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*J Immunol* 2013; 191:4640-4647; Prepublished online 25 September 2013;
doi: 10.4049/jimmunol.1300320

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Inhibition of NF-κB by Opioids in T Cells

Christine Börner and Jürgen Kraus

Opioids potently inhibit a number of physiological and pathophysiological effects such as pain and inflammation in the brain and the periphery. One of the targets of opioids mediating such effects is the proinflammatory transcription factor NF-κB. In neuronal cells, opioids inhibit this factor by inducing I-κB independently on calcium, involving the opioid-mediated activation of the transcription factor AP-1. However, when and how precisely NF-κB is modulated by opioids in T cells are unknown. By using the TNF-triggered, NF-κB–mediated induction of IL-8 mRNA in primary human T cells and Jurkat T cells, in this study we show that opioids inhibit NF-κB in T cells as well, but that the underlying mechanisms are different from those observed in neuronal cells. We found that stimulation of the T cells with opioids resulted in a significant inhibition of the TNF-triggered ubiquitination and degradation of I-κB. Additionally, an opioid-mediated induction of the deubiquitinating enzyme ubiquitin-specific protease 15 was observed, which is known to inhibit the NF-κB pathway by stabilizing I-κB. The induction of ubiquitin-specific protease 15 was dependent on calcium and the transcription factor NFAT. Activation of AP-1 and induction of I-κB in response to the opioids were not observed in the T cells. These results indicate that μ opioid receptors, which mediate the effects in both cell types, might be coupled to different effector cascades in the different cell types, which may then result in cell type–specific effects of the drugs.


Opioids are the most potent analgesics and are inevitable for the treatment of severe pain. In addition to their effects on neuronal functions, opioids modulate a large body of functions in the periphery, including immune functions. Often, the immunomodulatory effects of opioids result in immunosuppression, which is one of the adverse reactions of these drugs. Alternatively, opioids induce various anti-inflammatory effects, which may be beneficial in distinct opioid therapies (1–3). Opioids mediate their effects via three different receptors termed μ, δ, and κ opioid receptors. Among these, μ, δ opioid receptors are of special relevance, because they normally mediate effects of morphine and most of the opioids used in medicine (4).

The transcription factor NF-κB is an important mediator of numerous effects inside and outside the immune system (5, 6). Many effects mediated by NF-κB are proinflammatory, for example, the induction of the cytokine IL-8 (7–9). Several observations suggest that modulation of NF-κB is one of the mechanisms underlying the modulatory effects of opioids. However, although inhibition of NF-κB–mediated immune functions by morphine has been indicated earlier (10–12), the precise mechanisms underlying these effects are not fully understood. The activation of NF-κB, for example, by TNF, is a multistep cascade. In brief, in resting cells the inhibitory protein I-κB retains NF-κB in the cytoplasm, where the transcription factor is inactive. An early step in the activation of NF-κB is the stimulus (e.g., TNF)-triggered phosphorylation of the I-κB kinase (IKK) complex. It consists of two kinases (IκKα and IκKβ) and a regulatory subunit, termed IκKγ or NF-κB essential modulator (NEMO). The phosphorylation of the IKK complex in turn induces the phosphorylation of I-κB. Upon phosphorylation, I-κB is ubiquitinated and committed to proteasomal degradation. After degradation of I-κB, NF-κB, which may consist of several proteins among which p65 is most prominent, enters the nucleus and binds to cis-active DNA elements/transcriptional enhancers. Additionally, p65 is phosphorylated (for a review, see Ref. 13).

