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Nuclear Factor κB Signaling in Opioid Functions and Receptor Gene Expression

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Abstract

Opiates are the most powerful of all known analgesics. The prototype opiate morphine has been used as a painkiller for several thousand years. Chronic usage of opiates not only causes drug tolerance, dependence, and addiction, but also suppresses immune functions and affects cell proliferation and cell survival. The diverse functions of opiates underscore the complexity of opioid receptor signaling. Several downstream signaling effector systems, including adenylyl cyclase, mitogen-activated protein kinase, Ca^{2+} channels, K^+ channels, and phosphatidylinositol 3-kinase/Akt, have been identified to be critical in opioid functions. Nuclear factor-κB (NF-κB), one of the most diverse and critical transcription factors, is one of the downstream molecules that may either directly or indirectly transmit the receptor-mediated upstream signals to the nucleus, resulting in the regulation of the NF-κB-dependent genes, which are critical for the opioid-induced biological responses of neuronal and immune cells. In this minireview, we focus on current understanding of the involvement of NF-κB signaling in opioid functions and receptor gene expression in cells.

Keywords

Akt; NF-κB; gene expression; opioid receptor; PI3K

Introduction

Opiates have been used as medicine for thousands of years and are still major clinical analgesic and addictive drugs. The prototype opiate morphine is the main active component in opium, which was first isolated by the German chemist Friedrich Sertuner during the period of 1803–1805 (Schmitz 1985). Three opioid receptors have been cloned and belong to the rhodopsin family of the G protein-coupled receptor (GPCR) superfamily (Chen et al. 1993; Evans et al. 1992; Fukuda et al. 1993; Kieffer et al. 1992; Li et al. 1993; Meng et al. 1993; Yasuda et al. 1993). The opioid receptor subfamily is composed of three subsets of the gene products, the mu (μ), delta (δ), and kappa (κ) opioid receptors (MOR, DOR, and KOR, respectively), which play an important role in the pain control mechanism. Genetic knockout studies have demonstrated that almost all opioid functions, including analgesia, reward, withdrawal, respiratory depression, immunosuppression, and constipation, are mediated through these three opioid receptors (reviewed by Kieffer and Gaveriaux-Ruff 2002). The opioid receptors are generally coupled with G_i or G_o proteins that are sensitive to pertussis toxin (PTX) treatment. Binding of an opioid agonist to its receptor initiates THE activation

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of G protein-coupled opioid receptor signaling (reviewed by Law et al. 2000, 2004), resulting in the dissociation of $G\alpha_i$ from inhibitory $G\beta\gamma$ dimer (reviewed by Law et al. 2000; Standifer and Pasternak 1997). Dissociated $G\alpha_i$ and $G\beta\gamma$ subunits then mediate an array of downstream effectors, including inhibition of adenylyl cyclase (AC) activity (Ozawa et al. 1999; Prather et al. 1994; Smart et al. 1997; Wong et al. 1991) and both N- and L-type Ca^{2+} channels (Piros et al. 1995; Tallent et al. 1994), activation of inward rectifying K^+ channels (Henry et al. 1995), mitogen-activated protein kinase (MAPK) (Fukuda et al. 1996; Li and Chang 1996), phosphotidylinositol 3-kinase (PI3K) (Polakiewicz et al. 1998b), and phospholipase C (PLC) (Miyamae et al. 1993) as illustrated in Fig. 1. These effectors then control many important intracellular signal transduction pathways that regulate cellular responses and gene expression. Furthermore, the expression of the three opioid receptors is temporally and spatially regulated, and different mouse strains with varied receptor levels respond to morphine treatment differently (Law et al. 2004). Taken together, these observations indicate that both the receptor level and downstream signaling pathways play critical roles in opioid-mediated cellular responses.

Opioid-mediated immune responses have been investigated for two decades (Fischer 1988; Sibinga and Goldstein 1988), and there has been a wide appreciation of the effects of opioids on immune responses (Rogers and Peterson 2003). Recently, *mor*-null mouse studies have confirmed that MOR is involved in morphine immunosuppression (Gaveriaux-Ruff et al. 1998; Roy et al. 1998a). The transcription factor NF-κB is one of the most important transcription factors in immune cells (Li and Verma 2002). It may also be a critical transcription factor in neuronal cells (O'Neill and Kaltschmidt 1997). Various GPCRs mediate immune responses through the transcriptional regulation of NF-κB-dependent genes (Ye 2001). Moreover, NF-κB is either directly or indirectly involved in several GPCR-mediated downstream signaling pathways, including the cAMP/protein kinase A (PKA)/cAMP response element binding protein (CREB), PI3K/Akt/inhibitor of IκB kinase complex (IKK), and MAPK/IKK pathways (Ye 2001). Many research laboratories have reported that NF-κB may be involved in opiate-meditated immune functions (Azuma and Ohura 2002; Carr et al. 1995; Kraus et al. 2003; Roy et al. 1998b; Wang et al. 2003; Welters et al. 2000a). More recently, NF-κB has been found to be involved in the transcriptional regulation of *mor* gene expression in primary immune cells (Kraus et al. 2003) and *dor* gene expression during nerve growth factor (NGF)-induced differentiation of neuronal cells (Chen et al. 2006). NF-κB may also be involved in *kor* gene expression in P19 cells (Law et al. 2004). The opioid receptor expression level on the cell surface is detrimental to opioid functions (Law et al. 2004). Moreover, inhibition of NF-κB activation by pyrrolidine dithiocarbamate attenuates the effect of opiate withdrawal (Capasso 2001). Together, these data indicate that NF-κB may play a key role in the opioid-modulated neuronal and immune responses. This minireview focuses on the recent advances in the involvement of NF-κB signaling in opioid functions and receptor gene expression in neuronal and immune cells.

