP-CEU.

Morphine Conditioned Place preference (CPP)

To assess the rewarding effects of morphine, CPP paradigm was used in male PTN-/- (n=13) and WT (n=16) animals of 9–10 weeks (20–25 g). The CPP apparatus consisted of two chambers of the same size, one with black floor and walls and the other with black floor and white walls. The compartments were closed by a removable door during the morphine-paired session. This procedure was previously used in our laboratory [16, 40], and consisted of a 5-day schedule with three phases: preconditioning (PreC, day 1), conditioning (days 2–4) and testing (day 5). During preconditioning, mice were free to explore the two compartments for a 30-min period and their time spent in each compartment was recorded. The conditioning phase consisted of a 3-day schedule of double conditioning sessions. In the morning session starting at 8 am, animals received a single injection of saline i.p. (10 ml/kg) and were immediately confined to the initially preferred compartment for 30 min. In the evening session starting at 3 pm, the animals were injected with morphine sulphate (10 mg/kg, i.p., Alcaliber, Madrid, Spain) and confined to the initially less-preferred compartment for 30 min. During the following two days, the procedure used was the same but the order of the treatments (morning/evening) was changed to avoid the influence of circadian rhythms. The testing

phase was performed on day 5 of the schedule, where mice were allowed to freely move throughout the apparatus for 30 min, exactly as in the preconditioning phase (day 1). The time spent in each compartment was also registered, and the percentage of time-spent (stay) in the less-preferred (morphine-paired) compartment calculated. The difference between the time spent in the morphine-paired compartment in the testing phase and the time spent in the same compartment in the preconditioning was considered as indicative of the degree of conditioning induced by morphine.

Naloxone-precipitated withdrawal syndrome in morphine dependent mice

We wondered if PTN could be also involved in the physical dependence induced by morphine. To test this hypothesis, withdrawal syndrome was induced by naloxone administration in WT and PTN-/- morphine dependent mice. Due to the limited availability of the animals, and because opioid receptor antagonist-precipitated procedures like the one presented here do not show significant differences in the withdrawal syndrome in both genders [4, 21], 9-10 weeks old mice of either sex weighting 20-25 g were included in these experiments (PTN-/- male n=3, female n=4; WT male=3, female=4). Following known protocols to precipitate morphine withdrawal syndrome in mice [34, 35], morphine dependence was induced by morphine administration (5 mg/kg, i.p.) twice daily (8 am and 8 pm) for 5 days. On the 6^{th} day, 2 hours after the morphine administration of the morning session, naloxone (8 mg/kg, i.p., Sigma Aldrich, St. Louis, MO, USA) was injected to induce withdrawal syndrome. Immediately after the naloxone administration, mice were placed in an observation cylinder for a 30-min period, in which the appearance frequency of different withdrawal signs (rearings, forepaw licking and tremors, wet dog shakes, writhings, diarrhea) was recorded. Withdrawal Severity Score (WSS) was calculated with the addition of the incidences of forepaw licking, forepaw tremors, wet dog shakes and writhings of each

mouse during the 30 min of observation, and was employed to evaluate the magnitude of the withdrawal syndrome. The time spent in a standing position was also recorded. Mice were weighted before the naloxone administration and after the observation period, and the percentage of weight loss induced by naloxone was calculated for each mouse.

Statistical analysis

Statistical differences were analyzed using 2-way repeated measures ANOVA in the CPP experiment considering as factors the time (Pre-C/Test) and genotype (WT/PTN-/-). The morphine withdrawal data were analyzed by Student t-test. In the withdrawal experiment, statistical differences between genders were lacking in each genotype, so balanced groups of both genders were included and analyzed together. A p value less than 0.05 was considered a statistically significant difference. All statistical analyses were performed using Graphpad prism 5 program (La Jolla, CA, USA).

Results

Morphine conditioned place preference

ANOVA revealed a significant effect of time ($F(1, 27) = 6.83$; $P < 0.05$). The increase in the time spent in the morphine-paired compartment in the Test day compared with the Pre-C tended to be more pronounced in PTN-/- mice compared with WT (19% vs. 7%; Fig. 1). However, the effect of genotype or the interaction between factors were not found to be statistically significant.