Interference with this cascade, and thereby inhibition of NF-κB, may occur in several ways. One possibility is the inhibition of the stimulus-triggered degradation of I-κB, which has been reported for several drugs, as, for example, cannabinoids (14). Another mechanism that has been reported is the induction of the expression of I-κB, which retains NF-κB in the inactive, cytoplasmatic state (15, 16). In this context, our group has recently shown that a marked induction of I-κB by morphine, which is mainly dependent on the activation of the transcription factor AP-1, is underlying the inhibitory effect of the drug on TNF-triggered NF-κB signaling in primary striatal fetal neurons from rats and in the human neuroblastoma cell line SH SY5Y (17). Furthermore, we report in the same publication that the TNF-triggered degradation of I-κB is not affected by morphine in the neuronal cells. In this study, we used the well-established induction of IL-8 transcription in response to TNF in T cells, which is mediated by NF-κB (7–9), as a model to characterize the effect of morphine on NF-κB and investigate in detail how opioid signaling causes modulation of the activity of NF-κB in T lymphocytes. We report that morphine promotes a drastic inhibition of the TNF-triggered induction of IL-8 and NF-κB. Remarkably, however, the mechanisms of the inhibition of NF-κB by the drug in the T cells are completely different from those we recently observed in the neuronal cells (17).

Materials and Methods

Cell culture and transfection reagents

PBMCs were isolated from heparinized blood collected from healthy volunteers (18). The primary cells and cells of the human T cell line Jurkat were cultivated in RPMI 1640 medium supplemented with glutamine, penicillin/streptomycin (Lonza Verviers) and 10% FCS (PAN-Biotech, www.jimmunol.org/cgi/doi/10.4049/jimmunol.1300320
The transcription factor decoy oligonucleotide approach, its efficiency and specificity, has been described in detail in previous publications from our group (19–22). Briefly, short double-stranded oligonucleotides with specific binding sequences for transcription factors were introduced into living cells by passive uptake during an overnight incubation of the cells in the presence of 160 nM oligonucleotides. In the cells, transcription factors then rather interact with the excess of decoy oligonucleotides than bind to the natural regulatory motifs of genes. Thus, the decoys selectively disrupt the function of a desired transcription factor. Because the decoys act within living cells, they are highly specific. The sequences of the decoy oligonucleotides were (upper strand): NF-κB, 5'-GGGACTTTCC-3'; AP-1, 5'-TTGTACTGGT-3'; and 5'-CGAAAGTCTCGGAGCTCAGGATC-3'. The sequences of the binding sites as binding sites for NFAT, NF-κB, and AP-1 or as mutations that do not bind these factors are described elsewhere (19, 23). Decoy oligonucleotides were synthesized by Metabion ( Martinsried, Germany) as complementary single strands.

**Reporter gene analysis**

Construction of the reporter plasmid NF-κB/thymidine kinase/chloramphenicol acetyltransferase (CAT) containing the classic NF-κB binding site 5'-GGGACCTTCC-3' has been described earlier (23). The transfection of Jurkat cells was performed as described earlier (18, 24). The CAT reporter gene ELISA (Roche) was done according to the manufacturer’s suggestions.

**Western blot analysis**

Western blots were performed as previously described (18, 25). Briefly, prior to stimulation, cells were kept for 16 h in medium containing 1% FCS. Then, 10^6 cells per sample were incubated at 37°C with stimuli or the herpes simplex thymidine kinase minimal promoter (tk), and one copy of a binding sequence for NF-κB 5' to the promoter. The day after transfection, the medium was replaced and the cells were incubated with TNF (1 ng/ml) and morphine (1 μM) as indicated. Cells were lysed 72 h after transfection and a CAT-ELISA was performed. Results of at least two independent experiments performed in triplicate ± SEM are displayed. **p < 0.01.

