The IKK-NF-κB pathway: a source for novel molecular drug targets in pain therapy?

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ABSTRACT   Several studies indicate that the nuclear factor-kappa B (NF-κB) -activation cascade plays a crucial role not only in immune responses, inflammation, and apoptosis but also in the development and processing of pathological pain. Accordingly, a pharmacological intervention into this pathway may have antinociceptive effects and could provide novel treatment strategies for pain and inflammation. In this review we summarize the role of NF-κB in the nervous system, its impact on nociception, and several approaches that investigated the effects of various modulators of the classical I-κB-kinase-NF-κB signal transduction pathway in inflammatory nociception and neuropathic pain. The results indicate that NF-κB has an impact on nociceptive transmission and processing and that a number of substances that inhibit the NF-κB-activating cascade are capable of reducing the nociceptive response in different animal models. Therefore, a modulation of specific participants in the NF-κB signal transduction might exert a useful approach for the development of new painkillers.—Niederberger, E., Geisslinger, G. The IKK-NF-κB pathway: a source for novel molecular drug targets in pain therapy? *FASEB J.* 22, 3432–3442 (2008)

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THE TRANSCRIPTION FACTOR nuclear factor-kappa B (NF-κB) is composed of homo- and heterodimers of different Rel family proteins (p65, RelB, c-Rel, p52, and p50) and plays an important role in immune responses, inflammatory diseases, and cell death. All NF-κB subunits share a Rel homology domain that is crucial for dimerization, interaction with inhibitor of NF-κB (I-κB) proteins, nuclear translocation, and DNA binding. In most unstimulated cells, the majority of Rel/NF-κB dimers—the most common form is the p50/p65 heterodimer—are localized in the cytoplasm and sequestered by binding to the inhibitory subunit I-κB. On activation by a variety of stimuli, such as cytokines and bacterial lipopolysaccharides, I-κB is phosphorylated by I-κB kinases (IKKs), subsequently ubiquitinylated, and then degraded by a proteasome complex. Degradation of I-κB leads to the release of NF-κB from the trapping complex and translocation into the nucleus, where it binds to the promoter region of various genes, including cytokines [*e.g.,* tumor necrosis factor (TNF) -α, interleukin (IL) -1β], cyclooxygenase-2 (COX-2), inducible NO-synthase (iNOS), and proteases [*e.g.,* matrix metalloproteases (MMPs)], thereby activating their transcription (1, 2).

The activation of NF-κB can be induced by various pathways (**Fig. 1**). The classical, canonical pathway involves stimulation of TNF-, Toll-like, or T-cell receptors and leads to activation of an I-κB kinase complex consisting of the regulatory subunit IKKγ [also known as NF-κB essential modulator (NEMO)] and the catalytical subunits IKKα and β. This pathway is crucial for the activation of innate immunity and inflammation as well as inhibition of apoptosis. An alternative noncanonical pathway that is involved in B-cell activation, lymphoid organogenesis, and humoral immunity is activated by different stimuli, such as B-cell-activating factor, LT α/β, or CD40L, and depends on IKKα homodimers independently of IKKγ, thus activating p100/RelB. In contrast to the canonical pathway, this pathway shows slower activation kinetics. A third NF-κB-activating pathway is induced by DNA damage and activation of casein kinase 2 (2–4). Recently, a novel IKK complex has been elucidated that is activated by phorbol esters [phorbol 12-myristate 13-acetate (PMA)], lipopolysaccharide (LPS), and cytokines (5, 6). This pathway involves the activation of IKK epsilon (IKKε) and TANK-binding kinase (TBK), which are structurally similar to the classical IKKs α and β. It has been shown that these two kinases play major roles in the response to viral infection because both are involved in phosphorylation of interferon regulatory factors 3 and 7 and the subsequent activation of interferon-I (7, 8). However, they also appear to play a role in the NF-κB activation pathway by phosphorylation of several proteins including I-κBα, I-κB kinase β, p65, or c-Rel (5, 9–12). These pathways are still not fully understood, however.