Previous studies performed as a control in the same experimental conditions showed that saline treatment during conditioning phase does not induce a significant CPP neither in WT nor in PTN-/- mice [16].

The absence of endogenous PTN exacerbates morphine withdrawal syndrome

To test if PTN is involved in the physical dependence induced by morphine, withdrawal syndrome was induced by naloxone administration in WT and PTN-/- morphine dependent mice. Intensity and time course of morphine effects are correlated to the morphine treatment [37], so we used a low dose of morphine (5 mg/kg) to induce moderate effects that presumably avoid the influence of possible differences regarding the magnitude of the dependence induced by morphine in both genotypes. This protocol of morphine administration is demonstrated to be adequate to induce dependence in mice and has been successfully used by other to study naloxone-precipitation withdrawal syndrome [34, 35]. Agreeing with this, although no mouse of both genotypes did show any jumping, a widely considered withdrawal sign, unambiguous signs of morphine withdrawal were observed (standings, fore paw tremors and licking, wet dog shakes, writhings, diarrhea, teeth chattering, face and body grooming, ptosis, straightening, head tossing…) in both WT and PTN-/- naloxone-treated mice,

confirming the effective induction of a morphine withdrawal syndrome. As an additional withdrawal sign, the percentage of weight loss induced by naloxone was calculated subtracting the weight of each mouse before the naloxone administration and after the observation period. We found ~1-1.5% body weight decrease, similar in both genotypes (Fig. 2A).

When the main withdrawal signs were evaluated, we found that naloxone-induced rearing frequency was slightly higher in the PTN-/- genotype compared with WT morphine dependent mice (Fig. 2B; P=0.11). When the total time that each mouse spent standing was compared, we found that PTN-/- mice spent a significantly higher percentage of time standing than WT mice (Fig. 2C; P=0.01). Most of the WT mice spent less than 5% of the time on their hind paws (which corresponds to the sum of the time of each standing, without remaining more time in that position), while only one PTN-/- mouse showed a similar low value. In fact, most of the PTN-/- mice remained ~10% - 60% of the time on their hind paws, what was considered as an indicative of the severity of the withdrawal induced by naloxone in PTN-/- morphine dependent mice (Fig. 2C). This fact can mask differences between the frequencies of rearings, so the consideration of both parameters is crucial to better understand the severity of the morphine-induced withdrawal in these mice.

Due to the individual variability of the naloxone-induced withdrawal syndrome, other withdrawal signs registered were summarized in the Withdrawal Severity Score (WSS) [34, 35], calculated with the incidences of forepaw licking, forepaw tremors, wet dog shakes and writhings during the 30 min of observation. The frequencies of these individual withdrawal symptoms are summarized in Table 1. We found that the WSS of PTN-/- mice was significantly increased compared with WT mice (Fig. 2D; P=0.04), what indicates, along with the increased time standing, that naloxone-precipitated

withdrawal is more severe in PTN-/- morphine-dependent mice and that the physical dependence induced by morphine is enhanced when endogenous PTN is lacking.

Discussion

In the present work, we have used the CPP paradigm to test the rewarding effects of morphine in PTN-/- and WT mice. In this protocol, mice are conditioned to associate the initially less-preferred compartment with the rewarding effects of the drug. The increase in the time of stay in the morphine paired compartment in the Test day compared with the Pre-C tended to be higher in PTN-/- mice compared with WT mice, however statistical differences were not achieved. It is important to note that PTN-/- mice have been previously shown to exert similar locomotor or exploratory activities to those in the WT [2], what is essential to understand these results. On the other hand, even though 10 mg/kg morphine (i. p.) has been frequently used to induce an effective CPP in mice, some other reports failed to obtain an efficient CPP with this dose (reviewed in [38]), leading us to select it to potentially dissect the differences between genotypes in the vulnerability to develop morphine-induced CPP. These apparent discrepancies between previous reports using the same dose of morphine to induce CPP seem to depend on the experimental procedure used and on the individual variability. For instance, morphine-induced CPP is more likely to be relevant after longer conditioning phases or using different paradigms, including the three chambers-CPP [1, 36], whereas individual differences in the aggressiveness of the mice seem to determine the capacity of morphine to induce CPP [39]. In our experimental procedure, PTN-/- mice showed a 19% increase in the time of stay in the morphine-paired compartment in the Test day compared with Pre-C whereas WT mice increased their time of stay ~7%. The data could point to an increased vulnerability of PTN-/- mice to morphine rewarding effects. However, since statistical differences were not achieved, further studies, particularly additional experimental paradigms, i.e. autoadministration, are needed to demonstrate a possible role of PTN in morphine reward.

