

Short communication

Morphine and yohimbine regulate midkine gene expression in the rat hippocampus

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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.

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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 up-regulated 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

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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.

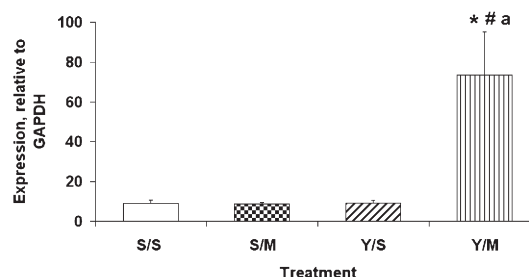
Frozen tissues were homogenized in 1 ml TRIZOL reagent (Invitrogen, Carlsbad, CA) per 50–100 mg tissue and total RNA extracted following the manufacturer's protocol. The concentration of RNA in each sample was measured by A_{260} and RNA integrity confirmed in 1.25% agarose gels after electrophoresis. 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'-TCAC-TTCCCAGAATCCCTTG-3'.

The relative expression of each gene was normalized against GAPDH (5'-TTCAACGGCACAGTCAAGGC-3'; 5'-CAC-CAGCATCACCCCATTTG-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.

A Midkine mRNA levels in hippocampus



B Midkine mRNA levels in hippocampus

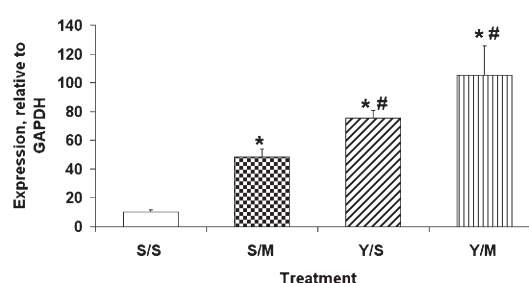


Fig. 1. Midkine gene expression in the hippocampus of rats treated with acute (panel A) or chronic (panel B) morphine (M) or saline (S). The animals also received before each saline or morphine injection a pre-treatment with yohimbine (groups Y/M and Y/S) or saline (groups S/M, S/S). Results are expressed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

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