Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 557 (2007) 147–150
www.elsevier.com/locate/ejphar

Short communication

Morphine and yohimbine regulate midkine gene expression in the rat hippocampus

Laura Ezquerra [a], Carmen Pérez-García [b], Elisa Garrido [b], Carmen Díez-Fernández [b], Thomas F. Deuel [a], Luis F. Alguacil [b], Gonzalo Herradón [a,b,*]

[a] *Department of Molecular and Experimental Medicine and Cell Biology, The Scripps Research Institute, La Jolla CA, USA*
[b] *Laboratory Pharmacology and Toxicology, Universidad San Pablo CEU, Urb. Montepríncipe, 28668 Boadilla del Monte, Madrid, Spain*

Received 2 October 2006; received in revised form 7 November 2006; accepted 8 November 2006
Available online 14 November 2006

Abstract

Pleiotrophin and midkine are two recently discovered growth factors that promote survival and differentiation of catecholaminergic neurons. Chronic opioid stimulation has been reported to induce marked alterations of the locus coeruleus–hippocampus noradrenergic pathway, an effect that is prevented when opioids are coadministered with the α_2-adrenoceptor antagonist yohimbine. The present work tries to examine a possible link between yohimbine reversal of morphine effects and pleiotrophin/midkine activation in the rat hippocampus by studying the levels of expression of pleiotrophin and midkine in response to acute and chronic administration of morphine, yohimbine and combinations of both drugs. Pleiotrophin gene expression was not altered by any treatment; however midkine mRNA levels were increased after chronic treatment with morphine. Chronic administration of yohimbine alone also increased midkine expression levels, whereas yohimbine and morphine administered together exhibited summatory effects on the upregulation of midkine expression levels. The data suggest that midkine could play a role in the prevention of opioid-induced neuroadaptations in hippocampus by yohimbine.

Keywords: Pleiotrophin; Midkine; Hippocampus; Opioid; Yohimbine

1. Introduction

Pleiotrophin, initially cited as heparin-binding growth factor-8 (HBGF-8) (Milner et al., 1989), and heparin-binding growth-associated molecule (HB-GAM) (Rauvala, 1989) is a secreted, highly conserved 136-amino acid cytokine (Milner et al., 1989; Li et al., 1990) that shares over 50% identity in amino acid sequence with midkine, the only other member of the pleiotrophin/midkine developmentally regulated gene family (Kadomatsu et al., 1988; Milner et al., 1989).

The pleiotrophin and midkine genes are widely expressed at different times in different cell types during development (see review by Deuel et al., 2002). During development, in the central nervous system, expression of pleiotrophin and midkine is found in discrete loci that correspond at the same time to peaks of growth and early differentiation of neurons and glia (Deuel et al., 2002; Kadomatsu and Muramatsu, 2004) suggesting that pleiotrophin may function in vivo in the differentiation functions of these cells. However, expression of both pleiotrophin and midkine genes is constitutive and limited to only a few cell types in adults (Kadomatsu et al., 1988; Li et al., 1990; Silos-Santiago et al., 1996). Both genes are upregulated at sites of injury and repair in inflammatory macrophages, microglia, dermal fibroblasts, endothelial cells and other cells (Yeh et al., 1998; Sakakima et al., 2004; Kikuchi-Horie et al., 2004), suggesting pleiotrophin and midkine signalling may be critical in different steps of differentiation of different cells both in development and in wound repair.

Pleiotrophin exhibits a trophic effect on survival of dopaminergic neurons in vitro (Hida et al., 2003) and induces the differentiation of dopaminergic neurons from embryonic stem

* Corresponding author. Laboratory Pharmacology and Toxicology, Universidad San Pablo CEU, Urb. Montepríncipe, 28668 Boadilla del Monte, Madrid, Spain. Tel.: +34 91 3724700; fax: +34 91 3510475.
E-mail address: herradon@ceu.es (G. Herradón).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.11.024

Table 1
Pleiotrophin gene expression in the hippocampus of rats treated with acute or chronic morphine (M) or saline (S)

Pleiotrophin mRNA levels in hippocampus		
Treatment	Acute	Chronic
S/S	145.4±31.7	135.7±29.5
S/M	117.3±24.3	133.9±22.8
Y/S	127.4±23.9	118.2±20.8
Y/M	141.4±31.7	130.5±32.1

The animals also received before each saline or morphine injection a pre-treatment with yohimbine (groups Y/M and Y/S) or saline (groups S/M, S/S). Results are expressed as mean±S.E.M.

