Available online at www.sciencedirect.com

SCIENCE DIRECT®

European Journal of Pharmacology 500 (2004) 331–349

www.elsevier.com/locate/ejphar

Review

Opioid abuse and brain gene expression

Ryszard Przewlocki*

Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Krakow, Poland
Institute of Applied Psychology, Jagiellonian University, Krakow, Poland

Accepted 1 July 2004
Available online 21 August 2004

Abstract

Opiate addiction is a central nervous system disorder of unknown mechanism. Neuronal basis of positive reinforcement, which is essential to the action of opioids, relies on activation of dopaminergic neurons resulting in an increased dopamine release in the mesolimbic brain structures. Certain aspects of opioid dependence and withdrawal syndrome are also related to the activity of noradrenergic and serotonergic systems, as well as to both excitatory and inhibitory amino acid and peptidergic systems. The latter pathways have been recently proven to be involved both in the development of dependence and in counteracting the states related to relapse. An important role in neurochemical mechanisms of opioid reward, dependence and vulnerability to addiction has been ascribed to endogenous opioid peptides, particularly those acting via the mu- and kappa-opioid receptors. Opiate abuse leads to adaptive reactions in the nervous system which occur at the cellular and molecular levels. Recent research indicates that intracellular mechanisms of signal transmission—from the receptor, through G proteins, cyclic AMP, MAP kinases to transcription factors—also play an important role in opioid tolerance and dependence. The latter link in this chain of reactions may modify synthesis of target genes and in this manner, it may be responsible for opiate-induced long-lasting neural plasticity.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Opioid addiction; Opioid peptide; Opioid receptor; Transcription factor; Gene expression

Contents

* Tel.: +48 12 6623218; fax: +48 12 6374500.
E-mail address: nfprzewl@cyf-kr.edu.pl.

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.036

1. Introduction

Addiction to drugs, including opiates, is a brain disease resulting in a loss of control over drug-taking or in compulsive drug-seeking, despite noxious consequences (Nestler, 2000). It is a serious social burden and has destructive influence on health and standard of living of an inflicted person. Costs of drug dependence are high both to an individual and to the society. Society bears partial or total costs of healthcare but a price paid by an addict is incomparably higher. He or she pays with devastated health on the one hand and on the other, with difficult to measure deterioration of their adaptive abilities.

Opioid dependence makes permanent physiological and psychological alterations leading to dramatic relapses of the disease. Uncontrollable compulsion to take the drug and anxiety of craving and relapse are characteristics of the use of opiates. Efficient treatment methods curing of the disease and fending off relapses and preventive measures counteracting development of dependence are not yet accessible. The present clinical treatment permits efficient detoxification, i.e. the removal of an addictive substances from the organism and liberation of a person from negative symptoms of drug withdrawal. In the case of opiate addiction, very high percentage of patient backslides to the drug use. Thus, the understanding of opioid addiction is critical for development of efficient pharmacological therapy. Addiction to morphine, heroin and other compounds acting via opioid receptors is a disease of unknown mechanisms. Numerous studies are based on animal models of various stages of addictive behavior since they well imitate phases of the development of the disease in humans. The results of animal behavioral experiments, correlated with molecular and clinical genetic studies, may lead to better understanding the disease. Many years of research led us to better understanding of a brain substrate and molecular mechanisms of opioid dependence. The main objective of investigations carried out by many research teams worldwide is to discover neuronal and molecular mechanisms of the disease. These adaptive changes underlie neuronal and molecular alterations that manifest themselves as behavioral syndromes, such as opioid dependence. Recent research has focused on a role of gene expression in mediating long-lasting opioid-induced neuronal plasticity. Understanding the molecular and genetic mechanisms of opioid abuse is critical to creation of a novel efficient therapy.

2. Neuronal bases of opioid dependence

The brain network mediating the rewarding properties of drugs and craving phenomena and the reinforcing properties of addictive drugs of abuse has been identified (Wise and Bozarth, 1987; Koob, 1992; Samson and Harris, 1992). The system was shown to involve such structures as the nucleus accumbens, ventral tegmental area, prefrontal cortex and limbic structures, in particular the so-called “extended” amygdala.

The mesolimbic system consists of dopaminergic neurons found in the ventral tegmental area and their projections to the nucleus accumbens. The nucleus accumbens is a heterogeneous structure with two distinct regions, core and shell. The nucleus accumbens core may be involved in the control of goal-directed behavior and acquisition of drug seeking behavior, while nucleus accumbens shell may be implicated in psychostimulant effects of drugs of abuse. The amygdala and prefrontal cortex are also linked to the mesolimbic network. Drug-associated cues and stress can activate the dopaminergic system via interaction with these structures. Thus, principal neural effect of drugs of abuse consists in the stimulation dopaminergic neurons in the ventral tegmental area and an increase in the release of dopamine in the nucleus accumbens, which in consequence leads to a