Opioid receptor signaling

The main side effects of opioid analgesic drugs are tolerance (diminished responsiveness to the drug), dependence (physically dependent on the drug), and craving for the drugs, which are the core features of drug addiction. It has been hypothesized that the transcriptional and post-translational modulation of receptor signaling is the basis of morphine tolerance and dependence (Law et al. 2004). The main opioid responses, including analgesic effect, tolerance and dependence, and immunosuppression, were abolished in opioid receptor gene-null mice (reviewed by Kieffer and Gaveriaux-Ruff 2002), further confirming that these opioid effects are mediated via opioid receptors. Activation of opioid receptor signaling leads to the modulation of several downstream effectors (Fig. 1), including AC, K^+ channels, Ca^{2+} channels, MAPK, and PI3K (reviewed by Law et al. 2000; Williams et al.

2001). Acute and chronic opioid treatments lead to changes in both the levels and activities of these effectors. These changes further lead to transcriptional regulation of other genes whose products may be associated with cellular responses to chronic opioid treatment such as tolerance (or receptor desensitization), dependence, and immunosuppression. The molecular mechanism and regulation of opioid receptor signaling have been subjected to several reviews (Law et al. 2000, 2004; Williams et al. 2001). The different cellular responses to acute and chronic use of opioids were also elegantly reviewed by Taylor and Fleming (2001). Readers who are interested in more information on how the opioid modulation of these downstream effector signaling pathways leads to the main cellular responses, including tolerance, dependence, and immunosuppression, are referred to review articles on these subjects (Kieffer and Gaveriaux-Ruff 2002; Law et al. 2000, 2004; McCarthy et al. 2001; Rogers and Peterson 2003; Sharp 2003; Williams et al. 2001; Zhang and Oppenheim 2005).

Among these opioid receptor downstream effectors, AC is one of the most studied biochemical markers and has been used to distinguish the difference between acute and chronic opioid treatments. Acute opioid treatment results in inhibition of AC activity and leads to the release of neurotransmitters, including gamma-aminobutyric acid, glutamate, and substance P (reviewed by Williams et al. 2001); chronic opioid treatment leads to diminished inhibition of AC activity (similar to desensitization or tolerance), whereas the administration of an opioid antagonist to the chronic opioid-treated cells results in activation of AC signaling (similar to dependence and withdrawal) (Collier and Francis 1975; Sharma et al. 1975). More recently, Nestler (2004) demonstrated similar changes in the activity of AC and AC-mediated downstream effectors in the locus coeruleus *in vivo* as shown in cell culture. Inhibition of AC activity by opioids reduces the cellular level of cAMP, resulting in inhibition of PKA, which eventually leads to inhibition of CREB, a critical transcription factor involved in learning, memory, and opioid tolerance and dependence (Nestler 1992). Thus, tolerance and dependence-like biochemical changes in the cAMP/PKA/CREB pathway in cells provide a cellular model for the study of opioid tolerance and dependence.

NF-κB signaling

NF-κB plays an important role in the development and maintenance of the immune system and in the coordinated response to infection (Li and Verma 2002; Pomerantz and Baltimore 2002). Many cytokines such as interleukin-1 (IL-1), IL-2, IL-6, tumor necrosis factor alpha (TNF-α), and granulocyte–macrophage colony-stimulating factor and chemokines such as IL-8, RANTES, and monocyte chemoattractant protein 1 are regulated by NF-κB (Ghosh and Karin 2002). For instance, binding of foreign antigens to the receptors on cells of the innate immune system leads to activation of NF-κB signaling, resulting in proinflammatory cytokine production and activation of T cells in the adaptive immune response. The proinflammatory cytokines TNF-α and IL-1 then activate NF-κB, resulting in the recruitment of other inflammatory and immune cells to the infection site (Pomerantz and Baltimore 2002). NF-κB is composed of homo- and heterodimers of five members of the Rel family, including NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, and c-Rel. The p50/p65 complex is the most common functional heterodimer found in cells (Li and Verma 2002). The canonical NF-κB signaling pathway is as follows: proinflammatory cytokines, growth factors, and hormones bind to their cell surface receptors to activate IKK; activated IKK complex, which contains IKKα, IKKβ, and IKKγ [also called NF-κB essential regulator (NEMO)] subunits, then phosphorylates IκBα, which is bound with the NF-κB p50/p65 heterodimer in an inactive state, mainly in the cytoplasm; phosphorylated IκBα is released from the complex and undergoes proteasome-dependent degradation; freed NF-κB then translocates to the nucleus to induce the expression of target genes (Baeuerle and Baltimore 1996). In addition, there is a noncanonical pathway for the activation of NF-κB

through NF-κB-induced kinase (NIK) signaling. In the noncanonical pathway, IKKα is phosphorylated by NIK, resulting in the translocation of the p100/RelB heterodimer complex into the nucleus and induction of NF-κB target genes (reviewed by Pomerantz and Baltimore 2002). The molecular mechanisms of activation of the IKK complex are complicated (reviewed by Chen and Greene 2004; Ghosh and Karin 2002; Li and Verma 2002; Pomerantz and Baltimore 2002). For instance, among many NF-κB signaling stimuli, TNF activates the IKK complex by activation of MAPK/ERK kinase kinase 3 (MEKK3) (Yang et al. 2001).

