

## Regulation of mu-opioid receptors by cytokines

Jurgen Kraus

Department of Pharmacology and Toxicology, Magdeburg University, 44 Leipzigerstrasse, 39120, Magdeburg, Germany

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Regulation of mu-opioid receptors by cytokines
  - 3.1. Interleukin-1
  - 3.2. Interleukin-4
  - 3.3. Interleukin-6
  - 3.4. Tumor necrosis factor
  - 3.5. Interferon-gamma
  - 3.6. Granulocyte/macrophage colony-stimulating factor
4. Perspective
5. Acknowledgements
6. References

## 1. ABSTRACT

Opioids are the most potent analgesics. However, their clinical use is limited by side effects like respiratory depression and their high potential for abuse. In addition, they modulate immune functions and cause immunosuppression. Effects of clinically important opioids like morphine are mediated by the mu-opioid receptor. Knowledge about the mechanisms controlling the expression of the mu-opioid receptor gene in neuronal and immune cells is crucial to understand the dynamics and the activity of this receptor. Cytokines, mediators typically released from cells of the immune system, are potent regulators of mu-opioid receptor gene expression. This emphasizes the importance of mu-opioid receptors in the neuro-immune-crosstalk and their role as a molecular basis for such interactions. In this review, the up-regulation of human mu-opioid receptor gene expression in neuronal and immune effector cells by interleukin-1, interleukin-4, interleukin-6 and tumor necrosis factor, as well as its down-regulation by interferon-gamma and granulocyte/macrophage colony-stimulating factor will be summarized along with molecular mechanisms, such as transcription factor-promoter-interactions. In addition, the physiological importance of these regulatory events will be discussed.

## 2. INTRODUCTION

The most prominent effects of opioids are those targeting neuronal systems. Opioids are the most powerful analgesics and are associated with euphoria (1-2). However, their clinical use is limited by side effects like respiratory depression and their high potential for abuse. In addition, opioids potentially modulate immune functions and often act in an immunosuppressive way at least *in vitro* and in animal experiments. (3-4). The effects of opioids ultimately depend on the expression of specific receptors. These are termed mu-, delta- and kappa-opioid receptors and belong to the family of G-protein-coupled receptors (5). The mu-opioid receptor will be in the focus of this review, because it is the main target for most of the commonly used opioid drugs, such as morphine. The human mu-opioid receptor gene consists of at least seven exons and is expressed in at least ten protein isoforms, which arise from alternative splicing (6-7). Nevertheless, among all these isoforms hMOR-1, consisting of exons one to four, is by far the most abundant isoform. The promoter sequences of the gene are located upstream of exon 1. They contain all the binding sites for the transcription factors involved in the gene's regulation described in this review. Since it was reported that the mouse mu-opioid receptor gene may be additionally regulated by a second, about ten

## Regulation of Mu-Opioid Receptors by Cytokines



**Figure 1.** Experimentally identified binding sites for inducible transcription factors on the promoter of the human mu-opioid receptor gene. References: AP-1 (47); GATA3 (36); NFAT (54) NF-kappaB (48); STAT1/3 (40); STAT6 (29).

kilobases more upstream promoter (8), it cannot be excluded that the human gene contains additional promoters as well. In the neuronal system, mu-opioid receptors are constitutively expressed at considerable high levels in a defined pattern in many brain areas, and in distinct spinal and peripheral neurons (9-12). In addition, they are expressed in the human neuroblastoma-derived cell line SH SY5Y (13). In contrast, in immune effector cells the expression of mu-opioid receptors is not constitutive. To our current knowledge, cytokines are the most powerful signals that induce a *de novo* synthesis of mu-opioid receptor expression in immune effector cells and modulate the expression of the receptors in neuronal cells. Cytokines are mediators, which are typically released from cells of the immune system and exert a plethora of effects in the nervous and the immune system (for an excellent and comprehensive review on cytokines see 14). The release of cytokines is regulated temporally and spatially in response to a great number of stimuli under physiological and pathophysiological conditions (14). Thus, the regulation of mu-opioid receptors by cytokines is a powerful tool, by which the expression of the receptors can be adapted according to numerous situations.