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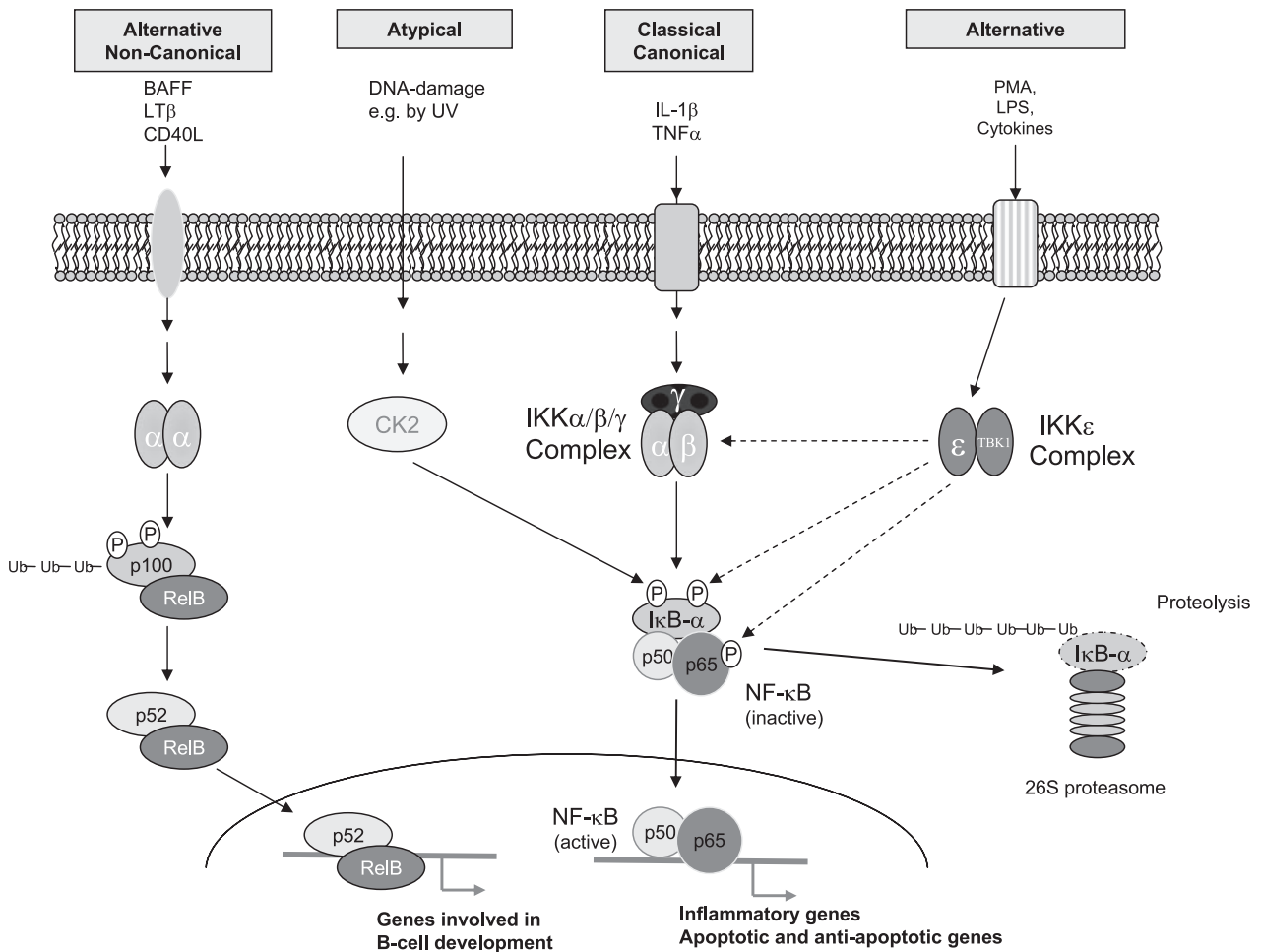
Figure 1. Schematic overview of different NF-κB activation pathways. The noncanonical pathway is activated by binding of the CD40 ligand, B-cell activating factor (BAFF), or lymphotoxin β (LTβ) to their receptors, leading to an activation of IKKα, which then induces the processing of the inhibitory NF-κB protein p100. p100 proteolysis releases p52, which can then translocate as a heterodimer with RelB into the nucleus and bind to the promoter of genes that are frequently involved in B-cell development. This pathway is independent of IKKγ but relies on IKKα homodimers. The atypical pathway, which is triggered by DNA damage such as by ultraviolet (UV) light, activates casein kinase 2 (CK2) and leads to phosphorylation and subsequent I-κBα degradation *via* an IKK-independent pathway. The classical canonical NF-κB-activating pathway can be induced by inflammatory stimuli such as chemokines and cytokines. This pathway is crucially dependent on activation of the classical IKKα,β,γ complex, which leads to the phosphorylation of I-κBα at serines 32 and 36 with subsequent ubiquitination (Ub, ubiquitin) and degradation of the inhibitory protein *via* the proteasome pathway. Then the heterodimer p50-p65 is released and migrates to the nucleus, where it binds to specific κB sites and activates a variety of NF-κB target genes, including COX-2, iNOS, or TNF-α. A novel alternative pathway is represented by an IKK complex consisting of IKKε and most likely the TANK-binding kinase 1 (TBK1). This complex is activated by different stimuli, such as phorbol esters (PMA, phorbol 12-myristate 13-acetate) or LPS, and may lead to phosphorylation of several targets in the NF-κB activation pathway leading to NF-κB activation. However, this activation pathway is not yet fully elucidated, which is indicated by the broken arrows.