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**FIGURE 1.** Treatment of T cells with morphine results in induction of the TNF/NF-κB-mediated induction of IL-8 mRNA. (A) The TNF-mediated induction of IL-8 mRNA is inhibited by morphine. IL-8 transcripts in primary human T cells (gray columns) and in Jurkat T cells (black columns) were assessed by quantitative real-time RT-PCR and normalized to those of b-actin. TNF (1 ng/ml) as indicated. Cells were incubated overnight with 160 nM decoy oligonucleotides directed against NF-κB (lanes 3, 8, and 10) or mutated control oligonucleotides (mutNF-κB, lanes 4 and 9) before stimulation with TNF and/or morphine. At least two independent experiments were performed in duplicate and are shown ± SEM. Samples were compared with TNF-treated samples shown in lane 2. *p < 0.05, **p < 0.01, ***p < 0.001. (B) The TNF-mediated induction of IL-8 mRNA is mediated by NF-κB and basal IL-8 mRNA is not regulated by morphine. The amounts of IL-8 transcripts in Jurkat T cells were assessed by quantitative real-time RT-PCR and normalized to those of b-actin. TNF (1 ng/ml) as indicated. Morphine (Mo) was used at a concentration of 1 μM for the indicated times. Some samples were incubated overnight with 160 nM decoy oligonucleotides directed against NF-κB (lanes 3, 8, and 10) or mutated control oligonucleotides (mutNF-κB, lanes 4 and 9) before stimulation with TNF and/or morphine. At least two independent experiments were performed in duplicate and are shown ± SEM. Samples were compared with untreated controls shown in lane 1. **p < 0.01, ***p < 0.001. (C) Effect of morphine on the TNF-mediated inducibility of an NF-κB reporter construct. Jurkat cells were transiently transfected with the reporter construct NF-κB/IL4/CAT containing the CAT reporter gene, Aidenbach, Germany). For all experiments, cells that endogenously express μ opioid receptors were used. To induce the expression of the receptors, the cells were prestimulated with IL-4 as described in detail previously (18).

TNF was obtained from R&D Systems (Wiesbaden, Germany). Morphine was obtained from SynPharm (Barsbüttel, Germany). β-endorphin, forskolin, and cycloheximide were obtained from Sigma-Aldrich (Taufkirchen, Germany). CTAP, cAMPS-RP, SB 203580, genistein, and U0126 were obtained from Tocris Bioscience (Bristol, U.K.). To avoid nonspecific effects, the smallest effective doses for all drugs and inhibitors were chosen according to our own observations or to published data.

**Quantitative real-time RT-PCR**

Total RNA was extracted using the NucleoSpin RNA II kit from Macherey-Nagel ( Düren, Germany). One microgram of total RNA was used for cDNA synthesis with Moloney murine leukemia virus reverse transcriptase, RNase H minus (Promega, Mannheim, Germany), and diluted to 50 μl. Two microliters of cDNA was used for real-time PCR reactions. Quantitative real-time RT-PCR was done in a total volume of 20 μl on a LightCycler instrument using the LightCycler FastStart DNA Master SYBR Green I kit (both from Roche, Mannheim, Germany) according to the manufacturer’s suggestions. PCR primers and conditions were as follows: b-actin, 5'-CGTCCACACCCGCCGCACTTC-3' and 5'-AGCCAGTCCAGACGAGGATGG-3' primers (preincubation for 8 min at 95°C; 50 cycles: 5 s at 95°C, 5 s at 60°C, and 22 s at 72°C); IL-8, 5'-GCCAACAGGTGCTAAAGAACATCG-3' and 5'-CTCCTAACCACATCCCAACACCCT-3' primers (preincubation for 8 min at 95°C; 50 cycles: 5 s at 95°C, 5 s at 68°C, and 9 s at 72°C); IL-8, 5'-GCCAACAGGTGCTAAAGAACATCG-3' and 5'-CTCCTAACCACATCCCAACACCCT-3' primers (preincubation for 8 min at 95°C; 50 cycles: 5 s at 95°C, 5 s at 66°C, and 11 s at 72°C); ubiquitin-specific protease 15 (Usp15), 5'-GCTAGTCACCCCAAGAGCTT-3' and 5'-TCGGGACACCACTTATCTGCGC-3' primers (preincubation for 8 min at 95°C; 50 cycles: 5 s at 95°C, 5 s at 64°C, and 10 s at 72°C).
with vehicle. The incubation was stopped by washing the cells with PBS and subsequent lysis. For protein detection, the following Abs were used: primary Abs: actin C-11, Usp15 (2D5), and Ub FL-76 from Santa Cruz Biotechnology; phospho–NF-κB p65 (Ser536; 93H1), phospho–IκB-α (Ser17/18), IKKγ (NEMO), phospho–IκKα/β (Ser17/18), and IκB-α from Cell Signaling Technology/New England Biolabs (Frankfurt, Germany). Primary Abs were from rabbits or mice. Secondary Abs included anti-rabbit IgG or anti-mouse IgG (both from GE Healthcare, Braunschweig, Germany).