### 3. REGULATION OF MU-OPIOID RECEPTORS BY CYTOKINES

#### 3.1. Interleukin-1

There is evidence for a relationship between the pro-inflammatory cytokine interleukin (IL)-1 and opioid analgesia and tolerance (15-18). In a cellular model, it was found that a combination of IL-1 alpha and beta induced transcription of the mu-opioid receptor gene in neural microvascular endothelial NMVEC cells, which under basal conditions do not express the gene (19). Mechanisms of this regulation were not determined. However, effects of IL-1 are often mediated by the transcription factors AP-1 and/or NF-kappaB, for which binding sites were localized on the promoter of the human mu-opioid receptor gene (Figure 1). It will be interesting to see, how IL-1 regulates mu-opioid receptor expression in neurons, which will contribute to the understanding of the role of IL-1 in opioid analgesia and tolerance. As a consequence of the induction of mu-opioid receptors in response to IL-1 in endothelial cells, mu-opioid receptor ligands may contribute to IL-1-regulated endothelial function, as e. g. the adhesion of circulating leukocytes (20).

#### 3.2. Interleukin-4

It is increasingly recognized that endogenous as well as exogenous opioids have anti-inflammatory

properties. Beneficial effects of mu-opioid receptor agonists have been demonstrated in hepatic (21) and intestinal (22) inflammation and arthritis (23, 24). In addition, it was shown that morphine inhibits the pro-inflammatory mediators IL-6 and tumor necrosis factor (25) and interferon-gamma (26). In addition, it was reported that opioids induce the expression and the release of IL-4 from lymphocytes (12,27-28). Anti-inflammatory effects of IL-4 and its inhibitory effects on pro-inflammatory processes are well known (14). Furthermore, it was reported that IL-4 itself up-regulates mu-opioid receptor gene expression in many immune effector cells, which may potentiate the anti-inflammatory effects caused by opioids and IL-4. Thus, incubation of primary and Jurkat T lymphocytes, Raji B lymphocytes, primary polymorphonuclear leukocytes, U937 monocytes, primary dendritic cells and HMEC-1 microvascular endothelial cells with IL-4 for 16 h caused a *de novo* induction of mu-opioid receptor mRNA (29). Another important function of IL-4 is the regulation of T helper (Th) cells. It is the prototypical cytokine, which is produced by Th2 cells and promotes the differentiation of Th2 cells. In contrast, interferon (IFN)-gamma is the physiological antagonist of this effect. It is produced by Th1 cells and promotes their differentiation (30). Expression of mu-opioid receptors is restricted to Th2 cells (31). Most probably, this is because Th2 cells produce IL-4, which had been shown to induce mu-opioid receptors in T cells (29). Interestingly, the regulation of mu-opioid receptors by IFN-gamma is opposite to that of IL-4, which is described in detail below. The above described results support earlier findings, which suggested an implication of mu-opioid receptor ligands in the regulation of the Th cell balance and in Th2 biology (32-33). With respect to neurons, an induction of mu-opioid receptor transcription by IL-4 was detected in primary embryonic cortical neurons from rats, which express mu-opioid receptors constitutively, within 30 min to 2 h (29). Effects of IL-4 are mediated by the transcription factors STAT6 and GATA3. Both transactivate the mu-opioid receptor gene promoter (29,34-36; Figure 1). The rat mu-opioid receptor gene promoter contains a functional STAT6 site at nt -727 (29). Interestingly, a polymorphism in the human mu-opioid receptor gene promoter directly affects binding of STAT6. Thus, a point mutation destroys the palindromic, STAT6-binding core sequence (37). However, the function of the mutated element is not destroyed completely, but its inducibility in response to IL-4 is significantly reduced, which may be of importance for persons carrying this mutation (29,34-35).