NF-κB has also been recognized as important in the central nervous system (CNS) and peripheral nervous system (PNS) (Meffert and Baltimore 2005; O'Neill and Kaltschmidt 1997). Several of signaling molecules have been identified to activate NF-κB signaling in neuronal cells, including TNF (Barger et al. 1995), glutamate (Guerrini et al. 1995), and NGF (Carter et al. 1996; Chen et al. 2006; Maggirwar et al. 1998; Rojo et al. 2004). Activation of the transcription factor NF-κB is required for peripheral myelin formation in Schwann cells (Nickols et al. 2003), in fear-potentiated startle (Yeh et al. 2002), and in synaptic signaling and behavior (Meffert et al. 2003). NGF may result in activation of the IKK complex via at least three possible mechanisms: activation of PI3K/Akt signaling (Chen et al. 2006; Rojo et al. 2004; Yuan and Yankner 2000), p62/aPKC (Wooten et al. 2001), and p75/TNF receptor associated factor 6 (TRAF6) (Carter et al. 1996; Yeiser et al. 2004). High constitutive activity of NF-κB has been observed in various neurons (Lezoualc'h et al. 1998; O'Neill and Kaltschmidt 1997).

Activation of GPCRs results in activation or inhibition of NF-κB signaling through various downstream effector pathways, including the cAMP/PKA/CREB, PI3K/Akt/IKK, and PLC/PKC/IKK signaling pathways (reviewed by Ye 2001). For instance, β2 agonist isopreterenol-induced activation of cAMP/PKA/CREB leads to inhibition of NF-κB signaling, while both α2 agonist-induced activation of PI3K/Akt/IKK and C5a-activated PLCβ/PKC/IKK signaling lead to activation of NF-κB (Ye 2001). NF-κB can also be activated by calcium/calmodulin-dependent kinase II signaling in synapses (Meffert et al. 2003) and by TNF-induced activation of potassium channel activity (Wang et al. 2005). It appears that these opioid receptor-mediated downstream effector pathways converge on NF-κB signaling in both neuronal and immune cells. Thus, the observations described above prompted us to hypothesize that modulation of NF-κB signaling by opioids may be one of the key factors that are associated with opioid-induced responses in cells.

Involvement of NF-κB signaling in opioid-mediated immune and neuronal responses

Opioids have a wide range of immune responses and the observed effects are very diverse and often cell-type-dependent. Some of immune responses are mediated via the direct interaction between the opioids and receptors, where others might be mediated through indirect pathways (reviewed by McCarthy et al. 2001; Roy et al. 1998b). Opioids and their receptors appear to play an important role in the communication between the nervous and immune systems (reviewed by Rogers and Peterson 2003; Salzet et al. 2000; Sharp 2003; Zhang and Oppenheim 2005). The chronic use of opiates leads to immunosuppression in animal models and human patients. Immunosuppression manifests with a decrease in the proliferative capacity of macrophage progenitor cells and lymphocytes (Roy et al. 1998b). The genetic knockout studies provide a definitive support to the notion that opiate-induced immunosuppression is mediated mainly via the opioid receptors (Gaveriaux-Ruff et al. 1998; Roy et al. 1998a). For instance, chronic morphine treatment resulted in a reduction of the ratio of CD4(+)/CD8(+) cells in the thymus and loss of natural killer activity in wild-type mice, whereas no effect was observed in MOR-deficient mice (Gaveriaux-Ruff et al. 1998; Roy et al. 1998a). Macrophage phagocytosis and macrophage secretion of TNF-α, which were modulated by morphine in wild-type mice, were not observed in *mor*-null mice

(Gaveriaux-Ruff et al. 1998; Roy et al. 1998a), suggesting that opioid receptor signaling plays a key role in opioid-mediated immune functions. Several reviews have extensively covered pharmacologic studies of opioid-mediated immune responses (Rogers and Peterson 2003; Salzet et al. 2000; Sharp 2003; Walker 2003). The molecular mechanism of opioid-mediated immune responses is not well defined. One of the hypotheses that have been put forward to account for opioid-mediated immune responses is the opioid-induced cross-heterologous desensitization of chemokine receptors (reviewed by Rogers and Peterson 2003; Zhang and Oppenheim 2005). Treatment of leukocytes with opioids leads to desensitization of chemokine receptors through a not-well-defined signaling pathway (Rogers and Peterson 2003). Another possible mechanism proposed by Sharp (2003) is that DOR activation in T cells leads to the inhibition of anti-CD3-induced ERK phosphorylation through the up-regulation of cAMP after chronic opioid treatment. Recent aggregate data have demonstrated that NF-κB signaling is involved in opioid-mediated immune and neuronal responses. Thus, this section focuses on the possible roles of NF-κB signaling in opioid-mediated effects in immune and neuronal cells.