With the aim to study if endogenous PTN is implicated in the physical dependence induced by opioids, withdrawal syndrome was precipitated by naloxone administration in PTN-/- and WT mice. Morphine withdrawal symptoms intensity depends on the dose of morphine used [37], so moderate effects were expected with the protocol used here. In fact, all the mice from both genotypes presented as main sign of withdrawal rearings instead of jumpings. Although jumpings frequency is the most widely used withdrawal sign, it is interesting to note that significant differences in the jumping frequency were found when naloxone-precipitated withdrawal was induced in different mouse strains. Specifically, C57BL/6J substrain showed lower jumping scores than other C57-derived substrains such as C57L/J or C57BR/cdJ [28]. The lack of jumpings, though uncommon, does not indicate the lack of withdrawal, clearly demonstrated by the presence of other unequivocal withdrawal symptoms after the naloxone administration, such as standings, fore paw tremors and licking, wet dog shakes, writhings, diarrhea, teeth chattering, ptosis, etc. Based on the inevitable variability of each withdrawal syndrome, global scores as the WSS used here are considered to be more useful for comparing the severity of the withdrawal [34, 35]. Our results show a significantly augmented WSS in PTN-/- mice, which correlates with the fact that most PTN-/- mice remain in standing position for minutes, an unnatural position for a rodent. These data demonstrate that the lack of endogenous PTN enhances the severity of the morphine withdrawal.

Although further studies are needed, the data presented here support that PTN may modulate some of the clinical adverse effects of morphine and, thus, suggest the modulation of the PTN signaling pathway to counterbalance morphine withdrawal. Pleiotrophin inactivates the Receptor Protein Tyrosine Phosphatase (RPTP)β/ζ [10, 27] through its binding to the receptor's D1 active domain. As a result of the blockade of

the phosphatase activity, PTN increases the tyrosine phosphorylation levels of the different substrates of RPTPβ/ζ, such as β-catenin [27], Fyn[31], β-adducin[32, 33], etc. Interestingly, morphine treatment has been shown to activate Wnt/β-catenin signaling in cell cultures [30], and more specifically, β-catenin is one of the factors that are induced by morphine withdrawal in the rat [7]. Based on the fact that PTN activates β-catenin via RPTPβ/ζ [27] and the implication of endogenous PTN in naloxone-induced morphine withdrawal demonstrated here, it is reasonable to propose that PTN may function during morphine withdrawal through the activation of β-catenin. On the other hand, several studies have proposed the relationship between the activation of ERK pathway in different brain areas implicated in drug addiction and naloxone precipitated withdrawal [5, 23]. Fyn kinase, one of the substrates of RPTPβ/ζ, activates ERK1/2 signaling pathway by increasing the phosphorylation levels of ERK1/2 [25]. Previous studies from our group showed that PTN protection against amphetamine-induced toxicity in vivo and in vitro is possibly mediated by the increased phosphorylation of Fyn and the consequent ERK activation [16, 17], and now we propose that this signaling pathway may be also mediating the involvement of PTN in naloxone induced morphine withdrawal. Anyway, studies directed to enlighten the possible contribution of each of these substrates to the PTN modulation of morphine withdrawal may contribute to the discovery of new drug targets in the future.

Conclusions

In summary, the data presented here demonstrate that PTN is a novel genetic factor that plays a role in the morphine withdrawal syndrome, and suggests the possibility of

pharmacological modulation of the PTN signaling pathway to presumably minimize some side effects associated to the clinical uses of morphine.

Acknowledgements

This work has been supported by grants SAF2009-08136 and CENIT CEN-20101023 from Ministerio de Ciencia e Innovación of Spain and by grant USP-BS-APP03/2014 from Universidad CEU San Pablo and Banco de Santander. EG and MV-R are supported by fellowships from Fundación Universitaria San Pablo CEU.