### 3.3. Interleukin-6

There is reason to believe that the regulation of mu-opioid receptors by IL-6 is important for the regulation of inflammatory pain. Up-regulation of mu-opioid receptors in neuronal cells in response to IL-6 was indirectly suggested in a model of peripheral inflammation. It was shown that injection of IL-6 increased antinociceptive mechanisms, which was blocked by a mu-opioid receptor-specific antagonist (38). The experiments indicate that either endogenous mu-opioid receptor ligands, or mu-opioid receptors, or both are up-regulated by the cytokine at the site of inflammation. Up-regulation of mu-opioid receptors in neuronal cells in response to IL-6 was also suggested in experiments with mice lacking the IL-6 gene. These mice show weaker analgesic responses, as well as a weaker mu-opioid receptor density in the midbrain (39). In the neuroblastoma cell line SH SY5Y up-regulation of mu-opioid receptor mRNA and protein by IL-6 was demonstrated directly (40). Furthermore, it was shown that the transcriptional induction is mediated by STAT1, STAT3 or both factors (40; Figure 1).

### 3.4. Tumor necrosis factor

Tumor necrosis factor (TNF), formerly designated as TNF-alpha, is originally known for its property to cause necrosis of tumors (14). TNF is secreted mainly by monocytes in response to inflammatory reactions and is currently discussed as a major player in the pathophysiology of several inflammatory diseases such as rheumatoid arthritis. It is, therefore, the target of several potent novel drugs for the treatment of rheumatoid arthritis. However, on the other hand, it was reported that TNF increases antinociception in a model of peripheral inflammation (38). This suggests a possible regulation of mu-opioid receptors by this cytokine, which physiologically could at least help to counteract the inflammatory pain known to be evoked by this pro-inflammatory cytokine. A large number of reports favour the model that inflammation leads to an induction of endogenous opioid peptides in the immune cells of the inflamed tissue, which cause antinociception after activation of opioid receptors on peripheral sensory neurons (15,41-44). In addition, it was shown that neuronal mu-opioid receptors are up-regulated in inflammation (11) and that their amount in the neurons innervating the site of inflammation is crucial for effective antinociception (45-46). On a cellular level, up-regulation of the receptor mRNA in neuronal cells was observed in SH SY5Y neuroblastoma cells (47), and in primary embryonic cortical neurons from rats (Börner and Kraus, unpublished data). In addition to neuronal cells, a *de novo* induction of mu-opioid receptors by TNF in immune cells was reported in primary T lymphocytes, Raji B lymphocytes, primary polymorphonuclear leukocytes, U937 monocytes, primary dendritic cells and HMEC-1 microvascular endothelial cells (48), which may cause immunomodulatory effects of mu-opioid receptor ligands in these cells. Effects of TNF are mediated by the receptors TNFR1 (p55, TNFRSF1A) and TNFR2 (p75, TNFRSF1B) and often involve the transcription factors AP-1 and/or NF-kappaB. The TNF-mediated induction of mu-opioid receptor mRNA in Raji cells is mediated by TNFR2 and NF-kappaB only (48). Three binding sites for NF-kappaB were localized on the

promoter of the mu-opioid receptor gene (48; Figure 1). For one of these sites (nt -557) an allelic variation has been described, which introduces a point mutation into the NF-kappaB binding motif (37). This polymorphism weakens the trans-activating potential of the element, as well as the binding affinity of the element to NF-kappaB, which may deteriorate responsivity to opioids in persons carrying the mutated allele (48). Interestingly, it was demonstrated recently that the HIV protein gp120 induces mu-opioid receptors in primary macrophages and in macrophages-like HL-60 cells via TNF and NF-kappaB (49). This sheds new light on the complex of HIV infections and opioid abuse, which often correlate, but so far could not be causally linked.

### 3.5. Interferon-gamma

Interferon (IFN)-gamma is a cytokine, which often has antagonistic effects to IL-4. It is produced by Th1 cells and promotes their differentiation (30). IFN-gamma is produced in response to viral infections, exerting various virus-unspecific, antiviral actions (14). It is a common observation that viral infections often are accompanied by hyperalgesia (50), which could be associated with reduced mu-opioid receptor activity. Interestingly, mu-opioid receptor transcription is indeed negatively regulated by IFN-gamma (31). In the neuronal SH SY5Y cells, it down-regulates transcription of the mu-opioid receptors gene. In T lymphocytes, IFN-gamma completely inhibits the IL-4-mediated induction of the gene. Consistently with the fact that Th2 cells express IL-4 and also mu-opioid receptors, Th1 cells, which typically express IFN-gamma, do not contain mu-opioid receptor mRNA (31). This further emphasises a possible role for the mu-opioid system in the regulation of the Th cell balance, which has been suggested earlier (32-33).