NF-κB IN THE NERVOUS SYSTEM

In contrast to extensive research on NF-κB in the immune system and in host defense, less is known about its activation and functions in the nervous system. NF-κB transcription factors are expressed throughout the peripheral and central nervous system in neurons and glial cells. Inducible NF-κB was described in astrocytes (13), microglia, and oligodendrocytes (14), and inducible as well as constitutively active NF-κB is reported in neurons (15, 16). The most common dimers in the nervous system are composed of the subunits p50-p50 and p50-p65 (17). Activation of NF-κB can occur through the same stimuli as in the immune system. The knowledge of specific activators in the nervous system is insufficient at the moment, but several specific stimuli are described; for example, β-amyloid (18), nerve growth factor (NGF) (19), and synaptic transmitters such as glutamate are able to activate NF-κB in neurons (20, 21). Because Ca^{2+}-influx is the most prominent postsynaptic response to excitatory transmitters it is suggested that, in many cases, NF-κB is activated in a Ca^{2+}-dependent manner in the synapses (15). This is supported by the fact that

NF-κB as well as the ubiquitin-proteasome system, which is crucial for NF-κB activation, are coexpressed in synaptic terminals of neurons (22, 23).

NF-κB target genes are also incompletely characterized in the nervous system. Already known regulated genes in neuronal tissues make up iNOS, μ-opiod receptors, brain-derived neurotrophic factor, and Ca^{2+}/calmodulin-dependent protein kinase II (24).

In the peripheral nervous system, NF-κB is suggested to play an essential role in myelination of peripheral axons and in differentiation of Schwann cells (25). The functional role of NF-κB in the central nervous system involves mechanisms of learning and memory (synaptic plasticity) as well as neurodegeneration and neuroprotection. Mice with a deletion of p65 (TNF-R$^{-/-}$ background) or p50, respectively, showed severe learning deficits that might be due to reduced synaptic plasticity (17, 26). Furthermore, inhibition of NF-κB in neurons by I-κB also resulted in a reduced long-term potentiation and long-term depression and in behavioral disturbances (27). Interestingly, NF-κB is described to have neuroprotective and neurodegenerative properties as well. It has been shown that survival of adult sensory neurons is dependent on TNF-induced NF-κB activity and that inhibition of NF-κB by decoy oligonucleotides abolishes this neuroprotection (28, 29). On the other hand, inhibition of NF-κB protected neurons against toxicity induced by chemical modulators such as glutamate or kainate (21, 30). It has been suggested that these contrary functions are due to antiapoptotic effects of NF-κB in neurons and proapoptotic properties in glial cells (31).

THE ROLE OF NF-κB IN PAIN

A number of recent studies indicate that NF-κB is involved in the pathogenesis of neuropathic pain and in inflammatory hyperalgesia. Dysregulations of NF-κB are associated with chronic inflammation (32, 33) and neurodegeneration (32), and it has been shown that a blockade of NF-κB alters synaptic plasticity (34). During inflammation and injury, respectively, the percentages of activated NF-κB immunoreactive neurons and astrocytes were significantly increased in dorsal root ganglia and the spinal cord of rats (35, 36). Furthermore, it is well known that NF-κB is activated in a number of diseases that are notable for severe pain in the affected tissues. In rheumatoid arthritis an enhanced activation of NF-κB in synovial tissue has been described in early and later stages of joint inflammation. Accordingly, inhibition of NF-κB in mouse models of RA resulted in a decreased production of cytokines, an inhibition of disease severity, and a later onset of the disease (37, 38). Interestingly, NF-κB activation appears to be involved in migraine by transcriptional regulation of iNOS, which has been detected in rodent models as well as in patients during acute migraine attacks (39, 40). In bone diseases, an essential role of the receptor activator of NF-κB (RANK) and its ligand (RANKL) has

been postulated. RANK and RANKL are crucially involved in the regulation of osteoclast development and function (41), and functional dysregulations are associated with severe and painful bone diseases such as osteoporosis. By using a monoclonal antibody against RANKL, a prolonged inhibition of bone resorption in postmenopausal women was demonstrated (42).

In rodent models of neuropathy, NF-κB reporter mice showed a clear up-regulation of NF-κB transactivation after sciatic nerve transection in spinal neurons ipsilateral to the side of transection (43). Peripheral axotomy of lumbar dorsal root ganglia (DRG) neurons by sciatic nerve crush led to increased NF-κB levels in neurons, and it has been suggested that this effect is due to adult sensory neuron survival (29).

In NF-κB p50 knockout mice we could demonstrate a reduced nociceptive response to acute and inflammatory noxious stimulation, which indicates that this subunit plays a role in nociceptive transmission. The reduced nociceptive response was in accordance with a reduced COX-2 expression in the spinal cord of the knockout mice. Because the inflammatory paw edema was indistinguishable between wild-type and knockout mice, these effects were not due to decreased inflammation in knockout mice (44). Another group found that the antinociceptive effect of electroacupuncture is decreased in these mice, which is also a hint for the role of this NF-κB subunit in nociception (45).

NF-κB AS A PHARMACOLOGICAL TARGET FOR THE TREATMENT OF PAIN

The crucial role of NF-κB in several pathologies that accompany pain provides evidence that intervention with the NF-κB signaling cascade might have beneficial antinociceptive effects. The NF-κB-activating pathways, in particular the canonical activation, offer several targets for various pharmacologically active molecules (**Fig. 2**), which are discussed in more detail below.