Some of the early observations on the involvement of NF-κB signaling in opioid-mediated immune responses came from the study on morphine-treated rhesus monkeys (Carr et al. 1995) and morphine-treated mice (Roy et al. 1998b). Stimulation of peripheral blood mononuclear cells (PBMCs) from morphine-treated animals with pokeweed mitogen (PWM) resulted in an approximately twofold increase in IL-2. Similarly, PBMCs from chronic morphine-treated animals resulted in an NF-κB increase by 43% after 72 h in culture with PWM (Carr et al. 1995). It was proposed that chronic morphine might mediate immune function via T cell activation and NF-κB-induced IL-2 production, because IL-2 is known to be an NF-κB-dependent gene (Carr et al. 1995). In activated macrophages, morphine treatment resulted in a bimodal modulation of lipopolysaccharide (LPS)-induced expression of IL-6 and TNF-α (Roy et al. 1998b). At nanomolar concentrations, the combinatory treatment of morphine and LPS induced a synergistic increase in the secretion of both IL-6 and TNF-α; at micromolar concentrations, the treatment resulted in a naloxone-independent decrease in NF-κB activity (Roy et al. 1998b).

Involvement of NF-κB signaling in opioid-mediated immune functions was also observed in the study of morphine-enhanced hepatitis C virus (HCV) replication and promotion of HCV disease progression (Li et al. 2003). In this study, morphine was found to activate NF-κB promoter and increase HCV RNA expression (Li et al. 2003), which was blocked by caffeic acid phenethyl ester, a specific inhibitor of NF-κB activation (Natarajan et al. 1996). In addition, in their study on the effects of alcohol on HCV and interferon α (IFN-α) treatment, Zhang et al. (2003) found that opioid signaling may be involved in alcohol-induced HCV RNA expression via NF-κB signaling. They further showed that the NF-κB signaling inhibitor caffeic acid phenethyl ester abolished alcohol-induced HCV RNA expression and the universal opioid receptor antagonist naltrexone abrogated the alcohol-enhanced HCV replicon expression (Zhang et al. 2003). To understand the mechanism of morphine-promoted human immunodeficiency virus (HIV) infection, Wang et al. (2003) investigated the effect of morphine on IFN-γ promoter activity in activated T cells from wild-type and *mor* knockout mice. Wang et al. found out that morphine attenuated anti-CD3/CD28-stimulated IFN-γ promoter activity and increased the intracellular cAMP level. Previous studies have shown that chronic morphine treatment resulted in activation of ERK1/2 in human CEMx174 lymphocytic cells (Chuang et al. 1997), cord blood $CD34^+/CD38^-$ cells (Rozenfeld-Granot et al. 2002), and the opioid receptor-transfected cells (Ai et al. 1999; Fukuda et al. 1996; Li and Chang 1996; Polakiewicz et al. 1998a). In contrast to the observations on chronic morphine treatment of these unstimulated cells, morphine treatment inhibited phosphorylation of ERK1/2 and p38 MAPK and down-regulated NF-κB signaling in activated T cells (Wang et al. 2003). This result is parallel with that from another study in

which DOR agonist DADLE inhibited anti-CD3-ε-induced phosphorylation of ERK1/2 in murine splenic T cells (Shahabi et al. 2000). Thus, opioid modulation of NF-κB signaling is cell-type-dependent. In agreement with other opioid-induced immune response studies on *mor*-null animals (Gaveriaux-Ruff et al. 1998; Roy et al. 1998a), the effects of morphine on IFN-γ promoter activity were not observed in *mor*-null murine T cells, indicating the involvement of MOR signaling in morphine modulation of IFN-γ promoter activity.

It has been noted that in comparison with MOR, KOR may play a different role in mediating some immune responses (Rogers and Peterson 2003). For instance, it has been known that HIV-1 nuclear protein Tat stimulated the production of the chemokine monocyte chemoattractant protein-1 and activated NF-κB in human astrocytes (Conant et al. 1996). Sheng et al. (2003) found that KOR agonist U50,488 down-regulated monocyte chemoattractant protein-1 production induced by Tat and potently inhibited Tat-induced NF-κB activation, suggesting that KOR agonists have an anti-inflammatory effect in the CNS and could be beneficial for the treatment of HIV-1-associated brain disorder.