### 3.6. Granulocyte/macrophage colony-stimulating factor

Inhibition of mu-opioid receptor transcription by granulocyte/macrophage colony-stimulating factor (GM-CSF) was observed in dendritic cells during their *in vitro* differentiation from human peripheral blood monocytes. It was observed that GM-CSF inhibited the IL-4-mediated induction of mu-opioid receptor transcription (29). At the moment, however, neither the molecular mechanisms underlying this inhibition, nor the physiological meaning of the effect of GM-CSF on mu-opioid receptor transcription are understood.

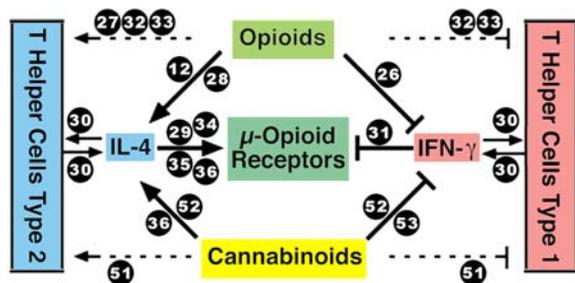
## 4. PERSPECTIVE

Cytokines are potent regulators of mu-opioid receptor gene expression in neuronal, as well as immune cells, which is summarized in Figure 2. A large number of reports established an important antinociceptive role for the mu-opioid system in inflammation (summarized in 2). The up-regulation of mu-opioid receptors by pro-inflammatory cytokines like IL-1, IL-6 and TNF, as well as anti-inflammatory cytokines like IL-4 in neurons innervating the site of inflammation and activation of these receptors is likely to contribute to an organism's management of inflammatory pain. Indeed, there is evidence for the hypothesis that an increased number of neuronal mu-opioid receptors, which are activated by an increased amount of

## Regulation of Mu-Opioid Receptors by Cytokines

Physiological Stimulus	Cytokine	Transcription Factor	$\mu$ -OR
	IL-1 <sup>E</sup>	(AP-1) (NF $\kappa$ B)	↑
Cannabinoids <sup>T</sup> Th2 <sup>T</sup>	IL-4 <sup>CTBMGDE</sup>	STAT6 GATA3	
(Inflammation <sup>P</sup> )	IL-6 <sup>S</sup>	STAT1/3	
(Inflammation <sup>P</sup> ) HIV <sup>M</sup>	TNF <sup>CTBMGDE</sup>	NF $\kappa$ B	
Th1 <sup>T</sup>	IFN- $\gamma$ <sup>ST</sup>		↓
	GM-CSF <sup>D</sup>		

**Figure 2.** Summary of reports providing evidence for up- and down-regulation of mu-opioid receptor transcription by cytokines, transcription factors and physiological stimuli. Letters indicate the cell types in which regulation was observed (B, B cells; C, central primary neurons; D, dendritic cells; E, endothelial cells; G, granulocytes; M, Monocytes/Macrophages; P, peripheral primary neurons; S, SH SY5Y cells; T, T cells). Terms in brackets indicate that there is indirect evidence only.



**Figure 3.** Interactions between opioids, cannabinoids, mu-opioid receptors, IL-4 and IFN-gamma. Arrows represent direct, dashed arrows indirect regulatory events. The numbers given refer to references describing these effects.

endogenous opioids in inflammation at least helps to counteract the inflammatory pain evoked by pro-inflammatory cytokines (45-46). While the expression of mu-opioid receptors on neurons is necessary for antinociceptive effects of opioids, their expression on immune effector cells allows immunomodulatory effects of opioids. Since several of these effects are anti-inflammatory (21-26), the use of opioids may be beneficial not only to treat the painful symptoms of an inflammation, but also its cause. Although controversially discussed earlier, it is now clearly established that mu-opioid receptors are expressed in immune effector cells. However, their expression in these cells is not constitutive, as it is the case in many neuronal cells, but inducible e. g. in response to cytokines. Since cytokines are expressed in immune tissue in response to many stimuli, there is reason to believe that a certain number of mu-opioid receptors is normally present in immune effector cells. In addition,