References

[1] M. Airavaara, H. Tuomainen, T.P. Piepponen, M. Saarma, L. Ahtee, Effects of repeated morphine on locomotion, place preference and dopamine in heterozygous glial cell line-derived neurotrophic factor knockout mice, Genes, brain, and behavior 6 (2007) 287-298.

[2] L.E. Amet, S.E. Lauri, A. Hienola, S.D. Croll, Y. Lu, J.M. Levorse, B. Prabhakaran, T. Taira, H. Rauvala, T.F. Vogt, Enhanced hippocampal long-term potentiation in mice lacking heparin-binding growth-associated molecule, Molecular and cellular neurosciences 17 (2001) 1014-1024.

[3] M. Basille-Dugay, M.M. Hamza, C. Tassery, B. Parent, E. Raoult, M. Benard, R. Raisman-Vozari, D. Vaudry, D.C. Burel, Spatio-temporal characterization of the pleiotrophinergic system in mouse cerebellum: evidence for its key role during ontogenesis, Experimental neurology 247 (2013) 537-551.

[4] K. Blum, J.D. Eubanks, B. Wiggins, J.E. Wallace, Morphine withdrawal reactions in male and female mice, The American journal of drug and alcohol abuse 3 (1976) 363-368.

[5] A. Ciccarelli, A. Calza, F. Santoru, F. Grasso, A. Concas, M. Sassoe-Pognetto, M. Giustetto, Morphine withdrawal produces ERK-dependent and ERK-independent epigenetic marks in neurons of the nucleus accumbens and lateral septum, Neuropharmacology 70 (2013) 168-179.

[6] T.F. Deuel, N. Zhang, H.J. Yeh, I. Silos-Santiago, Z.Y. Wang, Pleiotrophin: a cytokine with diverse functions and a novel signaling pathway, Archives of biochemistry and biophysics 397 (2002) 162-171.

[7] S.A. Dunbar, I. Karamian, L. Roberts, J. Zhang, Increased prostaglandin E2 release and activated Akt/beta-catenin signaling pathway occur after opioid withdrawal in rat spinal cord, Anesthesiology 105 (2006) 154-159.

[8] L. Ezquerra, C. Perez-Garcia, E. Garrido, C. Diez-Fernandez, T.F. Deuel, L.F. Alguacil, G. Herradon, Morphine and yohimbine regulate midkine gene expression in the rat hippocampus, European journal of pharmacology 557 (2007) 147-150.

[9] T. Flatscher-Bader, P.A. Wilce, Impact of alcohol abuse on protein expression of midkine and excitatory amino acid transporter 1 in the human prefrontal cortex, Alcoholism, clinical and experimental research 32 (2008) 1849-1858.

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

[11] D. Garcia-Perez, M. Luisa Laorden, C. Nunez, M. Victoria Milanes, Glial activation and midkine and pleiotrophin transcription in the ventral tegmental area are modulated by morphine administration, Journal of neuroimmunology 274 (2014) 244-248.

[12] C. Gonzalez-Castillo, D. Ortuno-Sahagun, C. Guzman-Brambila, M. Pallas, A.E. Rojas-Mayorquin, Pleiotrophin as a central nervous system neuromodulator, evidences from the hippocampus, Frontiers in cellular neuroscience 8 (2014) 443.

[13] E. Gramage, N. Del Olmo, A. Fole, Y.B. Martin, G. Herradon, Periadolescent amphetamine treatment causes transient cognitive disruptions and long-term changes in hippocampal LTP depending on the endogenous expression of pleiotrophin, Addiction biology 18 (2013) 19-29.

[14] E. Gramage, G. Herradon, Genetic deletion of pleiotrophin leads to disruption of spinal nociceptive transmission: evidence for pleiotrophin modulation of morphine-induced analgesia, European journal of pharmacology 647 (2010) 97-102.

[15] E. Gramage, Y.B. Martin, G. Herradon, The heparin binding growth factors midkine and pleiotrophin regulate the antinociceptive effects of morphine through alpha(2)-adrenergic independent mechanisms, Pharmacology, biochemistry, and behavior 101 (2012) 387-393.