For coimmunoprecipitation, 10⁶ cells per sample were incubated at 37°C with stimuli or with vehicle. Then, cells were lysed in RIPA buffer containing protease inhibitors (bacitracin, pepstatin, leupeptin, aprotinin, PMSF, and trypsin inhibitor, all from Sigma-Aldrich), incubated on ice for 10 min, and treated with ultrasound for 20 s. Then the lysates were incubated with ubiquitin Abs by shaking overnight at 4°C. Wheat germ agarose beads (lecitin from Triticum vulgaris/agarose; Sigma-Aldrich) were added and incubated with shaking for 4 h at 4°C. The beads were pelleted, washed five times, and the protein was eluted in Western blot sample buffer for 20 min at 60°C. After centrifugation, a third of each sample was separated on a 10% polyacrylamide gel and further processed as described for Western blots.

Small interfering RNA approach

The commercially available small interfering RNAs (siRNAs) “Usp15 siRNA (h)” and “control siRNA-A” were used (Santa Cruz Biotechnology).

Results

Treatment of T cells with morphine resulted in an inhibition of the TNF-mediated induction of IL-8 mRNA

The induction of IL-8 mRNA in response to TNF, which is known to depend on NF-κB (7–9), was used as a model to investigate the effect of morphine on NF-κB in T lymphocytes (Fig. 1A). Simultaneous addition of the drug together with TNF had no significant effect on the induction of IL-8 mRNA. Interestingly, however, when primary human T cells or Jurkat cells were preincubated for 5 or 24 h with morphine prior to TNF stimulation, the TNF-mediated induction of IL-8 mRNA was significantly inhibited (compare lanes 4 and 5 with the TNF-triggered IL-8 mRNA shown in lane 2). Furthermore, the inhibitory effect of morphine on the TNF-induced IL-8 mRNA was reversible with the μ opioid receptor-specific antagonist CTAP. Additionally, the inhibitory effect of morphine was abolished in the presence of the protein biosynthesis inhibitor cycloheximide. The pivotal role of NF-κB in the TNF-triggered induction of IL-8, which already has been described (7–9), was confirmed using decoy oligonucleotides directed against NF-κB (Fig. 1B, lanes 1–4). Whereas the NF-κB decoy oligonucleotides abolished the TNF-triggered induction of
IL-8 mRNA (compare lane 3 with the TNF-triggered IL-8 mRNA shown in lane 2), mutated control oligonucleotides (lane 4), which, as previously shown, do not bind NF-κB (19), had no effect on the induction of IL-8 mRNA. In additional controls it was shown that the basal IL-8 mRNA was not regulated by morphine, nor had NF-κB decoy oligonucleotides any effect on this (Fig. 1B, lanes 5–9). Also, there was no significant effect on IL-8 mRNA when morphine, TNF, and the NF-κB decoy oligonucleotides were applied together (Fig. 1B, lane 10).

Transient expression of an NF-κB–driven reporter construct in Jurkat cells was used to directly demonstrate the inhibitory effect of morphine on the TNF-induced activity of this transcription factor (Fig. 1C).