Nonsteroidal antiinflammatory drugs (NSAIDs) and glucocorticoids

NSAIDs are first-choice drugs for the treatment of inflammatory pain and inflammation. Their mechanism of action is mainly based on inhibition of cyclooxygenase enzymes, which convert arachidonic acid into prostaglandins. In particular the COX-2 isoform is accepted as a proinflammatory enzyme that is induced by inflammatory stimuli and responsible for the generation of proinflammatory PGE_2. However, since aspirin and sodium salicylate were shown to inhibit the NF-κB activation pathway by competing with ATP at the ATP-binding site of IKKβ (46), it is clear that some NSAIDs are also capable of inhibiting the NF-κB pathway and that this may also contribute to their analgesic and antiinflammatory properties. Remarkably, these effects occur mainly at concentrations that exceed that of
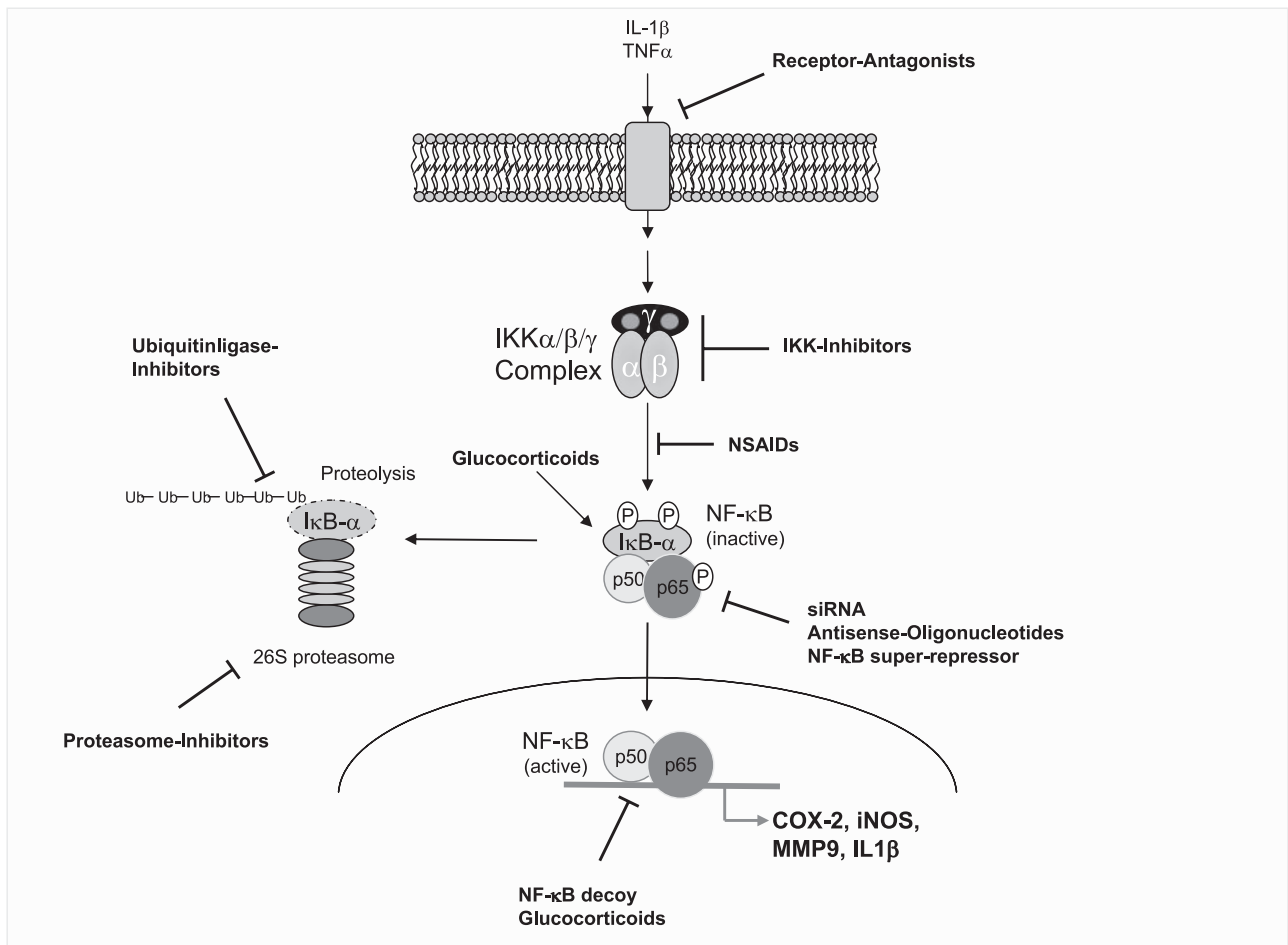
Figure 2. Potential pharmacological targets for analgesics in the classical NF-κB activation pathway.

cyclooxygenase inhibition, indicating COX-independent mechanisms.