[16] E. Gramage, A. Putelli, M.J. Polanco, C. Gonzalez-Martin, L. Ezquerra, L.F. Alguacil, P. Perez-Pinera, T.F. Deuel, G. Herradon, The neurotrophic factor pleiotrophin

modulates amphetamine-seeking behaviour and amphetamine-induced neurotoxic effects: evidence from pleiotrophin knockout mice, Addiction biology 15 (2010) 403-412.
[17] E. Gramage, L. Rossi, N. Granado, R. Moratalla, G. Herradon, Genetic inactivation of pleiotrophin triggers amphetamine-induced cell loss in the substantia nigra and enhances amphetamine neurotoxicity in the striatum, Neuroscience 170 (2010) 308-316.
[18] G. Herradon, C. Perez-Garcia, Targeting midkine and pleiotrophin signalling pathways in addiction and neurodegenerative disorders: recent progress and perspectives, British journal of pharmacology 171 (2014) 837-848.
[19] K. Kadomatsu, M. Tomomura, T. Muramatsu, 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, Biochemical and biophysical research communications 151 (1988) 1312-1318.
[20] Y.B. Keleta, J.L. Martinez, Brain Circuits of Methamphetamine Place Reinforcement Learning: The Role of the Hippocampus-VTA Loop, Brain and behavior 2 (2012) 128-141.
[21] B. Kest, C.A. Palmese, E. Hopkins, M. Adler, A. Juni, Assessment of acute and chronic morphine dependence in male and female mice, Pharmacology, biochemistry, and behavior 70 (2001) 149-156.
[22] P. Le Greves, Pleiotrophin gene transcription in the rat nucleus accumbens is stimulated by an acute dose of amphetamine, Brain research bulletin 65 (2005) 529-532.
[23] T. Li, Y. Hou, W. Cao, C.X. Yan, T. Chen, S.B. Li, Naloxone-precipitated withdrawal enhances ERK phosphorylation in prefrontal association cortex and accumbens nucleus of morphine-dependent mice, Neuroscience letters 468 (2010) 348-352.
[24] Y.S. Li, P.G. Milner, A.K. Chauhan, M.A. Watson, R.M. Hoffman, C.M. Kodner, J. Milbrandt, T.F. Deuel, Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity, Science 250 (1990) 1690-1694.
[25] M. Lovatt, A. Filby, V. Parravicini, G. Werlen, E. Palmer, R. Zamoyska, Lck regulates the threshold of activation in primary T cells, while both Lck and Fyn contribute to the magnitude of the extracellular signal-related kinase response, Molecular and cellular biology 26 (2006) 8655-8665.
[26] Y.B. Martin, E. Gramage, G. Herradon, Maintenance of amphetamine-induced place preference does not correlate with astrocytosis, European journal of pharmacology 699 (2013) 258-263.
[27] K. Meng, A. Rodriguez-Pena, T. Dimitrov, W. Chen, M. Yamin, M. Noda, T.F. Deuel, 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, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 2603-2608.
[28] P. Metten, J.C. Crabbe, J.K. Belknap, Genetic correlates of morphine withdrawal in 14 inbred mouse strains, Drug and alcohol dependence 99 (2009) 123-131.
[29] P.G. Milner, Y.S. Li, R.M. Hoffman, C.M. Kodner, N.R. Siegel, T.F. Deuel, A novel 17 kD heparin-binding growth factor (HBGF-8) in bovine uterus: purification and N-terminal amino acid sequence, Biochemical and biophysical research communications 165 (1989) 1096-1103.
[30] D.G. Niu, F. Peng, W. Zhang, Z. Guan, H.D. Zhao, J.L. Li, K.L. Wang, T.T. Li, Y. Zhang, F.M. Zheng, F. Xu, Q.N. Han, P. Gao, Q.P. Wen, Q. Liu, Morphine promotes cancer stem cell properties, contributing to chemoresistance in breast cancer, Oncotarget 6 (2015) 3963-3976.
[31] H. Pariser, L. Ezquerra, G. Herradon, P. Perez-Pinera, T.F. Deuel, Fyn is a downstream target of the pleiotrophin/receptor protein tyrosine phosphatase beta/zeta-signaling pathway: regulation of tyrosine phosphorylation of Fyn by pleiotrophin, Biochemical and biophysical research communications 332 (2005) 664-669.
[32] H. Pariser, G. Herradon, L. Ezquerra, P. Perez-Pinera, T.F. Deuel, Pleiotrophin regulates serine phosphorylation and the cellular distribution of beta-adducin through