recent data from our laboratories demonstrated that mu-opioid receptors are induced in response to activation of the T cell receptor complex (54). Additional signals, which lead to induction of mu-opioid receptors in immune cells, are likely to be elucidated in the future. A rather low amount of specific transcripts may be one of the reasons, why it had been difficult to detect mu-opioid receptors in immune effector cells. We recently showed that the amount of cytokine-induced mu-opioid receptor mRNA in immune cells is about one to two orders of magnitude lower than that in neuronal cells. Nevertheless, it was also shown that even this small amount of mRNA is enough to produce functional mu-opioid receptor proteins in the immune cells (12). Interestingly, the amount of mu-opioid receptor-specific mRNA induced by TNF or IL-4 in Jurkat T cells is very similar (31), although the two cytokines exert quite different effects, with TNF as a pro-inflammatory, and IL-4 as an anti-inflammatory cytokine. Among other immunomodulatory effects of opioids (reviewed in 3-4), they modulate the Th cell balance and promote Th2 cell differentiation (32-33). Most probably, this is due to the effect of opioids to induce IL-4 and inhibit IFN-gamma expression in T cells (12,26-28). In this scenario, a positive regulatory circuit is established, since the opioid-induced release of IL-4 further induces expression of mu-opioid receptors. Interestingly, cannabinoids act very similar to opioids in this respect by increasing the expression of IL-4 and decreasing that of IFN-gamma (36,51-53). To further increase this complexity, it has been shown that cannabinoids induce mu-opioid receptors, which is mediated by IL-4, and which explains synergistic immunomodulatory effects of cannabinoids and opioids (36). The complex regulatory circuits involving opioids, cannabinoids, mu-opioid receptors, IL-4 and IFN-gamma are depicted in Figure 3. Although far from being understood, the data from our laboratory showing mu-opioid receptor induction by IL-4 not only in immune cells, but also in neurons (29) suggest a possible mechanism for combined anti-inflammatory and antinociceptive effects of IL-4 together with opioids. In conclusion, a lot more of research is needed in the future to fully understand the complex neuro-immune-interactions involving the mu-opioid system and cytokines.

## 5. ACKNOWLEDGEMENTS

The author likes to thank Volker Höllt and Christine Börner for critical review of this manuscript.

## 6. REFERENCES

1. M. J. Kreek: Opiates, opioids and addiction. *Mol Psychiatry* 1, 232-254 (1996)
2. C. Stein, M. Schafer and H. Machelkska: Attacking pain at its source: new perspectives on opioids. *Nat Med* 9, 1003-1008 (2003)
3. P. Sacerdote: Opioids and the immune system. *Palliat Med* 20 Suppl 1, s9-15 (2006)

