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Interferon-γ down-regulates transcription of the μ-opioid receptor gene in neuronal and immune cells

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Abstract

Earlier investigations demonstrated up-regulated μ-opioid receptor expression in neuronal and immune cells in response to IL-1, IL-4, IL-6 and TNF-α. We herein report that μ-opioid receptor expression is down-regulated in SH SY5Y neuroblastoma cells by IFN-γ, and that IL-4-mediated induction of μ-opioid receptor expression is inhibited in Jurkat T cells by IFN-γ. Additionally, μ-opioid receptor transcripts were found in IL-4-expressing human primary T helper cells type 2, but not in type 1 cells, which typically express IFN-γ. This indicates that μ-opioid receptor expression may be altered under conditions like inflammation, viral infections or neurological diseases associated with imbalanced cytokine expression.

Keywords: Opioid receptors; MOPr; Gene regulation; Cytokines; T helper cells

1. Introduction

Opioids act at the interface between the nervous and the immune system. On the one hand, opioid drugs like morphine are the most potent clinically used analgesics. Opioid peptides like β-endorphin also act antinociceptive inhibiting neuronal transmission of pain, for example in inflammation (Stein et al., 2001, 2003). On the other hand, opioids markedly modulate immune cell functions. It is well known, for example, that drug addicts using morphine-related drugs have a higher risk for infectious diseases. Other immunomodulatory effects of opioids include lymphoid organ atrophy, reduced natural killer cell and macrophage activity, and reduced secretion of several cytokines (Gaveriaux-Ruff et al., 1998; Roy et al., 1998). Effects of opioids are mediated by three different receptors, termed μ, δ and κ (Kieffer, 1995; Pol and Puig, 2004). From the pharmacological point of view, μ-opioid receptors are especially important due to their high affinity to morphine and related drugs. μ-Opioid receptors are expressed constitutively and in considerable levels in defined neurons (Mansour et al., 1995). In contrast, expression of the receptors in immune cells is normally repressed, but inducible by various stimuli (Kraus et al., 2001, 2003). From our current knowledge, the most powerful substances, which up-regulate μ-opioid receptor expression in neuronal cells and induce expression of the gene in immunocytes, are cytokines. Thus far, up-regulation of the μ-opioid receptor gene has been observed after stimulation with IL-1 (Vidal et al., 1998), IL-4 (Kraus et al., 2001), IL-6 (Borner et al., 2004) and TNF-α (Kraus et al., 2003). For example, μ-opioid receptor expression is up-regulated by IL-4 in embryonic neurons from rats and induced de novo in various immune effector cells from humans including B and T lymphocytes (Kraus et al., 2001). In addition to regulation by cytokines themselves, μ-opioid receptor expression can be regulated indirectly by substances, which induce cytokine release. For example, peripheral cannabinoids induce μ-opioid receptor expression in Jurkat T cells via secretion of IL-4 (Borner et al., 2006). IL-4 is the prototypical cytokine released by T helper (Th) 2 cells. Many physiological effects of IL-4 are regulated in an antagonistic

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manner by IFN-γ, the prototypical cytokine released by Th1 cells. For example, during Th cell differentiation IFN-γ and IL-4 promote the direction of differentiation towards Th1 and Th2 cells, respectively, and simultaneously inhibit mechanisms underlying differentiation of the opposite direction (O'Garra and Arai, 2000).

In this report, we investigated whether IL-4 and IFN-γ antagonistically regulate transcription of the μ-opioid receptor gene. We demonstrated that IFN-γ down-regulates μ-opioid receptor transcription in neuronal cells and inhibits IL-4-induced transcription of the gene in T cells. Furthermore, μ-opioid receptor transcripts were detected in primary human T cell models for Th2 cells, but not in Th1 cells. In conclusion, these results provide a first example for a cytokine with a strong inhibitory effect on μ-opioid receptor gene expression, which may have consequences for opioid effects mediated by these receptors.

2. Materials and methods

2.1. Cell culture and reagents

SH SY5Y cells were cultivated in DMEM (Cambrex Bio Science, Verviers, Belgium) supplemented with 15% fetal calf serum (Biochrom, Berlin, Germany) and antibiotics (100 units/ml penicillin and 100 mg/ml streptomycin, Cambrex Bio Science). Jurkat (E6.1) cells were cultivated in RPMI-1640 medium (Cambrex Bio Science) supplemented with 10% fetal calf serum (Biochrom) and antibiotics (see above). IL-4, IFN-γ and TNF-α were purchased from R&D Systems (Wiesbaden, Germany). Schemes for stimulation of cells are given for the individual experiments in the legends to the figures.