activation of protein kinase C, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 12407-12412.
[33] H. Pariser, P. Perez-Pinera, L. Ezquerra, G. Herradon, T.F. Deuel, Pleiotrophin stimulates tyrosine phosphorylation of beta-adducin through inactivation of the transmembrane receptor protein tyrosine phosphatase beta/zeta, Biochemical and biophysical research communications 335 (2005) 232-239.
[34] A.K. Rehni, T.G. Singh, Pharmacological modulation of geranylgeranyltransferase and farnesyltransferase attenuates opioid withdrawal in vivo and in vitro, Neuropharmacology 71 (2013) 19-26.
[35] P. Singh, B. Sharma, S. Gupta, B.M. Sharma, In vivo and in vitro attenuation of naloxone-precipitated experimental opioid withdrawal syndrome by insulin and selective KATP channel modulator, Psychopharmacology 232 (2015) 465-475.
[36] W. Solecki, A. Turek, J. Kubik, R. Przewlocki, Motivational effects of opiates in conditioned place preference and aversion paradigm--a study in three inbred strains of mice, Psychopharmacology 207 (2009) 245-255.
[37] T. Suzuki, M. Shimada, T. Yoshii, J. Uesugi, S. Yanaura, Development of physical dependence on and tolerance to morphine in rats treated with morphine-admixed food, Progress in neuro-psychopharmacology & biological psychiatry 7 (1983) 63-71.
[38] T.M. Tzschentke, Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade, Addiction biology 12 (2007) 227-462.
[39] O.Y. Vekovischeva, S.G. Semenova, E.V. Verbitskaya, E.E. Zvartau, Effects of morphine and cocaine in mice with stable high aggressive and nonaggressive behavioral strategy, Pharmacology, biochemistry, and behavior 77 (2004) 235-243.
[40] M. Vicente-Rodriguez, C. Perez-Garcia, M. Ferrer-Alcon, M. Uribarri, M.G. Sanchez-Alonso, M.P. Ramos, G. Herradon, Pleiotrophin differentially regulates the rewarding and sedative effects of ethanol, Journal of neurochemistry 131 (2014) 688-695.
[41] A. Wanaka, S.L. Carroll, J. Milbrandt, Developmentally regulated expression of pleiotrophin, a novel heparin binding growth factor, in the nervous system of the rat, Brain research. Developmental brain research 72 (1993) 133-144.

Table legend

Table 1. Incidences of naloxone-induced morphine withdrawal symptoms in PTN-/- and WT mice. Results are presented as the mean ± S.E.M. of the individual frequencies of each symptom included in the Withdrawal Severity Score (WSS).

Figure legends

Figure 1. Morphine Conditioned Place Preference in PTN-/- mice. Results are presented as the mean ± S.E.M. of the percentage of the time spent by WT and PTN-/- mice in the morphine (10 mg/kg)-paired, least-preferred compartment during preconditioning (PreC, day 1) and testing phases (Test, day 5) of the CPP procedure.

Figure 2. Naloxone induces more severe acute morphine withdrawal syndrome in PTN-/- mice. Withdrawal syndrome was induced in morphine dependent PTN-/- and WT mice by naloxone (8 mg/kg) injection, and immediately, each mouse was observed during 30 min. The mean ± S.E.M. of the percentage of weight loss induced by naloxone (A), the rearing frequency, expressed as mean of number of standings observed in 30 minutes ± S.E.M (B) and the mean ± S.E.M. of the percentage of the time that mice spent on their hind paws (C) are represented for both genotypes. Other signs of withdrawal (forepaw licking, forepaw tremors, wet dog shake and writhing) were also recorded, and the mean ± S.E.M. of the addition of their incidences is summarized in the Withdrawal Severity Score (WSS) (D). * $P<0.05$ vs. WT.