cell-derived nestin-positive cells (Jung et al., 2004). Midkine is known to promote survival of mesencephalic neurons and to induce tyrosine hydroxylase-positive neurons (Kikuchi et al., 1993). Pleiotrophin is upregulated in striatum of patients with Parkinson's disease (Marchionini et al., 2004) suggesting a trophic effect of pleiotrophin in dopaminergic neurons in this pathological condition. The possible roles of pleiotrophin and/or midkine in other pathologies compromising catecholaminergic neurons (e.g. drug addiction) remains to be studied. Pleiotrophin expression levels are upregulated in nucleus accumbens after an acute administration of amphetamine (Le Greves, 2005). To the best of our knowledge, this is the only study relating the pleiotrophin/midkine developmentally regulated gene family and drug abuse. We have now studied the effects of morphine on the expression of pleiotrophin and midkine in the hippocampus, a brain area that exhibits marked changes in α_2-adrenoceptor expression after chronic morphine exposure (Alonso et al., in press). In that study it was also shown that yohimbine, an α_2-adrenoceptor antagonist known to prevent the development of behavioural dependence and neuronal plasticity induced by opioids in rodents and humans (Iglesias et al., 1998; Morales et al., 2001; Hameedi et al., 1997; Garrido et al., 2005), also prevents morphine-induced changes in α_2-adrenoceptor expression in hippocampus (Alonso et al., in press). Thus, we have also studied the effects of yohimbine alone and in combination with morphine, on pleiotrophin and midkine gene expression in this brain area.

2. Materials and methods

Male Sprague–Dawley rats (San Pablo CEU University breeding) weighing 300–350 g were used. The animals were housed under controlled environmental conditions (22 °C and a 12-h light/dark cycle) with free access to food and water. All experiments were carried out in accordance with the NIH guidelines for care and use of laboratory animals. The rats were i.p. treated with acute (50 mg/kg) morphine (Alcaliber, Madrid, Spain), chronic morphine or equivalent injections of saline. Chronic morphine treatment consisted in two daily injections according to the following schedule: days 1 and 2: 2×10 mg/kg; days 3 and 4: 2×20 mg/kg; days 5 and 6: 2×30 mg/kg; days 7 and 8: 2×40 mg/kg; days 9 and 10: 2×50 mg/kg; day 11, 1×50 mg/kg. Thirty minutes prior to each of these injections, the rats were pre-treated either with yohimbine (2 mg/kg; Sigma, Spain) or saline (n=3/experimental group). Animals were sacrificed 2 h after the last injection, the hippocampus dissected and frozen until the analysis of gene expression followed.

Frozen tissues were homogenized in 1 ml TRIZOL reagent (Invitrogen, Carlsbad, CA) per 50–100 mg tissue and total RNA extracted following the manufacturer's protocol. The concentration of RNA in each sample was measured by A_{260} and RNA integrity confirmed in 1.25% agarose gels after electrophoresis. RNA samples were treated with a preparation of DNAses (Ambion, Austin, TX) following manufacturer's protocol.

Complementary DNAs were synthesized from total RNA using a cDNA synthesis kit (BIO-RAD, Hercules, CA, USA). The SYBR green RT-PCR method (BIO-RAD, Hercules, CA, USA) was used, as previously described (Ezquerra et al., 2004), to determine the relative expression of pleiotrophin and midkine mRNAs in the different tissues. The following primer sets (forward and reverse) were used: pleiotrophin: 5′-GAAAATTTGCAGCTGCCTTC-3′; 5′-CACACACTCCATTGCCATTC-3′; midkine: 5′-CCACCAGTGCCTTTTGCTTT-3′; 5′-TCACTTCCCAGAATCCCTTG-3′.

The relative expression of each gene was normalized against GAPDH (5′-TTCAACGGCACAGTCAAGGC-3′; 5′-CACCAGCATCACCCCATTTG-3′), the reference standard, as described by the manufacturer's user bulletin # 2 of ABI prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA).

Statistical differences were determined using ANOVA followed by Newman Keuls post-hoc test. Comparisons with $P<0.05$ were considered significant.