Treatment with the MOR-specific agonist, [D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin (DAMGO), increased the DNA binding activity of NF-κB in primary cultures of rat cerebral cortex neurons (Hou et al. 1996). Such DNA binding activity of NF-κB increased with time of DAMGO treatment and was naloxone-dependent, suggesting that the induction of NF-κB binding activity is mediated by MOR (Hou et al. 1996). Moreover, inhibition of NF-κB activation by pyrrolidine dithiocarbamate is involved in opiate withdrawal (Capasso 2001). NF-κB has been implicated in regulating memory and neuroplasticity (Mattson 2003). Because certain opiate effects are associated to memory and neuroplasticity (Nestler 1997), it would be very interesting to examine how NF-κB signaling is involved in opiate functions both in immune and neuronal cells. A recent study showed that morphine treatment led to NF-κB promoter activation in NT2-N neurons and that substance P antagonist CP-96345 abolished this activation (Wang et al. 2004). Because substance P activates NF-κB in this cell line, it is possible that morphine induces NF-κB activation through up-regulation of substance P (Wang et al. 2004). It is known that both morphine and substance P also modulate the expression of proinflammatory cytokines and chemokines that are transcriptionally controlled by NF-κB (Ghosh and Karin 2002). These observations suggest that one alternative mechanism of NF-κB involvement in opioid-mediated immune and neuronal responses is via the crosstalk between substance P receptor signaling and opioid receptor signaling. A recent study on β-arrestin-2-null mice demonstrated that chronic morphine treatment did not induce desensitization of μ-opioid receptor and these animals did not develop antinociceptive tolerance (Bohn et al. 2000). Interestingly, deletion of β-arrestin-2 did not have an effect on superactivation of AC activity (Bohn et al. 2000), which is a biochemical marker of dependence (Collier and Francis 1975; Sharma et al. 1975). More recently, the study of β-arrestin-2 involvement in immune responses demonstrated that the direct interaction between β-arrestin-2 and IκBα resulted in stabilization of IκBα, leading to inhibition of NF-κB signaling (Gao et al. 2004). It has yet to be determined whether opioid-induced inhibition of NF-κB signaling results from the interaction between β-arrestin-2 and IκBα.

Physiological NF-κB activity is regulated by several protein modification steps, including phosphorylation (Viatour et al. 2005) and nitrosylation (Marshall et al. 2004). It has been known that nitric oxide (NO) inactivates NF-κB signaling in a variety of cells through *S*-nitrosylation of cysteine residues and nitrosation of tyrosine residues (Colasanti and Persichini 2000; DelaTorre et al. 1997; Matthews et al. 1996; Park et al. 2005). Effects of morphine on NO production have been observed in neutrophils, monocytes, and endothelial cells (Magazine et al. 1996; Stefano et al. 1995). A variant μ receptor, μ-3, was proposed to be involved in morphine-induced NO production (Stefano et al. 1995). Morphine attenuated

LPS-induced NF-κB nuclear binding in human blood neutrophils and monocytes in a naloxone-sensitive-dependent manner (Welters et al. 2000b). The NO synthase-specific inhibitors were able to block the effect of morphine on NF-κB nuclear binding. Thus, Welters et al. (2000a) proposed that morphine modulates NF-κB activation via NO in part to cause immunosuppression. On the other hand, opioid peptides endomorphines 1 and 2, which are the MOR endogenous ligands (Zadina et al. 1997), inhibited LPS-stimulated IL-10 and IL-12 production, but potentiated LPS-induced NF-κB binding activity in human monocytic cells (Azuma and Ohura 2002). One of the explanations is that the increase in NF-κB DNA binding activity does not necessarily translate to an increase in NF-κB *trans*-activating activity (Azuma and Ohura 2002). This could be true because it has been found that certain NF-κB dimers such as the p50/50 homodimer have an inhibitory effect on NF-κB-dependent gene expression (Zhong et al. 2002). It is also possible that other DNA binding proteins bind to the NF-κB consensus binding site (Hirano et al. 1998), and such possibility has not been ruled out yet in this study (Azuma and Ohura 2002).

All the data discussed above have demonstrated that NF-κB signaling may be involved in opioid-mediated immune responses and neuronal functions. Furthermore, the cross-talk between opioid receptor signaling and NF-κB signaling may be cell-type-dependent. Indeed, this phenomenon is typical for NF-κB whose functions are known to be cell-type-dependent (Dixit and Mak 2002).

Involvement of NF-κB in opioid receptor gene expression

The intracellular mechanism in activation of NF-κB by certain cytokines such as TNF-α has been well established (Pomerantz and Baltimore 2002). Recently, the important roles of NF-κB in the CNS and PNS have also been recognized (Meffert and Baltimore 2005; O'Neill and Kaltschmidt 1997). For instance, peripheral myelin formation in Schwann cells requires activation of NF-κB (Nickols et al. 2003). The requirement of NF-κB/p65 for learning has been demonstrated by the spatial maze test using NF-κB/p65-null mice (Meffert et al. 2003). Furthermore, the opioid receptor expression levels have been established to be critical for opioid functions (Law et al. 2004). In the previous section, we reviewed the involvement of NF-κB signaling in opioid functions both in immune cells and neuronal cells. This section focuses on the involvement of NF-κB signaling in the transcriptional regulation of opioid receptor genes (Chen et al. 2006; Kraus et al. 2003; Park and Wei 2003; Wang et al. 2004).

It has been reported that the proinflammatory cytokine TNF induced *mor* gene transcription in various immune cells, including human T lymphocytes, leukocytes, and mature dendritic cells (Kraus et al. 2003). NF-κB was identified to be responsible for TNF-induced *mor* gene expression through three NF-κB *cis* elements on the human *mor* promoter (Kraus et al. 2003). This study provides a possible molecular mechanistic explanation for the earlier observations that inflammation led to an increase in the levels of opioids and receptors in cells (Czlonkowski et al. 1993; Pol et al. 2001; Stein et al. 1990), indicating that a regulatory feedback mechanism between the inflammation sites and CNS may exist (Murphy 2003). More recently, in a study on the potential pharmacological effects of certain opiates in human inflammatory bowel diseases, it was found that ex vivo DALDA treatment dampened TNF-α mRNA expression in the colon of active inflammatory bowel disease patients (Philippe et al. 2006). Moreover, *mor* gene expression was significantly enhanced by cytokines and repressed by the NF-κB inhibitor in myeloid and lymphocytic cell lines (Philippe et al. 2006). This result appears to be consistent with the previous finding that expression of the *mor* gene was enhanced in TNF-induced NF-κB activation in human T lymphocytes (Kraus et al. 2003). Interestingly, in the study on the roles of morphine and substance P in the modulation of immune responses and inflammation, Wang et al. (2004) demonstrated that morphine and DAMGO significantly up-regulated NF-κB-specific luciferase reporter activity in NT2-N neurons, suggesting that morphine activates NF-κB