Treatment of T cells with morphine resulted in an inhibition of the TNF-triggered degradation of IκB

Next, the effects of morphine on distinct steps of the TNF-triggered NF-κB activation cascade were investigated (Fig. 2). Morphine treatment of Jurkat cells for up to 24 h prior to TNF stimulation did not interfere significantly with the TNF-induced phosphorylation of the proteins IKK, IκB, and p65 (p-IKK, p-IκB, and p-p65; Fig. 2A). However, the TNF-induced degradation of IκB was significantly inhibited by morphine (Fig. 2A, third row from top). In fact, preincubation of the cells with the drug for 8 and 24 h abolished the TNF-induced degradation of IκB (Fig. 2B). In all Western blot experiments, TNF was applied for 5 min, because TNF stimulation of the cells produced a rapid degradation of IκB (Fig. 2C). Similar to the inhibition of the TNF-triggered induction of IL-8 mRNA, the inhibition of the degradation of IκB by morphine was mediated by μ opioid receptors, as demonstrated with the specific antagonist CTAP (Fig. 2D). When applying 100 nM morphine, we observed a less drastic, but considerable inhibition of the TNF-induced degradation of IκB (TNF, 18% of controls; TNF plus morphine (100 nM), 55% of controls; TNF plus morphine (1 μM), 105% of controls; Fig. 2E). Furthermore, the inhibition of the TNF-induced degradation of IκB by morphine was also observed in primary human T cells (Fig. 2F). We have recently shown that morphine-mediated induction of IκB is underlying the inhibitory effect of the drug on the TNF-triggered NF-κB activity in neuronal cell models (17). However, in sharp contrast to the neuronal cells, no significant induction of IκB mRNA or protein could be observed in the Jurkat T cells in response to morphine (Fig. 2G, 2H).

Calcium is necessary for the inhibition of the TNF-triggered degradation of IκB by morphine

Next, the role of calcium in the inhibition of the TNF-induced degradation of IκB by morphine was investigated (Fig. 3). When Jurkat cells were incubated in a calcium-free medium, the effect of the drug was no longer observed (Fig. 3A). However, the effect could be restored by addition of 500 μM calcium to the medium, suggesting that calcium is necessary for the inhibition of the TNF-induced degradation of IκB by morphine (Fig. 3A). Augmenting the calcium concentration in normal medium by extra addition of CaCl2 did not influence the effect of morphine (Fig. 3B).

Treatment of T cells with morphine resulted in an inhibition of the TNF-triggered ubiquitination of IκB, but not of NEMO

To further investigate mechanisms underlying the inhibition of the TNF-induced degradation of IκB by morphine, we addressed the ubiquitination of IκB, which is a prerequisite for its degradation (Fig. 4). Using coimmunoprecipitation it was found that treatment of Jurkat cells with morphine prevented the ubiquitination of IκB. In contrast, the ubiquitination of NEMO was not inhibited by the drug.
experiments as described above were performed with forskolin (For; 25 μM), which was applied simultaneously with morphine, and cAMPS-Rp (cRp; 100 μM), U0126 (10 μM), SB203580 (SB; 1 μM) and genistein (66 μM), which were applied 1 h prior to morphine. At least two independent experiments performed in duplicate are shown ±SEM. (**p < 0.01, ***p < 0.001). (D) The upregulation of Usp15 by morphine in Jurkat T cells is independent of cAMP and MAPK pathways. Similar quantitative RT-PCR experiments as described above were performed with forskolin (For; 25 μM), which was applied simultaneously with morphine, and cAMPS-Rp (cRp; 100 μM), U0126 (10 μM), SB203580 (SB; 1 μM) and genistein (66 μM), which were applied 1 h prior to morphine. At least two independent experiments performed in duplicate are shown ±SEM. (E) Western blot performed in primary human T cells showing the induction of Usp15. Cells were stimulated with morphine (1 μM) for 5 h.