2.2. Preparation and stimulation of Th1 and Th2 cells

Human mononuclear cells were harvested from peripheral blood of healthy human donors and cultured at a density of 2×10^6 cells/ml in AIM-V medium (Gibco BRL, Eggenstein, Germany) supplemented with 10% (v/v) fetal calf serum (Gibco) at 37 °C over a total period of 9 days. The leukocytes were stimulated by the staphylococcal enterotoxin A (50 ng/ml, Sigma, Deisenhofen, Germany) in the presence of either 0.2 ng/ml IL-4, or 1 μg/ml anti-IL-4 antibody (R&D Systems). At days 3 and 5 after stimulation, cells were exposed to 5 ng/ml IL-2 (R&D Systems) and again to either IL-4 or anti IL-4 antibody. The obtained cell populations were characterized for the presence of IL-18-receptor mRNA and protein (Breit et al., 1996; Maggi et al., 1992). Quantitative RT-PCR was performed in the iCycler (Bio-Rad, Munich, Germany). A typical 25-μl reaction mixture contained 12.5 μl HotStarTaq Master Mix (Qiagen), 0.3 μl of a 1:1000 dilution of SYBR Green I (Molecular Probes, Eugene, Oregon), and 0.5 μmol of the specific primers (5′-CGTGTTCACTTGCACATGAG-3′ and 5′-ATGCACG CAGGAGTAATACC-3′). An initial denaturation/activation step (15 min 95 °C) was followed by 40 cycles (30 s, 95 °C; 30 s, 58 °C; 45 s, 72 °C). The amounts of mRNA were normalized to that of GAPDH. Cytofluorimetric analyses were performed with an Epics XL-MCL (Coulter, Germany) using monoclonal antibody to IL-18 receptor alpha chain, clone H44 (Acris Antibodies, Hiddenhausen, Germany). Goat anti-mouse IgG (Fab specific fragment-TRITC conjugate; Sigma) was used for secondary staining. Cytofluorimetric analyses revealed the presence of 48.7±7.1% IL-18Rα positive cells in the Th1 population, whereas in the Th2-enriched population only 24.8±6.8% of the cells expressed the IL-18Rα ($n=12$, $p<0.05$). Similarly, the amounts of IL-18α-mRNA in cells enriched for Th1 were more than 2-fold higher than those in Th2-enriched cells (100±27.7% vs. 46.7±21.0%, $n=12$, $p<0.05$).

2.3. RNA isolation, cDNA synthesis and PCR

Total RNA from 2×10^7 primary human cells was prepared using the RNeasy Mini kit (Qiagen, Hilden, Germany). Contaminating DNA was removed by two rounds of DNase I digestion (RNase-free, Roche, Mannheim, Germany; 20 U/50 μl reaction, 30 min at 37 °C). One microgram of total RNA was converted into first strand cDNA using AMV reverse transcriptase (Promega) and random hexanucleotides (Boehringer Mannheim). RNA from SH SY5Y and Jurkat cells was isolated using the NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany). Complementary DNA synthesis and PCR strategies have been described in detail earlier (Borner et al., 2006; Kraus et al., 2001, 2003). Briefly, quantitative real-time PCR was performed in a total volume of 20 μl on a LightCycler instrument using the "LightCycler-Fast Start DNA Master SYBR Green I" kit (both from Roche, Mannheim, Germany). For GAPDH, a 163 bp fragment was amplified (forward primer 5′-CAACTACATGGTTTACATGTTC-3′, reverse primer 5′-GCCAGTGGACTCCACGAC-3′). μ-Opioid receptor transcripts (342 bp) were amplified as follows: (I) preincubation for 8 min at 95 °C, (II) 50 cycles of 5 s at 95 °C, 5 s at 65 °C and 18 s at 72 °C. The sequences of μ-opioid receptor primers were: 5′-GATCATGGCCCTCTACTCCA-3′ (forward primer) and 5′-GCATTTCGGGGAGTACG GAA-3′ (reverse primer). Conventional PCR was performed as follows: to check the integrity of the cDNA a 564 bp fragment of the human β-actin gene was amplified (forward primer 5′-GGTCCACACCCGCCACCAG-3′ and reverse primer 5′-CAGGTCCAGACGCAGGATGG-3′). Two consecutive reactions (nested PCR) were performed to amplify μ-opioid receptor transcripts: a first PCR reaction obtaining a 853 bp amplificate with the forward primer 5′-CTTGGCGTA CTCAAGTTGCT-3′ and the reverse primer 5′-AATGT GAATGGGAGTCCAGC-3′ using 25 cycles of 40 s at 94 °C, 60 s at 63 °C and 90 s at 72 °C with an initial denaturation step (150 s at 94 °C) and a final prolongation step (120 s at 72 °C). Five microliters of the first reaction were used for a second PCR reaction with the primers reported