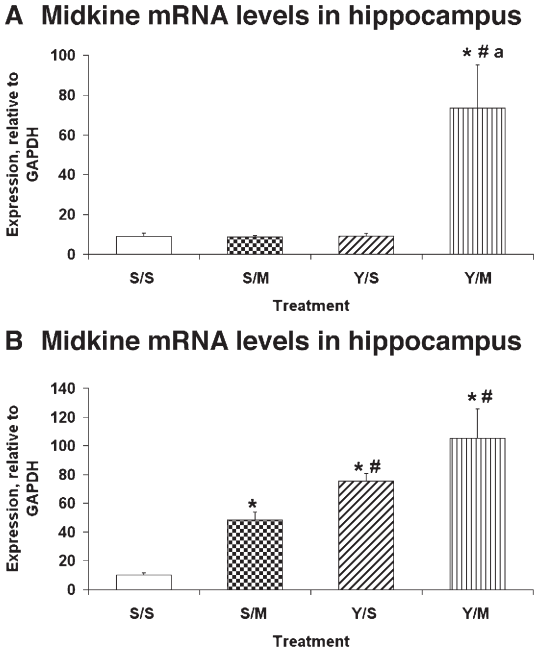

Fig. 1. Midkine gene expression in the hippocampus of rats treated with acute (panel A) or chronic (panel B) morphine (M) or saline (S). The animals also received before each saline or morphine injection a pre-treatment with yohimbine (groups Y/M and Y/S) or saline (groups S/M, S/S). Results are expressed as mean±S.E.M. *$P<0.05$ *vs.* S/S; $^{\#}P<0.05$ *vs.* S/M; $^{a}P<0.05$ *vs.* Y/S.

3. Results

To further study the role of the pleiotrophin/midkine developmentally regulated gene family in drug abuse we have determined the levels of expression of pleiotrophin and midkine in rat hippocampus in response to acute and chronic morphine treatments alone and in combination with yohimbine. We found that pleiotrophin gene expression was not altered after acute or chronic administration of morphine alone or in combination with yohimbine (Table 1). Acute doses of morphine or yohimbine alone did not show any effect on midkine expression levels; however, when acute doses of morphine and yohimbine were administered together a significant increase in the levels of midkine was observed (Fig. 1A). In contrast, midkine mRNA levels were increased after chronic administration of morphine (Fig. 1B). Chronic administration of yohimbine alone also increased midkine expression levels, whereas yohimbine and morphine administered together showed the highest upregulation of midkine mRNA levels (Fig. 1B).

4. Discussion

Opioid drugs produce neuroadaptations altering several neurotransmitter systems in the brain which lead to tolerance, dependence and addiction. This is the case of the noradrenergic system, which has been shown to be deeply involved in the pharmacological effects of opioid drugs (see review by Alguacil and Morales, 2004). Thus, identification of factors involved in survival and differentiation of catecholaminergic neurons and regulated by opioid administration is of critical importance. In this work, we have studied the levels of pleiotrophin and midkine, known to promote the survival and differentiation of tyrosine hydroxylase-positive neurons (Kikuchi et al., 1993; Hida et al., 2003; Jung et al., 2004), after acute and chronic administration of morphine. Morphine administration did not show any effect on pleiotrophin expression levels, thus suggesting that pleiotrophin upregulation after treatment with amphetamine previously reported (Le Greves, 2005) could be considered a drug specific effect. In contrast, we found a significant increase in the levels of midkine after chronic (but not acute) administration of morphine. Pleiotrophin and midkine overlap many functions and consistently show redundant effects (Deuel et al., 2002; Herradon et al., 2005). Thus, identification of differences concerning the regulation of the expression of pleiotrophin and midkine is important since it may help characterize functional differences between the only two members of this family of cytokines.

Midkine is known to promote survival of mesencephalic neurons and to induce tyrosine hydroxylase-positive neurons (Kikuchi et al., 1993). Importantly, pleiotrophin and midkine genetically deficient mice exhibit deficits of tyrosine hydroxylase mRNA in the brain (Ezquerra et al., 2005). Recently, it was shown that chronic administration of morphine induces significant changes in α_2-adrenoceptor expression in the hippocampus (Alonso et al., in press). Upregulation of midkine levels in hippocampus after chronic administration of morphine could be opposing opioid-induced neuroadaptations since midkine is known to be upregulated at sites of injury in the central nervous system (Sakakima et al., 2004; Kikuchi-Horie et al., 2004) and to prevent hippocampal neuronal death following forebrain ischemia (Yoshida et al., 2001). Interestingly, we have found that yohimbine increased midkine levels in the hippocampus at the same dose that prevents morphine-induced changes of α_2-adrenoceptor gene expression in this brain area (Alonso et al., in press), thus suggesting that midkine may be involved in the prevention of opioid-induced neuroadaptations by yohimbine. This idea is in agreement with the known positive effect of midkine on survival and differentiation of catecholaminergic neurons (Kikuchi et al., 1993) and, more importantly, with the finding that both pleiotrophin and midkine play significant roles in injury-induced and activity-dependent plasticity in rat hippocampus (Rauvala and Peng, 1997).