R-flurbiprofen is considered the "inactive" isomer of the nonselective COX inhibitor S-flurbiprofen, because it at best only marginally inhibits cyclooxygenase (COX) activity. Nevertheless, R-flurbiprofen showed antinociceptive and antiinflammatory activity in paw inflammation models in rats (47, 48). Interestingly, it was shown that R-flurbiprofen effectively inhibits NF-κB activation and the transcription of several NF-κB-dependent genes such as cyclooxygenase 2. From these data it was suggested that the observed antinociceptive and antiinflammatory effects of R-flurbiprofen are at least partially mediated by inhibition of NF-κB (49). These results have been interpreted as particularly interesting because R-flurbiprofen has not been reported for the typical side effects of NSAIDs, such as gastrointestinal ulcerations, and might therefore be useful for the treatment of patients who are dependent on long-term analgesic therapy in painful pathologies that involve NF-κB activation.

COX-2-selective nonsteroidal antiinflammatory drugs (coxibs) also showed an impact on NF-κB activation, but not always in the same manner. While rofecoxib (50), etoricoxib, and lumiracoxib (51) inhibited LPS-stimulated NF-κB activation in RAW 264.7 mouse macrophages, celecoxib stimulated NF-κB activation at higher concentrations, which finally led to a loss of its antiinflammatory efficacy at higher doses (≥100 mg/kg) in the zymosan-induced inflammation model in rats (33).

Glucocorticoids are mainly used for the treatment of inflammatory diseases comprising allergies and autoimmune diseases. It has been suggested that their beneficial effects are due to inhibition of NF-κB at distinct stages of the NF-κB activation pathway. On the one hand, glucocorticoid treatment leads to an up-regulation of the NF-κB inhibiting protein I-κB (52, 53), and, on the other hand, glucocorticoid-activated glucocorticoid receptors bind to NF-κB subunits and thus prevent binding of NF-κB to the DNA and the subsequent transcription of NF-κB-dependent genes (54). A number of studies have been performed to investigate analgesic effects of glucocorticoids in the context of inflammatory and neuropathic pain. Because of their antiinflammatory properties, these drugs inhibit inflammatory hyperalgesia as expected (55). Interestingly, in several animal models of neuropathic pain, administration of glucocorticoids also led to an inhibition of allodynia and hyperalgesia (56, 57). A recent study that investigated betamethason in a model of spinal nerve injury showed that this glucocorticoid

inhibits NF-κB and reduces hyperalgesia after mechanical and thermal stimulation, indicating that there may be a link among glucocorticoids, NF-κB, and analgesia (58).

Although these results are all of general interest, it is unlikely that NSAIDs or glucocorticoids will be applied as specific NF-κB inhibitors in clinical practice. To inhibit NF-κB activation, NSAIDs have to be used at severalfold higher doses than necessary for COX inhibition. Because these drugs already exert a number of side effects at the physiological COX-inhibiting doses, it is clear that higher doses will increase these unwanted effects. Glucocorticoids are also connected to a number of severe concomitants, such as glaucoma or osteoporosis. Moreover, it must be mentioned that in the context of neuropathic pain, studies also showed that neuropathic pain can be inhibited by glucocorticoid receptor antagonists and that glucocorticoids can restore neuropathic pain behavior (59, 60).

Thalidomide as an immunomodulatory drug

Although the primary target of the immunomodulatory drug thalidomide is the tumor necrosis factor, a number of studies have reported a direct inhibition of NF-κB through this drug (61–63). In addition to TNF-α reduction, this NF-κB inhibition might contribute to antinociceptive effects of thalidomide, which have been shown several times. Thalidomide reduces endoneurial TNF-α up-regulation as well as thermal hyperalgesia and mechanical allodynia in chronic constrictive sciatic nerve injury (64, 65). Hyperalgesic responses in inflammatory models of carrageenan- and bradykinin-induced paw inflammation were dose dependently inhibited by thalidomide (66). However, use of thalidomide is limited because of its neurotoxic and teratogenic effects. Newer analogues may improve the benefit/risk ratio for such immunomodulators. Other immunomodulatory drugs, such as FK506 (tacrolimus), have also been linked to NF-κB inhibition and showed antiinflammatory and antinociceptive properties in carrageenan-induced inflammation in rats (67).

Receptor antagonists

Inhibition of membrane receptors that can induce NF-κB activation on ligand binding is the earliest possible interference in the NF-κB activation cascade and an attractive target for that reason. However, it is clear that these inhibitions will not be very specific for the NF-κB pathway but will also interfere with other signaling pathways, such as MAP-kinase signaling.

Inhibition of TNF receptors

Recent evidence suggests that tumor necrosis factor plays a crucial role in the pathophysiology of inflammatory and neuropathic pain. Peripheral nerve injury leads to the up-regulation of TNF-α and TNF-R1 in the DRG and the spinal dorsal horn (68, 69), whereas inhibition of TNF-α synthesis (64, 66) or antagonism of TNF-α receptors (70) reduces neuropathic pain behaviors. Because a NF-κB inhibitor (PDTC) blocked TNF-alpha-induced long-term potentiation in models of neuropathic pain, it must be suggested that the TNF-induced pain is at least partially mediated by NF-κB (71). Antagonism of the TNF-signaling pathway also revealed antinociceptive effects in inflammatory models. In clinical settings, TNF receptor signaling is already interrupted by soluble TNF-R or anti-TNF-antibody, which both bind the TNF receptor ligand and thus interrupt the subsequent signal transduction (72).