FIGURE 5. Treatment of T cells with morphine results in the induction of the deubiquitinating enzyme Usp15. (A) Detection of Usp15 protein in Jurkat T cells in response to morphine by Western blot analysis. The cells were treated with morphine (Mo; 1 μM) for different times as indicated. The blot was reprobed for actin as a control. A representative example out of two experiments is depicted. (B) Detection of Usp15 transcripts in response to morphine and β-endorphin by quantitative real-time RT-PCR. Jurkat T cells were stimulated with the opioids (1 μM each) for the indicated times. Then, cells were lysed and RT-PCR was performed. The amounts of the Usp15-specific transcripts are normalized to those of β-actin. Two to three independent experiments performed in duplicate are shown ±SEM. *p < 0.05, **p < 0.01, ***p < 0.001. (C) The upregulation of Usp15 by morphine is dependent on calcium and the transcription factor NFAT and mediated by μ opioid receptors. Levels of Usp15 transcripts in Jurkat T cells were assessed by quantitative real-time RT-PCR and normalized to those of β-actin. Morphine (Mo) was used at a dose of 1 μM. CTAP (250 nM) was applied 1 h prior to morphine. Some samples were incubated overnight with 160 nM decay oligonucleotides directed against NF-κB, AP-1, and NFAT or mutated control oligonucleotides (mu−) prior to stimulation with morphine. As shown, some experiments were performed in a calcium-free medium. At least two independent experiments performed in duplicate are shown ±SEM. *p < 0.01, ***p < 0.001. (D) The upregulation of Usp15 by morphine in Jurkat T cells is independent of cAMP and MAPK pathways. Similar quantitative RT-PCR experiments as described above were performed with forskolin (For; 25 μM), which was applied simultaneously with morphine, and cAMPS-Rp (cRp; 100 μM), U0126 (10 μM), SB203580 (SB; 1 μM) and genistein (66 μM), which were applied 1 h prior to morphine. At least two independent experiments performed in duplicate are shown ±SEM. (E) Western blot performed in primary human T cells showing the induction of Usp15. Cells were stimulated with morphine (1 μM) for 5 h.

The NFAT-mediated induction of Usp15 is vital for the inhibitory effect of morphine on the TNF-triggered induction of IL-8 and NF-κB in T cells

To more conclusively demonstrate the vital roles of NFAT and Usp15 in the effect of morphine we investigated 1) the inhibition of the TNF-triggered IL-8 induction by morphine in the presence of decoy oligonucleotides directed against NFAT (Fig. 6A), and 2) the inhibition of the TNF-induced degradation of I-κB by morphine in the presence of siRNA directed against Usp15 (Fig. 6B). In the experiments using the decoy oligonucleotides directed against NFAT, we first performed controls to guarantee that these oligonucleotides do not interfere with the induction of IL-8 by TNF itself. The experiments indicated that NFAT is not involved in this effect (Fig. 6A, lanes 1–4). However, NFAT decoy oligonucleotides highly significantly blocked the effect of morphine, namely the inhibition of the TNF-induced transcription of IL-8 (Fig. 6A, lane 6, compare with lane 5). Mutated control oligonucleotides that do not bind NFAT did not interfere with the effect of morphine (Fig. 6A, lane 7, compare with lane 5).

Transfection of Jurkat cells with Usp15-specific siRNA resulted in a significant blocking of the effect of morphine to inhibit the TNF-triggered degradation of I-κB (Fig. 6B). In contrast, in cells transfected with non-Usp15–matching control siRNA the effect of morphine was not affected, emphasising the role of Usp15 in the effect of the opioid.

Discussion

We have recently demonstrated that the TNF-triggered NF-κB signaling in neuronal cells is inhibited by opioids via an AP-1–mediated induction of I-κB (17). In this study we used the TNF-triggered,
NF-κB–mediated induction of IL-8 as a well-established model (7–9) to investigate mechanisms underlying the modulation of NF-κB by opioids in T cells. At first glance, the effects of opioids on the transcription factor appear to be similar in the T cells and the neuronal cells. However, underlying mechanisms in the two cell types were found to be different, which is summarized in Fig. 7. We demonstrated that opioids induce the deubiquitinating enzyme Usp15 in a calcium- and NFAT-dependent manner in the T cells. Consequently, the TNF-triggered degradation of I-κB is inhibited by the opioids in T cells. In the cells of both origins, the effects of the opioids were mediated by μ opioid receptors, as demonstrated with the specific antagonist CTAP (see Figs. 1, 2, 5).