In summary, the data demonstrate a differential regulation of pleiotrophin and midkine expression levels by morphine and yohimbine. Further studies are needed to demonstrate the possible involvement of midkine in the positive effects of yohimbine on opioid-induced neuroadaptations.

Acknowledgements

This study was supported by grants from Laboratorios ESTEVE and USP (11/03).

References

Alguacil, L.F., Morales, L., 2004. Alpha-2 adrenoceptor ligands and opioid drugs: pharmacological interactions of therapeutic interest. Curr. Neuropharmacol. 2, 343–352.

Alonso, E., Garrido, E., Díez-Fernández, C., Pérez-García, C., Herradón, G., Ezquerra, L., Deuel, T.F., Alguacil, L.F., in press. Yohimbine prevents morphine-induced changes of glial fibrillary acidic protein in brainstem and α_2-adrenoceptor gene expression in hippocampus. Neurosci. Letters, doi: 10.1016/j.neulet.2006.11.002.

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, 162–171.

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, 512–517.

Ezquerra, L., Herradon, G., Nguyen, T., Silos-Santiago, I., Deuel, T.F., 2005. Pleiotrophin regulates tyrosine hydroxylase gene expression in mouse nervous system. Program No. 1006.13. 2005 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience. Online.

Garrido, E., Pérez-García, C., Alguacil, L.F., Díez-Fernández, C., 2005. The alpha-2 adrenoceptor antagonist yohimbine reduces glial fibrillary acidic protein upregulation induced by chronic morphine administration. Neurosci. Lett. 383, 141–144.

Hameedi, F.A., Woods, S.W., Rosen, M.I., Pearsall, H.R., Kosten, T.R., 1997. Dose dependent effects of yohimbine on methadone maintained patients. Am. J. Drug Alcohol Abuse 23, 327–333.

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.

Iglesias, V., Alamo, C., Cuenca, E., Morales, L., Pérez-García, C., Alguacil, L.F., 1998. Effect of oral yohimbine on withdrawal jumping behaviour of morphine-dependent mice. Addict. Biol. 3, 459–463.

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., Muramatsu, T., 2004. Midkine and pleiotrophin in neural development and cancer. Cancer Lett. 204, 127–143.

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.

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, 678–687.

Kikuchi, S., Muramatsu, H., Muramatsu, T., Kim, S.U., 1993. Midkine, a novel neurotrophic factor, promotes survival of mesencephalic neurons in culture. Neurosci. Lett. 160, 9–12.

Le Greves, P., 2005. Pleiotrophin gene transcription in the rat nucleus accumbens is stimulated by an acute dose of amphetamine. Brain Res. Bull. 65, 529–532.

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, 1690–1694.

Marchionini, D.M., Chu, Y., Kordower, J.H., Collier, T.J., 2004. Up-regulation of pleiotrophin expression in the substantia nigra in Parkinson's Disease. Program No. 1014.14. 2004 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience. Online.

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, 1096–1103.

Morales, L., Perez-Garcia, C., Alguacil, L.F., 2001. Effects of yohimbine on the antinociceptive and place conditioning effects of opioid agonists in rodents. Br. J. Pharmacol. 133, 172–178.

Rauvala, H., 1989. An 18-kD heparin-binding protein of developing brain that is distinct from fibroblast growth factors. EMBO J. 8, 2933–2941.

Rauvala, H., Peng, H.B., 1997. HB-GAM (heparin-binding growth-associated molecule) and heparin-type glycans in the development and plasticity of neuron-target contacts. Prog. Neurobiol. 52, 127–144 (Review).

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, 251–260.

Silos-Santiago, I., Yeh, H.J., Gurrieri, M.A., Guillerman, R.P., Li, Y.S., Wolf, J., Snider, W., Deuel, T.F., 1996. Localization of pleiotrophin and its mRNA in subpopulations of neurons and their corresponding axonal tracts suggests important roles in neural–glial interactions during development and in maturity. J. Neurobiol. 31, 283–296.

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, 3699–3707.

Yoshida, Y., Ikematsu, S., Moritoyo, T., Goto, M., Tsutsui, J., Sakuma, S., Osame, M., Muramatsu, T., 2001. Intraventricular administration of the neurotrophic factor midkine ameliorates hippocampal delayed neuronal death following transient forebrain ischemia in gerbils. Brain Res. 894, 46–55.