IL-1β receptor antagonists

IL-1 plays a major role in a wide range of inflammatory and autoimmune diseases, including rheumatoid arthritis, osteoarthritis, atherosclerosis, and diseases of the central nervous system such as multiple sclerosis (MS), Alzheimer's disease, and stroke. A number of different approaches are being used to target the IL-1 pathway and its receptor. IL-1Ra [anakinra (Kineret; Amgen, Inc., Thousand Oaks, CA, USA)] is approved for the treatment of RA and is used in clinical studies for other inflammatory diseases. IL-1 trap, anti-IL-1 antibody, and soluble IL-1 receptor II (sIL1R-II) are also under study in this context (73). Although all these drugs have been mainly developed to treat inflammatory diseases, a number of indications suggest that they may have pharmacological efficacy in pain. It has been shown that the IL1 receptor antagonist IL-1Ra attenuated hyperalgesia induced by IL-1β, formalin, and capsaicin (74–76), respectively, as well as dynorphin-induced allodynia (77). Because NF-κB is a major target in the IL-1β signal transduction pathway, inhibition of IL-1β also inhibits NF-κB activation.

RAGE inhibitors/antagonists

The receptor of advanced glycation end products (RAGE) is associated with NF-κB in the field of diabetic neuropathy. Binding of ligands to RAGE results in activation of the transcription factor NF-κB and subsequent expression of NF-κB-regulated cytokines and is a possible pathomechanism in diabetic and vasculitic polyneuropathies (78). Studies using RAGE knockout mice showed that these mice exhibit decreased NF-κB activation, accompanied by prevention of a loss in pain perception in diabetic neuropathy (79). Increased RAGE expression has been observed in synovial cells of patients with rheumatoid arthritis or osteoarthritis (80, 81), and application of soluble RAGE could effectively block inflammatory responses (82).

IKK inhibitors

Inhibitors of the catalytical subunits

The development of selective, small-molecule I-κB kinase inhibitors has been a focus of the pharmaceutical

industry in the last few years. Although no potent specific IKKα inhibitor has been developed so far, most of the generated IKK inhibitors selectively inhibit the β-subunit of the kinase complex. At least 3 of these IKKβ inhibitors have reached clinical phase II studies and showed antiinflammatory activities in rodent inflammatory models. SPC 839 (Celgene) inhibits IKKβ with an IC_{50} of 62 nM and was effective in reducing paw edema in an arthritis model. Another IKKβ inhibitor, BMS345541, has also shown efficacy in a mouse model of collagen-induced arthritis without signs of major toxicology (reviewed in ref. 1).

In our group we investigated the antiinflammatory and antinociceptive effects of the I-κB kinase inhibitor S1627, which also preferentially inhibits the β-subunit of the IKK complex. Specific inhibition of I-κB kinase by S1627 modulates the nociceptive response in various nociceptive models in rats. S1627 inhibited the activation of NF-κB in the spinal cord and thereby thermal and mechanical hyperalgesia in the zymosan-induced paw inflammation model and the inflammatory edema. It also reduced tactile and cold allodynia in a model of neuropathic pain (83). These data are in accordance with a study that showed that spinal application of S1627 in rat acute knee joint inflammation before and early in the development of inflammation totally prevented spinal hyperexcitability (84).

The role of the novel IKKε in pain and inflammation is not yet clear; however, expression of IKKε in fibroblast-like synoviocytes in patients with rheumatoid arthritis or osteoarthritis might indicate a potential role of this kinase in chronic inflammatory diseases as well (85, 86).

It has been suggested for a long time that IKKs are the most specific and effective pharmacological targets in the NF-κB activation pathway, because it appeared very likely that all of their target proteins are exclusively involved in NF-κB activation. In the meantime, this point of view has changed, and it is well known that IKKs are also able to phosphorylate proteins that are participants in distinct signal transduction pathways such as 14–3-3β or insulin receptor substrate-1 for IKKβ, and β-catenin or histone H3 for IKKα (reviewed in ref. 87). Furthermore, at least one promising specific IKKβ inhibitor has been shown to lead to dramatic loss of bone marrow B cells (88). For their therapeutic use, it is likely that they will initially be used as anticancer drugs because of the antiapoptotic properties of NF-κB. It is suggested that selective inhibition of IKKβ might sensitize cancer cells to undergo apoptotic cell death after chemotherapy (89).

However, because these effects also affect all the other cells of the body, inhibition of IKKβ might furthermore bear the danger of side effects, in particular liver toxicity due to enhanced apoptosis in liver cells (1). This is supported by the fact that IKKβ knockout mice are embryonically lethal (90). Furthermore, recently it has been shown that IKKβ seems to be crucial as a negative regulator of IL-1β secretion. Mice lacking the β subunit of IKK in myeloid cells were more susceptible to endotoxin shock than control mice, which might be a serious challenge for long-term IKKβ inhibition (91).