The inhibition of the TNF-triggered degradation of I-κB depends on the time of preincubation of the cells with morphine (see Fig. 2). Although incubation of the cells with the drug for 3 and 5 h a significantly inhibits the TNF-triggered degradation of I-κB, incubation of the cells with the drug for 8 and 24 h abolishes it. This time course is well in line with the time course of the induction of Usp15 (see Fig. 5). In contrast to the neuronal cells, an induction of I-κB was not observed in the Jurkat cells (see Fig. 2G, 2H). Astonishingly, it was reported that morphine inhibited the doxorubicin-induced NF-κB activity by inhibition of I-κB degradation in human neuroblastoma SH SY5Y cells (26), which were also used in our earlier investigation (17). However, Lin et al. (26) used an extremely high dose of morphine (200 μM) and the effect was not reversible by opioid receptor antagonists, which puts the specificity of this effect into question. We nevertheless took such a possibility into account and monitored the effect of morphine (in the common dose of 1 μM) on the TNF-induced degradation of I-κB in SH SY5Y cells during a period of 24 h, but we found no indication that morphine acted in such a way (data not shown).

The effect of the opioid in the T cells was dependent on calcium (see Figs. 3, 5) and the calcium-dependent transcription factor NFAT (see Fig. 5), but not on the cAMP and MAPK pathways. Coupling of the G protein–coupled μ opioid receptors to the calcium pathway, for example, to calmodulin, may occur in the canonical way via phospholipase C and inositol trisphosphate. Alternatively, it has been shown that μ opioid receptors interact directly with calmodulin and activate it via its binding to the third intracellular loops of the receptors (27).

The different opioid-induced mechanisms of NF-κB inhibition in the different cell types might be explained by cell type–specific coupling of μ opioid receptors to different effector/second messenger systems. In accordance with this hypothesis we observed that the induction of I-κB in the neuronal cells was not dependent on calcium, but involved the cAMP and MAPK pathways (17). Another explanation for the different opioid-induced mechanisms in the different cell types could be that different receptor subtypes are expressed in the different tissues. Such subtypes had been postulated earlier; however, there is no experimental evidence thus far that the immune cells would express μ opioid receptors that are structurally different from those expressed in neuronal cells. Rather, the receptors in the different tissues seem identical (24, 28).

In addition to the inhibition of the TNF-triggered degradation, the ubiquitination of I-κB, which is necessary for its degradation,
was also inhibited by morphine in the T cell model. In contrast, the ubiquitination of NEMO was not influenced by the drug (see Fig. 4). These findings, together with those that demonstrated that protein synthesis is needed for the inhibitory effect of morphine in the Jurkat cells (see Fig. 1), suggested consideration of the involvement of the deubiquitinating enzyme Usp15 in the effect of the opioid (29, 30). Whereas there are several reports showing that Usp15 stabilizes IκB by inhibiting its ubiquitination and degradation (e.g. Ref. 31), Usp15 seems not involved in the regulation of the ubiquitination of NEMO. This is in contrast to other deubiquitinating enzymes such as A20 (32) and CYLD (33), which are known to inhibit the ubiquitination of both IκB and NEMO. In fact, we found a remarkable induction of Usp15 in the Jurkat cells in response to morphine, whereas A20 and CYLD were not regulated by the drug. Significantly induced Usp15 protein levels were observed at the 3 h and later time points, which fits well with the kinetics of the morphine-mediated inhibition of the TNF-triggered degradation of IκB.