## Regulation of Mu-Opioid Receptors by Cytokines

4. S. Roy, J. Wang, J. Kelschenbach, L. Koodie and J. Martin: Modulation of immune function by morphine: implications for susceptibility to infection. *J Neuroimmune Pharmacol* 1, 77-89 (2006)
5. O. Pol and M. M. Puig: Expression of opioid receptors during peripheral inflammation. *Curr Top Med Chem* 4, 51-61 (2004)
6. L. Pan, J. Xu, R. Yu, M. M. Xu, Y. X. Pan and G. W. Pasternak: Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm. *Neuroscience* 133, 209-220 (2005)
7. Y. X. Pan: Diversity and complexity of the mu opioid receptor gene: alternative pre-mRNA splicing and promoters. *DNA Cell Biol* 24, 736-750 (2005)
8. J. Xu, M. Xu and Y. X. Pan: Characterizing exons 11 and 1 promoters of the mu opioid receptor (Oprm) gene in transgenic mice. *BMC Mol Biol* 7, 41 (2006)
9. A. Mansour, C. A. Fox, R. C. Thompson, H. Akil and S. J. Watson: mu-Opioid receptor mRNA expression in the rat CNS: comparison to mu-receptor binding. *Brain Res* 643, 245-265 (1994)
10. A. Mansour, C. A. Fox, H. Akil and S. J. Watson: Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci* 18, 22-29 (1995)
11. C. Zollner, M. A. Shaqura, C. P. Bopaiah, S. Mousa, C. Stein and M. Schafer: Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. *Mol Pharmacol* 64, 202-210 (2003)
12. C. Borner, R. Stumm, V. Holtt and J. Kraus: Comparative analysis of mu-opioid receptor expression in immune and neuronal cells. *J Neuroimmunol* 188, 56-63 (2007)
13. J. E. Zadina, S. L. Chang, L. J. Ge and A. J. Kastin: Mu opiate receptor down-regulation by morphine and up-regulation by naloxone in SH-SY5Y human neuroblastoma cells. *J Pharmacol Exp Ther* 265, 254-262 (1993)
14. J. H. Curfs, J. F. Meis and J. A. Hoogkamp-Korstanje: A primer on cytokines: sources, receptors, effects, and inducers. *Clin Microbiol Rev* 10, 742-780 (1997)
15. M. Schafer, L. Carter and C. Stein: Interleukin 1 beta and corticotropin-releasing factor inhibit pain by releasing opioids from immune cells in inflamed tissue. *Proc Natl Acad Sci U S A* 91, 4219-4223 (1994)
16. H. Gul, O. Yildiz, A. Dogrul, O. Yesilyurt and A. Isimer: The interaction between IL-1beta and morphine: possible mechanism of the deficiency of morphine-induced analgesia in diabetic mice. *Pain* 89, 39-45 (2000)
17. I. N. Johnston, E. D. Milligan, J. Wieseler-Frank, M. G. Frank, V. Zapata, J. Campisi, S. Langer, D. Martin, P. Green, M. Fleshner, L. Leinwand, S. F. Maier and L. R. Watkins: A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine. *J Neurosci* 24, 7353-7365 (2004)
18. Y. Shavit, G. Wolf, I. Goshen, D. Livshits and R. Yirmiya: Interleukin-1 antagonizes morphine analgesia and underlies morphine tolerance. *Pain* 115, 50-59 (2005)
19. E. L. Vidal, N. A. Patel, G. Wu, M. Fiala and S. L. Chang: Interleukin-1 induces the expression of mu opioid receptors in endothelial cells. *Immunopharmacology* 38, 261-266 (1998)
20. S. L. Chang, R. L. Moldow, S. D. House and J. E. Zadina: Morphine affects the brain-immune axis by modulating an interleukin-1 beta dependent pathway. *Adv Exp Med Biol* 402, 35-42 (1996)
21. D. Chakass, D. Philippe, E. Erdual, S. Dharancy, M. Malapel, C. Dubuquoy, X. Thuru, J. Gay, C. Gaveriaux-Ruff, P. Dubus, P. Mathurin, B. L. Kieffer, P. Desreumaux and M. Chamaillard: micro-Opioid receptor activation prevents acute hepatic inflammation and cell death. *Gut* 56, 974-981 (2007)
22. D. Philippe, L. Dubuquoy, H. Groux, V. Brun, M. T. Chuoi-Mariot, C. Gaveriaux-Ruff, J. F. Colombel, B. L. Kieffer and P. Desreumaux: Anti-inflammatory properties of the mu opioid receptor support its use in the treatment of colon inflammation. *J Clin Invest* 111, 1329-1338 (2003)
23. Y. Takeba, N. Suzuki, A. Kaneko, T. Asai and T. Sakane: Endorphin and enkephalin ameliorate excessive synovial cell functions in patients with rheumatoid arthritis. *J Rheumatol* 28, 2176-2183 (2001)
24. S. Kyrkanides, P. M. Fiorentino, J. N. Miller, Y. Gan, Y. C. Lai, S. S. Shaftel, J. E. Puzas, M. G. Piacino, M. K. O'Banion and R. H. Tallents: Amelioration of pain and histopathologic joint abnormalities in the Col1-IL-1beta(XAT) mouse model of arthritis by intraarticular induction of mu-opioid receptor into the temporomandibular joint. *Arthritis Rheum* 56, 2038-2048 (2007)
25. M. P. Bonnet, H. Beloeil, D. Benhamou, J. X. Mazoit and K. Asehnoune: The mu opioid receptor mediates morphine-induced tumor necrosis factor and interleukin-6 inhibition in toll-like receptor 2-stimulated monocytes. *Anesth Analg* 106, 1142-1149 (2008)
26. J. Wang, R. A. Barke, R. Charboneau, H. H. Loh and S. Roy: Morphine negatively regulates interferon-gamma promoter activity in activated murine T cells through two distinct cyclic AMP-dependent pathways. *J Biol Chem* 278, 37622-37631 (2003)
27. S. Roy, J. Wang, S. Gupta, R. Charboneau, H. H. Loh and R. A. Barke: Chronic morphine treatment differentiates