Inhibitors of the regulatory subunit (NBD inhibitors)

The complex consisting of IKKα/β and γ (NEMO) is an essential regulator of NF-κB activation in all inflammatory processes. The binding of the regulatory subunit NEMO to the catalytic subunits is coordinated by a very small peptide sequence in the C-terminal sequence of both IKKα (L738-L743) and β (L737 and L742), a region that has been termed the NEMO binding domain (NBD). NBD binding inhibitors prevent binding of the regulatory to the catalytic subunits and therefore inhibit NF-κB activity. In the carageenan-induced model of acute paw inflammation, i.p. administration of a cell-permeable NBD binding peptide reduced inflammation significantly comparable with the effect of dexamethasone (92). Furthermore, in collagen-induced arthritis in mice, peptide-injected mice showed a lower incidence, delayed onset, and lower severity of the disease. It is important to note that no signs of toxicity, organ damage, or death were observed throughout this study (93).

Taken together, modulation of NEMO appears to be a truly promising approach to inhibit NF-κB activation selectively under pathological conditions. Because NEMO is involved only in the classical NF-κB activation pathway, alternative physiological pathways will not be affected. The problem in this case is that so far only peptides are available as NEMO inhibitors, which does not allow for practical oral administration in patients. However, the small size of the NBD raises the possibility of designing small molecule inhibitors. The development of such molecules would present a crucial progress in the development of pharmacologically useful NBD inhibitors.

Proteasome inhibitors

Degradation of I-κBα by the proteasome is an important step in the NF-κB activation cascade and may therefore also serve as a tool for pharmacological NF-κB inhibition. The proteasome inhibitor bortezomib is already approved for the treatment of multiple myeloma, and its mechanism of action is linked to NF-κB. A second proteasome inhibitor, PR171, has been developed to treat patients who are resistant to bortezomib (94). However, because neuropathic pain has been reported as one of the side effects of bortezomib (95), it is not very likely that these drugs will be applied for the treatment of pain.

Ubiquitin-ligase inhibitors

The metal chelator pyrrolidine dithiocarbamate (PDTC) has been shown to suppress reversibly the release of the inhibitory subunit I-κB from the latent cytoplasmic form of NF-κ B at micromolar concentra-

tions in cells treated with phorbol ester, interleukin-1, tumor necrosis factor alpha, and bacterial lipopolysaccharide (96). PDTC was originally thought to inhibit NF-κB activity by its antioxidative properties. However, it is now known that inhibition of I-κB ubiquitin-ligase in an unspecific manner is the underlying mechanism of NF-κB inhibition rather than antioxidation (97, 98). Administration of PDTC in a model of TNF-induced mechanical allodynia in rats resulted in inhibition of the TNF-induced up-regulation of TNF-α and TNF-R1 in dorsal root ganglia and the spinal cord and subsequently in reduced mechanical allodynia, thus suggesting that NF-κB is involved in the TNF-response (99).

Inhibitors of NF-κB nuclear translocation

Overexpression of I-κBα

Phosphorylation and degradation of I-κBα is an important step in the NF-κB activation cascade by enabling NF-κB to nuclear translocation. The phosphorylation occurs on two N-terminal serines positioned at residues 32 and 36. To prevent I-κB phosphorylation, subsequent degradation, and the release of NF-κB in response to activating signals, an I-κBα protein mutated at these residues has been generated (I-κB superrepressor) (100). This I-κBα super-repressor showed beneficial effects in a rat model of rheumatoid arthritis (101). Furthermore, selective inhibition of NF-κB activation in glial cells of the spinal cord by lentiviral delivery of the I-κB super-repressor resulted in prolonged antihyperalgesic and antiallodynic effects in a model of neuropathic pain (chronic constriction injury) (102). These results were in accordance with data from transgenic mice that overexpress I-κBα. These mice exhibited a reduced expression of proinflammatory proteins, such as CCL2 and TGF-β2, as well as decreased pathological signs and a dramatic improvement of the functional recovery after spinal cord injury (103, 104). Moreover, a decreased nociceptive behavior has been observed in the formalin assay (103).

SN50

SN50 is a small cell-permeable peptide consisting of the nuclear localization sequence of the p50 subunit of NF-κB, which is fused to a linker sequence that facilitates cell permeability. This peptide effectively inhibits NF-κB activation to a variety of stimuli (105). Intrathecal application of SN50 in rats attenuated allodynia induced by the HIV envelope protein gp120 (106).

Oligonucleotide NF-κB inhibitors

DNA technology, such as the antisense or decoy oligonucleotides or siRNA, respectively, has been shown as another possible approach to regulate transcription of NF-κB-dependent genes *in vivo* and might provide important therapeutic potential because of its high specificity.