signaling. However, morphine activation of NF-κB signaling may be indirectly mediated by activation of substance P gene expression because substance P at nanomolar concentrations can activate NF-κB signaling and induce NF-κB-dependent gene expression (Lieb et al. 1997). Thus, morphine modulation of NF-κB-mediated immune responses may act through substance P signaling in the CNS.

Expression of the *kor* gene in immune cells has been demonstrated by varieous groups (Gaveriaux et al. 1995; Lawrence et al. 1995; Suzuki et al. 2001). No direct studies have been carried out to determine whether NF-κB signaling is involved in *kor* gene expression in immune cells. However, in the mouse embryonic carcinoma P19 cells, the NO donor sodium nitroprusside (SNP) was able to block c-Myc-mediated *kor* gene expression in retinoic acid-treated cells (Park et al. 2002). Furthermore, nitration of NF-κB (Park et al. 2005) is involved in the inactivation of c-*myc* gene expression in P19 cells (Park and Wei 2003), indicating that inhibition of NF-κB signaling could be the mechanism of SNP-mediated *kor* gene expression in retinoic acid-treated P19 cells (Law et al. 2004; Park et al. 2002).

Like MOR and KOR, DOR is also involved in both neuronal and immune functions (reviewed by Rogers and Peterson 2003). DOR transcripts have been identified in certain immune cells (reviewed by Sharp 2003). In the study on the intracellular signaling mechanism of NGF-induced delta opioid receptor gene expression, we found that NF-κB signaling is involved in *dor* gene expression in NGF-differentiated rat adrenal pheochromocytoma PC12h cells (Chen et al. 2006). NGF/TrkA signaling is critical in the development and maintenance of the CNS and PNS (Sofroniew et al. 2001). During neuronal development, NGF and other neurotrophins play a detrimental role in the expression of many neuronal survival-associated genes, including the NF-κB-dependent *bcl-xL* gene (Middleton et al. 2001). NGF activation of PI3K/Akt signaling leads to phosphorylation of the downstream transcription factors, including NF-κB (Brunet et al. 2001; Yuan and Yankner 2000). Furthermore, the DOR agonists have been shown to promote survival of neurons in the CNS and preserved peripheral organs (Borlongan et al. 2004). Opioid receptor agonists can activate PI3K/Akt survival signaling in the opioid receptor-transfected HEK293 cells (Polakiewicz et al. 1998b), suggesting that opioid survival effects may be attributable to activation of PI3K/Akt signaling. Previously, a putative NF-κB *cis* element on the *dor* promoter was also indicated according to a computer database search (Augustin et al. 1995). Moreover, expression of the *dor* gene is temporally and spatially controlled at developmental stages (Georges et al. 1998; Zhu et al. 1998). Taking these considerations into account, we hypothesized that NGF-mediated PI3K/Akt signaling may be involved in the temporal and spatial expression of the survival-associated *dor* gene during neuronal differentiation. We found that constitutive phosphorylation of Akt and IκBα correlates with NGF-induced *dor* expression. Through a series of biochemical studies including transfection of cells with NF-κB signaling super inhibitor mutant IκBα (S32A/S36A), NF-κB/p65-expressing vector, and small interference RNA oligos, we were able to establish that the molecular mechanism of NGF-induced *dor* expression is mainly via the sustained activation of PI3K/Akt signaling, which leads to the activation of the transcriptional factor NF-κB to induce *dor* expression (Chen et al. 2006). Thus, this molecular mechanism provides not only a new mechanistic explanation of how NGF induces *dor* expression, but also insights into the molecular mechanism of the temporal and spatial expression of the *dor* gene and the roles of DOR during neuronal differentiation. In the future, we need to evaluate whether such mechanism is applicable to the immune system where the *dor* gene can be induced by concanavalin A (a T cell mitogen), anti-CD3 in murine $CD4^+$ T cells (Li et al. 1999), and PHA in EL-4 thymoma cells (Sun and Loh 2002).

Perspective

Activation of NF-κB signaling plays a critical role in the expression of both the *mor* and *dor* genes in different cell types; NF-κB signaling is also likely involved in *kor* gene expression in P19 cells. Ample data indicate that NF-κB signaling is involved in opioid functions in both the neuronal and immune systems. In acute opioid treatment, activation of the opioid receptors leads to the release of Gβγ, which activates PI3K/Akt signaling (Polakiewicz et al. 1998b) that is known to activate the downstream effector NF-κB (Brunet et al. 2001). Inhibition of the cAMP/PKA/CREB pathway may also enhance NF-κB activity because activation of this signaling leads to inhibition of NF-κB (Parry and Mackman 1997). Chronic opioid treatment results in activation of AC, leading to activation of cAMP/PKA/CREB signaling and inhibition of NF-κB (Wang et al. 2003). TNF treatment activates MEKK3, which activates NF-κB (Yang et al. 2001). NGF treatment of PC12h with NGF leads to sustained PI3/Akt/NF-kB signaling, resulting in the induction of *dor* gene expression (Chen et al. 2006). With these facts, we proposed a model for the involvement of NF-κB signaling in opioid functions in opioid receptor-expressing cells, i.e., NF-κB serves as the nexus through which opioids mediate neuronal and immune functions (Fig. 2).