## Regulation of Mu-Opioid Receptors by Cytokines

T helper cells to Th2 effector cells by modulating transcription factors GATA 3 and T-bet. *J Neuroimmunol* 147, 78-81 (2004)

28. S. Roy, J. Wang, R. Charboneau, H. H. Loh and R. A. Barke: Morphine induces CD4+ T cell IL-4 expression through an adenylyl cyclase mechanism independent of the protein kinase A pathway. *J Immunol* 175, 6361-6367 (2005)

29. J. Kraus, C. Borner, E. Giannini, K. Hickfang, H. Braun, P. Mayer, M. R. Hoehe, A. Ambrosch, W. Konig and V. Hollt: Regulation of mu-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. *J Biol Chem* 276, 43901-43908 (2001)

30. A. O'Garra and N. Arai: The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol* 10, 542-550 (2000)

31. J. Kraus, C. Borner, U. Lendeckel and V. Hollt: Interferon-gamma down-regulates transcription of the mu-opioid receptor gene in neuronal and immune cells. *J Neuroimmunol* 181, 13-18 (2006)

32. P. Sacerdote, V. E. di San Secondo, G. Sirchia, B. Manfredi and A. E. Panerai: Endogenous opioids modulate allograft rejection time in mice: possible relation with Th1/Th2 cytokines. *Clin Exp Immunol* 113, 465-469 (1998)

33. P. Sacerdote, B. Manfredi, L. Gaspani and A. E. Panerai: (Blood)The opioid antagonist naloxone induces

a shift from type 2 to type 1 cytokine pattern in BALB/cJ mice. *Blood* 95, 2031-2036 (2000)

34. J. Kraus, C. Borner and V. Hollt: Distinct palindromic extensions of the 5'-TTC...GAA-3' motif allow STAT6 binding in vivo. *Faseb J* 17, 304-306 (2003)

35. C. Borner, M. Woltje, V. Hollt and J. Kraus: STAT6 transcription factor binding sites with mismatches within the canonical 5'-TTC...GAA-3' motif involved in regulation of delta- and mu-opioid receptors. *J Neurochem* 91, 1493-1500 (2004)

36. C. Borner, V. Hollt and J. Kraus: Cannabinoid Receptor Type 2 Agonists Induce Transcription of the  $\mu$ -Opioid Receptor Gene in Jurkat T Cells. *Mol Pharmacol* 69, 1486-1491 (2006)

37. M. R. Hoehe, K. Kopke, B. Wendel, K. Rohde, C. Flachmeier, K. K. Kidd, W. H. Berrettini and G. M. Church: Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. *Hum Mol Genet* 9, 2895-2908 (2000)

38. A. Czlonkowski, C. Stein and A. Herz: Peripheral mechanisms of opioid antinociception in inflammation: involvement of cytokines. *Eur J Pharmacol* 242, 229-235 (1993)

39. M. Bianchi, R. Maggi, F. Pimpinelli, T. Rubino, D. Parolaro, V. Poli, G. Ciliberto, A. E. Panerai and P. Sacerdote: Presence of a reduced opioid response in interleukin-6 knock out mice. *Eur J Neurosci* 11, 1501-1507 (1999)

40. C. Borner, J. Kraus, H. Schroder, H. Ammer and V. Hollt: Transcriptional regulation of the human mu-opioid receptor gene by interleukin-6. *Mol Pharmacol* 66, 1719-1726 (2004)

41. C. Stein, A. H. Hassan, R. Przewlocki, C. Gramsch, K. Peter and A. Herz: Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. *Proc Natl Acad Sci U S A* 87, 5935-5939 (1990)