NF-κB antisense oligonucleotides

Antisense oligonucleotides that target the mRNA sequence of NF-κB have already been studied in a number of *in vitro* and *in vivo* inflammatory models and were shown to inhibit the expression of several NF-κB-dependent genes (107, 108). However, little is known about their potential use in models of inflammatory and neuropathic pain. Until now, only one study using the chronic constriction injury model of neuropathy showed an attenuation of NF-κB expression and a reduced mechanical as well as thermal hyperalgesia after intrathecal injection of p65 antisense oligonucleotides (109). These data indicate that NF-κB antisense application might also exhibit a useful approach for the therapy of pain.

NF-κB decoy

NF-κB decoy oligonucleotides are synthetic double-strand oligonucleotides with the NF-κB specific consensus sequence that bind to activated NF-κB and thus prevent the binding of the transcription factor to the promoter of NF-κB-regulated genes (110). Several studies have been performed using NF-κB decoy oligonucleotides to determine their effects on inflammatory and neuropathic pain. Injection of NF-κB decoy into the sciatic nerve of rats suppressed the inflammatory response as well as mechanical allodynia and thermal hyperalgesia in complete Freund's adjuvant-induced paw inflammation (111). Intrathecal administration of NF-κB decoy led to a decreased expression of COX-2 and iNOS in the spinal cord and an attenuation of mechanical allodynia and thermal hyperalgesia (112). Moreover, local intraplantar treatment of rats with NF-κB decoy oligonucleotides inhibited paw edema formation and thermal hyperalgesia in response to chemically induced peripheral inflammation paralleled by decreased NF-κB p65 and COX-2 protein levels in DRG and paw tissue, respectively (113, 114). In a model of nerve injury, injection of NF-κB decoy, at the site of a nerve lesion, significantly alleviated thermal hyperalgesia for up to 2 wk and suppressed the expression of mRNA of inflammatory cytokines, iNOS, and adhesion molecules at the site of nerve injury (115). In synovial cells from patients with rheumatoid arthritis, NF-κB decoy decreased the expression of inflammatory cytokines and MMPs (116).

NF-κB siRNA

Small interfering RNA directed against NF-κB or other participants in the NF-κB activation cascade are also considered as novel therapeutics for the treatment of inflammatory diseases. *In vitro* studies have already shown an inhibitory effect of p65-siRNA on transcription of NF-κB-regulated proteins such as COX-2 or MMPs (117) that are also involved in pain. However, until now no study has been conducted that investigated the effects of such molecules *in vivo*.

In general the use of oligonucleotides appears to be a promising pharmacological tool because of their high specificity. However, some obstacles must be circumvented. First, the problem of delivery, especially the specific delivery into organs and cells, is still not solved. Several approaches have been raised using viral transfection methods or chemical modifications of the oligonucleotides, but these modifications are often accompanied by novel adverse effects that restrict clinical safety. Second, stability is also a great problem because most ODN are degraded very quickly *in vivo*. Moreover, at least antisense oligonucleotides and decoy oligonucleotides are quite large molecules, which also constrains their cellular uptake and bioavailability. Because their main target in the NF-κB cascade is inhibition of NF-κB DNA binding, it is unlikely that small molecules can be found for this purpose because of the large interaction surface of NF-κB and the DNA. Taken together, great affords have to be raised to improve these drugs concerning the above mentioned issues before a clinical application can be considered.

CONCLUSIONS

NF-κB is an essential and ubiquitously expressed transcription factor whose function is involved in numerous diseases including inflammatory and neuropathic pain. Therefore, inhibition of NF-κB activity might provide a functional tool in the development of novel therapeutics for patients with pain and inflammation. However, because NF-κB also fulfils a number of physiological functions, unselective and complete inhibition might lead to several serious side effects. This assumption is confirmed by approaches to develop knockout mice for several components of the NF-κB activation pathway. Animals with complete knockout of p65, IKKα, β, or γ, respectively, die during embryonic development or perinatally, indicating crucial functions of NF-κB in development and survival (90, 118–120). Therefore, drugs that selectively inhibit pain-related NF-κB activity while leaving basal activity unaffected would be of great clinical benefit with reduced unwanted effects.

Based on these facts, a number of the discussed NF-κB inhibitors will probably be excluded from the list of suitable NF-κB inhibitors. NSAIDs, glucocorticoids, and receptor antagonists as well as proteasome inhibitors and ubiquitin-ligase inhibitors will most probably interfere with signal transduction pathways besides NF-κB and therefore provide unexpected effects. At the moment, inhibition of NEMO and the use of oligonucleotide inhibitors are the most promising approaches to specifically impair pain-related NF-κB activation.

It is obvious that most of the cited studies in which the IKK-NFκB pathway was inhibited have been performed in an inflammatory context and that the inhibition of inflammation often also contributes to antinociceptive effects. However, the studies that concentrated on inflammatory pain or, in particular, neuropathic pain showed that the antinociceptive properties of the respective inhib-

itors are not only due to their antiinflammatory effects. Therefore, inhibition of specific parts of the NF-κB activation pathway might exert a useful approach for the development of new painkillers. Nevertheless, a number of further and more detailed studies will be necessary to reach this target in the future. ▣

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