It is well established that the NF-κB family proteins play a critical role in inflammation, immunity, cell proliferation, and apoptosis (Li and Verma 2002). NF-κB has been implicated in regulating memory and neuroplasticity (Mattson 2003). Recent studies on NF-κB in opioid functions and receptor gene expression in both immune cells and neuronal cells provide some insights into the connection between these two families of signaling molecules. It would be greatly beneficial to understand how NF-κB activity is regulated via opioid receptor signaling and how NF-κB signaling controls opioid receptor gene expression (a feedback control mechanism) in both immune and neuronal cells. Some researchers have suggested that opioids be called cytokines (Rogers and Peterson 2003). After all, NF-κB is one of the key molecules that control the expression of many important cytokine genes (Li and Verma 2002). Understanding the molecular mechanism of involvement of NF-κB signaling in opioid functions and receptor gene expression would shed some light into the development of new interventions for immune system-related diseases and drug addiction. NF-κB may hold one of the keys to this endeavor.

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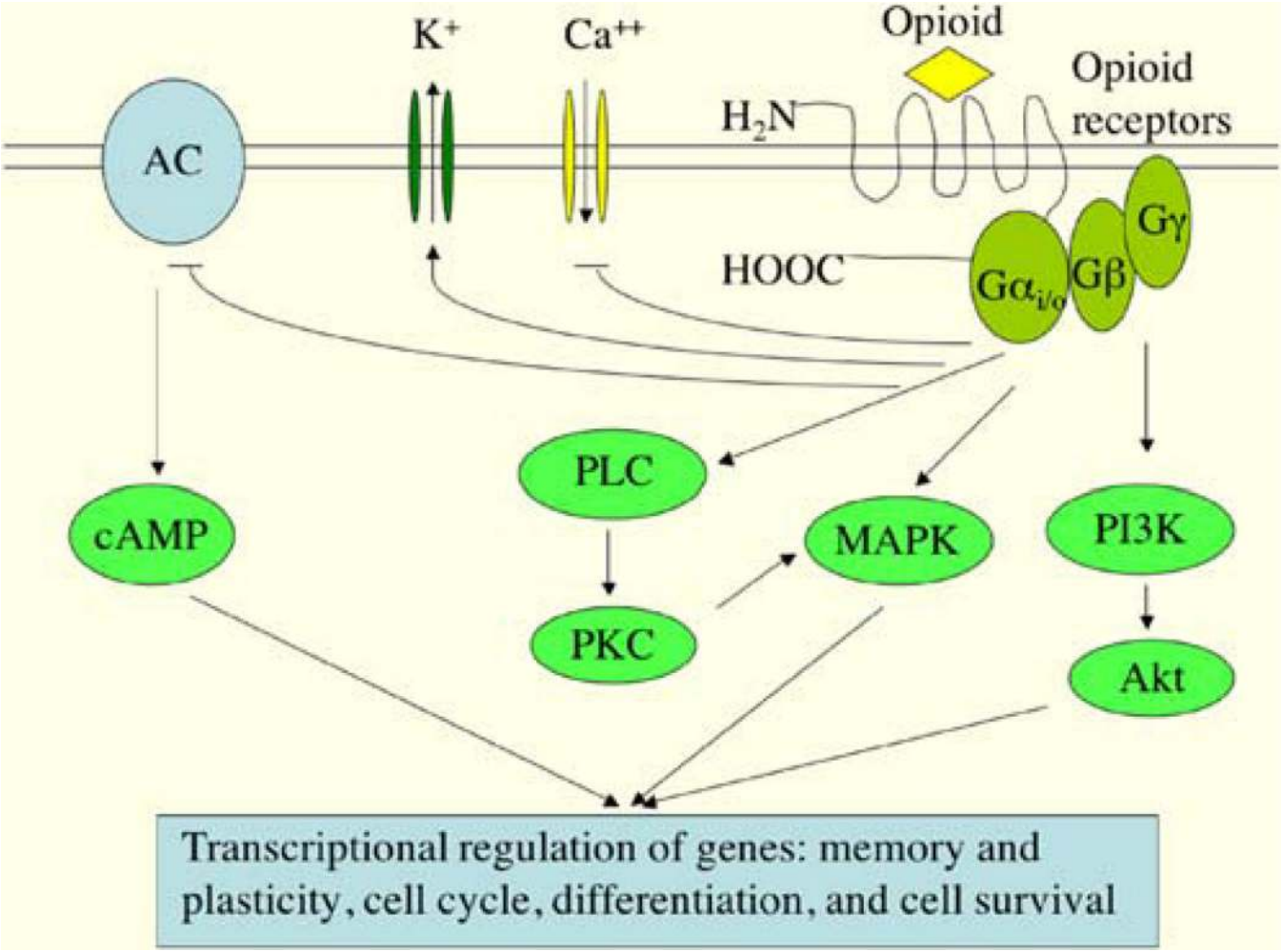

Fig. 1.