Earlier investigations in nonimmune cells have demonstrated ligand-biased signaling of μ opioid receptors, meaning that different ligands produce different effects on the same receptor in the same cell (34). Interestingly, it was reported that morphine acted in a different way compared with other agonists. Similarly, we have shown recently that the opioid-mediated induction of IL-4 in T cells depends strongly on the opioid. Thus, a strong induction of IL-4 was observed using fentanyl, loperamide, methadone, and β-endorphin, whereas only a moderate, ~10-fold lower induction of IL-4 was observed using morphine (C. Börner and J. Kraus, unpublished observations). Therefore, the induction of Usp15 was also determined for the endogenous opioid β-endorphin. However, morphine and β-endorphin produced similar mRNA induction patterns (see Fig. 5B), suggesting that this μ opioid receptor-mediated effect is not dependent on the opioid. Our observation is in good accordance with an earlier report suggesting that β-endorphin inhibits IL-8 in human placenta cells (35).

The above experiments suggested that the NFAT-mediated up-regulation of Usp15 by opioids is a key mechanism in the inhibitory effect of the drugs on NF-κB. In favor of this hypothesis were experiments using transcription factor decoy oligonucleotides directed against NFAT, as well as siRNA, that inhibited Usp15, which both abolished the inhibitory effect of morphine on NF-κB. The fact that morphine activated NFAT in the T cells raises the question of whether this factor regulated IL-8 itself. We could not find evidence in literature for a direct transactivation of IL-8 by NFAT in T cells. Few reports demonstrated regulation of IL-8 by NFAT in other cells, for example, it was suggested that NFAT and AP-1, but not NFκB, upregulate this cytokine in response to prokineticin 1 in endometrial epithelial Ishikawa cells stably expressing the prokineticin receptor 1 (36). Our own experiments do not suggest direct transactivation of IL-8 by NFAT in T cells, because morphine, which activated NFAT, did not regulate IL-8 (see Fig. 1B). In contrast to this, there is a large body of evidence in the literature indicating that NFκB is induced by IL-8 (e.g., Refs. 7–9). Also, binding of NFκB to regulatory IL-8 promoter sequences has been demonstrated: IL-8 promoter sequences ranging from nt −94 to −71 were shown to confer inducibility of the gene by TNF and to harbor a binding site for NFκB (37).

Finally, note that it has been suggested that some effects of opioids on T cells are not mediated by the classical opioid receptors. For example, it has been reported that morphine, at concentrations of up to 100 μM, inhibited the induction of IL-2 and the phosphorylation of IκB in activated T cells and induced phosphorylation of p42/44 MAPK, which was not reversible with the opioid receptor antagonist naloxone (38). In principle, two of these observations are similar to earlier observations from our group. In contrast, however, both the morphine (1 μM)-mediated inhibition of the induction of IL-2 in activated T cells (18) and the induction of phosphorylation of p42/44 MAPK (28) were blocked with CTAP in our experiments, and therefore they were very likely mediated by μ opioid receptors. Although there appears to be a tendency, the phosphorylation of IκB (see Fig. 2) was not significantly inhibited by morphine in our hands. Note that Mizota et al. (38) observed this effect at a concentration of morphine of 100 μM, which is at least 100-fold more than usual. More research will be necessary to understand, first, what effects opioids have on T cells, and second, how these effects are mediated. The low expression rate of opioid receptors on T cells (see Ref. 28 for a discussion on this) may make it even more difficult to answer this question.

In summary, the inhibition of the TNF-triggered activation of NFκB by morphine involves a complex interplay of mechanisms, which are different in T cells and neuronal cells. A different coupling of the μ opioid receptors to second messenger systems in different cell types might be the reason for this and offers interesting views for future investigations. Because NFκB mediates a bulk of functions in humans, better knowledge of its modulation, and especially its inhibition, is crucial to better understand its physiology. Additionally, better knowledge of the inhibition of NFκB is pharmacologically interesting toward a better understanding of novel (adverse) drug reactions.

Acknowledgments

We thank Dana Mayer for excellent technical assistance. Primary human T cells were provided by Burkhart Schraven from the Department of Molecular and Clinical Immunology, University of Magdeburg.

Disclosures

The authors have no financial conflicts of interest.

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