42. S. A. Mousa, Q. Zhang, N. Sitte, R. Ji and C. Stein: beta-Endorphin-containing memory-cells and mu-opioid receptors undergo transport to peripheral inflamed tissue. *J Neuroimmunol* 115, 71-78 (2001)

43. H. Machelska, J. K. Schopohl, S. A. Mousa, D. Labuz, M. Schafer and C. Stein: Different mechanisms of intrinsic pain inhibition in early and late inflammation. *J Neuroimmunol* 141, 30-39 (2003)

44. N. Sitte, M. Busch, S. A. Mousa, D. Labuz, H. Rittner, C. Gore, H. Krause, C. Stein and M. Schafer: Lymphocytes upregulate signal sequence-encoding proopioidmelanocortin mRNA and beta-endorphin during painful inflammation in vivo. *J Neuroimmunol* 183, 133-145 (2007)

45. Y. Xu, Y. Gu, G. Y. Xu, P. Wu, G. W. Li and L. Y. Huang: Adeno-associated viral transfer of opioid receptor gene to primary sensory neurons: a strategy to increase opioid antinociception. *Proc Natl Acad Sci U S A* 100, 6204-6209 (2003)

46. A. Brack, H. L. Rittner, H. Machelska, M. Shaqura, S. A. Mousa, D. Labuz, C. Zollner, M. Schafer and C. Stein: Endogenous peripheral antinociception in early inflammation is not limited by the number of opioid-containing leukocytes but by opioid receptor expression. *Pain* 108, 67-75 (2004)

47. C. Borner, V. Hollt and J. Kraus: Involvement of activator protein-1 in transcriptional regulation of the human mu-opioid receptor gene. *Mol Pharmacol* 61, 800-805 (2002)

48. J. Kraus, C. Borner, E. Giannini and V. Hollt: The Role of Nuclear Factor  $\kappa$ B in Tumor Necrosis Factor-Regulated Transcription of the Human mu-Opioid Receptor Gene. *Mol Pharmacol* 64, 876-884 (2003)

49. J. A. Beltran, A. Pallur and S. L. Chang: HIV-1 gp120 up-regulation of the mu opioid receptor in TPA-differentiated HL-60 cells. *Int Immunopharmacol* 6, 1459-1467 (2006)

## Regulation of Mu-Opioid Receptors by Cytokines

50. R. Eccles: Understanding the symptoms of the common cold and influenza. *Lancet Infect Dis* 5, 718-725 (2005)

51. T. W. Klein, C. Newton, K. Larsen, J. Chou, I. Perkins, L. Lu, L. Nong and H. Friedman: Cannabinoid receptors and T helper cells. *J Neuroimmunol* 147, 91-94 (2004)

52. M. Yuan, S. M. Kiercher, Q. Cheng, R. Zoumalan, D. P. Tashkin and M. D. Roth: Delta 9-Tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. *J Neuroimmunol* 133, 124-131 (2002)

53. T. W. Klein, C. A. Newton, N. Nakachi and H. Friedman: Delta 9-tetrahydrocannabinol treatment suppresses immunity and early IFN-gamma, IL-12, and IL-12 receptor beta 2 responses to *Legionella pneumophila* infection. *J Immunol* 164, 6461-6466 (2000)

54. C. Borner, J. Kraus, A. Bedini, B. Schraven and V. Höllt: T cell receptor/CD28-mediated activation of human T lymphocytes induces expression of functional  $\mu$ -opioid receptors. *Mol Pharmacol* in press

**Abbreviations:** GMCSF: granulocyte/macrophage colony-stimulating factor; HIV: human immunodeficiency virus; IFN: interferon; IL: interleukin; Th: T helper; TNF: tumor necrosis factor

**Key Words** Opioid, Mu-Opioid Receptor, Cytokine, Gene Regulation, Transcription Factor, Cannabinoid; Review

**Send correspondence to:** Jurgen Kraus, Dept. of Pharmacology and Toxicology, Magdeburg University, 44 Leipzigerstrasse, 39120 Magdeburg, Germany, Tel: 49 391 6715372, Fax: 49 391 6715869, E-mail: juergen.kraus@med.ovgu.de

<http://www.bioscience.org/current/volS1.htm>