Possible cellular responses and transcriptional regulation initiated by activation of opioid receptor signaling. Arrows indicate activation of the signaling pathway; the "⊣" signs indicate inhibition of the signaling pathway. AC, adenylyl cyclase; Akt, also called protein kinase B (PKB); cAMP, cyclic AMP; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C.

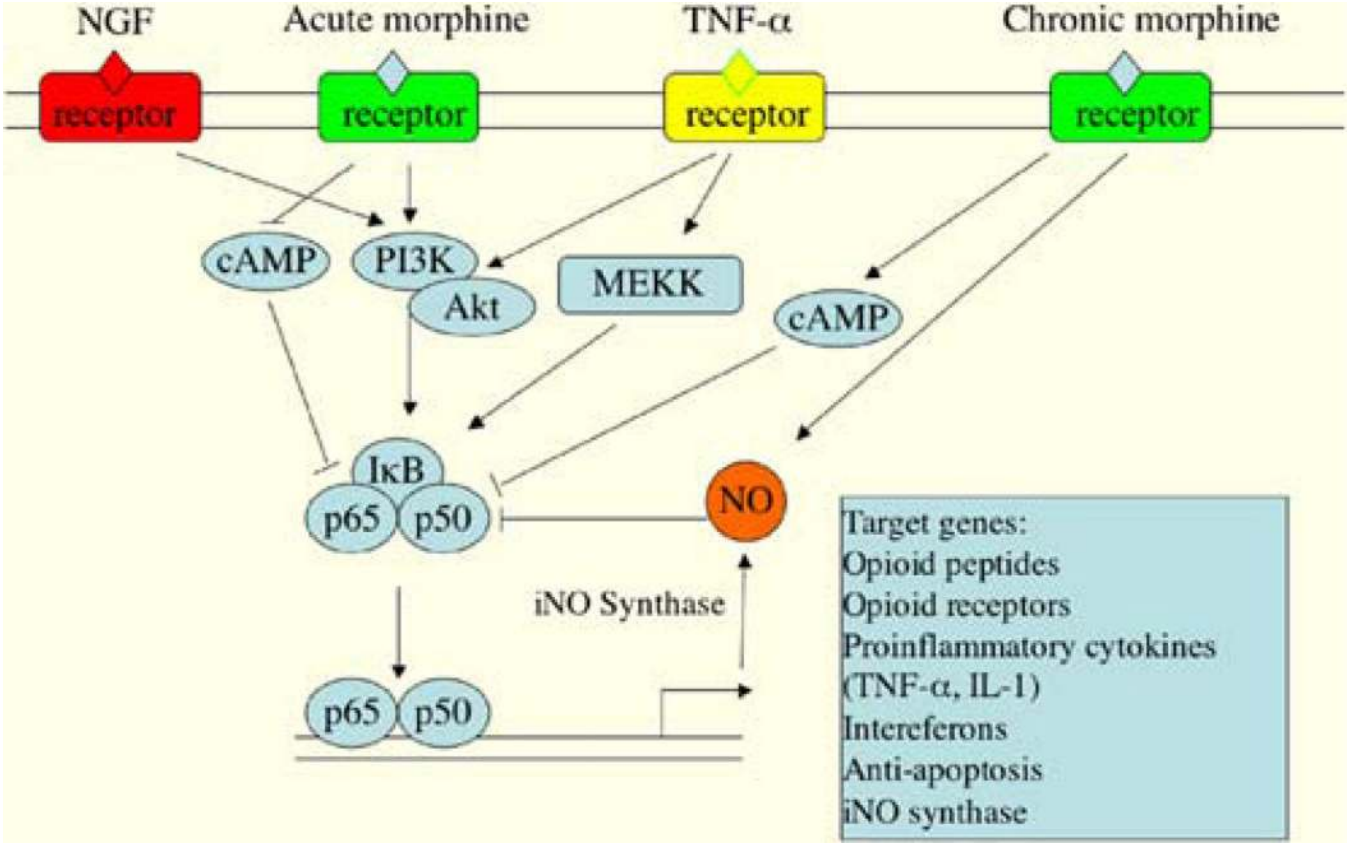

Fig. 2.

Proposed model for the involvement of NF-κB signaling in opioid receptor-expressing immune and neuronal cells. Arrows indicate activation of the signaling pathway; the "⊣" signs indicate inhibition of the signaling pathway. Both immune and neuronal cells express opioid receptors. Modulation of NF-κB signaling leads to the regulation of a variety of genes, including the opioid receptor genes and proinflammatory genes. Nitration or nitrosylation of NF-κB may be one of the feedback mechanisms of controlling homeostasis of NF-κB signaling *in vivo*. Deregulation of NF-κB signaling could result in opioid-mediated immunosuppression, cell survival, morphine tolerance, and physical dependence. MEKK, mitogen-activated protein kinase kinase; IκB, inhibitor of κB protein; p65, NF-κB/p65 subunit; p50, NF-κB/p50 subunit; iNO, inducible NO.