reward response (Di Chiara and Imperato, 1988; Di Chiara, 1998; Koob and Swerdlow, 1988; Wood and Rao, 1991; Koob, 1992; Herz, 1998; Xie et al., 1998; Piepponen et al., 1999). The enhancement of dopamine secretion in the nucleus accumbens is a common effect of different pharmacological classes of drugs of abuse: opiates, cocaine, amphetamine, ethanol, nicotine and cannabinoids. This effect can result from both direct action on dopaminergic neurons (cocaine and amphetamine), and an indirect effect (ethanol, opioids). The mechanisms underlying this effect are dependent on the type of the taken drug which can either directly affect dopamine release (cocaine) or interact with dopamine transporter influencing re-uptake mechanism, or can act indirectly, by modifying activity of certain populations of neurons such as GABAergic interneurons that interact with dopaminergic ventral tegmental area neurons (ethanol, opioids). Therefore, the application of addictive drugs with different pharmacological mechanisms can produce similar behavioral changes (such as craving, locomotor sensitization, etc.). This further suggests that various drugs of abuse can cause similar cellular and molecular adaptations in the brain. The precise function of particular structures of the reward system in drug action is difficult to establish. However, it seems clear that the ventral tegmental area–nucleus accumbens dopaminergic neurons are responsible for the rewarding response to drugs of abuse. On the other hand, Hyytia and Koob (1995) emphasize an important role of a complex of structures localized within the ventral forebrain structures, exhibiting morphological and neurochemical similarity, so-called extended amygdala, in development of drug dependence. This set of structures comprises the external part (shell) of nucleus accumbens, responsible for the positive reinforcement, the central–medial nucleus of the amygdala and bed nucleus of stria terminalis. The limbic structures appear to be responsible for emotional components of drug abuse effects. Primary substrate of the opioid action is the mesolimbic dopaminergic system (Koob, 1992; Davidson and Stamford, 1993; Kornetsky and Porrino, 1992; Koob et al., 1998). Opioids interact directly with various molecular targets thereby modulating activity of a number of brain structures, within the reward system.

3. Endogenous opioid systems and reward

The endogenous opioid systems play a key role in modulating reward, mood and regulate the brain hedonic homeostasis (Koob and Le Moal, 1997). Alterations of the endogenous systems following exposure to drugs of abuse appear to contribute to the dysregulation of the reward processes which may participate in the development of addiction (Przewlocka et al., 1996; Koob and Le Moal, 1997; Herz, 1997; Turchan et al., 1997, 1999; Van Ree et al., 2000; Gianoulakis, 2004).

4. A family of endogenous opioid peptides

Opioid peptides derive from three different precursor proteins: proopiomelanocortin (POMC), proenkephalin and prodynorphin. POMC is the precursor of endorphins (β-endorphin, α-endorphin) and a family of non-opioid peptides, melanocortins: α, β and γ melanocyte stimulating hormone (MSH) and adrenocorticotropin releasing hormone (ACTH). proenkephalin is a source of both enkephalins and several longer opioid peptides, such as Met-enkephalin-Arg-Phe. Dynorphin A, dynorphin B, α- and β-neoendorphin and several larger molecules can be generated from prodynorphin. Another group of opioid peptides discovered in the central nervous system was named endomorphins. Their prohormon molecule is not known. Endomorphins are unique in comparison with other endogenous opioid peptides, having a characteristic, atypical structure and high selectivity towards the mu-opioid receptor. Finally, another group of peptides related to the endogenous opioid peptides, which do not bind, however, to the classic opioid receptors is a family of pronociceptin/preproorphaninFQ, cleaved to yield nociceptin/orphaninFQ.

Opioid peptides are found in neuronal networks involved in reward and in responses to drug-associated cues. Among them, the ventral tegmental area–nucleus accumbens system, prefrontal cerebral cortex and, extended amygdala are interconnected and essential for opioid action (Koob and Nestler, 1997; Nestler, 2001). The nucleus accumbens shell shares morphological and anatomical features and has connections with some other areas of the limbic forebrain, such as the bed nucleus of the stria terminalis and the central nucleus of the amygdala. All these structures are rich in neurons producing multiple opioid peptides. The endogenous opioid system seems to play a key role in modulating mood and well-being and to regulate the brain hedonic homeostasis (Koob and Le Moal, 1997). Activation of opioid receptors therein produces reward response but also alters expression and functions of endogenous opioid peptide systems. In fact, number of evidence indicate that opiates and several drugs of abuse modify activity of endogenous opioid system (Gianoulakis, 2004; Herz, 1997; Przewlocka et al., 1996, 1997; Turchan et al., 1997, 1998; Van Ree et al., 2000; Spangler et al., 1996; Bronstein et al., 1988, 1990). The alterations of endogenous opioids and disturbance of brain opioid homeosthasis may contribute to the dysregulation of reward processes and participate in the development of addiction.

5. Effect of morphine on opioid peptide systems in the brain reward circuit

Proenkephalin- and POMC-containing neurons localized within the ventral tegmental area dopamine system modulate activity of dopaminergic neurons. The increase of their activity may indirectly enhance dopamine release. The

prolonged administration of exogenous opiates appears to inhibit the biosynthesis of the opioid peptides which in consequence may lead to the shortage of endogenous opioid peptide agonists at the mu-opioid receptors localized in the reward system and therefore inhibition of dopaminergic activity. In fact, biochemical experiments showed a small but consistent decrease in the level of opioid peptides and in the expression of genes encoding proenkephalin and POMC in the nucleus accumbens striatum pathways as well as in the hypothalamus and medulla, respectively, after prolonged application of morphine (Uhl et al., 1988; Mocchetti et al., 1989; Bronstein et al., 1990; Przewlocka et al., 1997; Turchan et al., 1997; Van Bockstael et al., 2000).

In contrast, opioid peptides derived from prodynorphin and kappa-opioid receptor agonists inhibit dopamine neurons of the mesolimbic system. Therefore, they inhibit secretion of dopamine within the nucleus accumbens and induce an aversive behavior (Rattan et al., 1992; Herz, 1998), characteristic of the state of withdrawal. The prodynorphin messenger RNA hybridization signal in the nucleus accumbens was enhanced at 3 h after acute morphine injection, whereas repeated morphine administration decreased the messenger RNA level at that time point in rats (Przewlocka et al., 1996). In contrast, in mice a single dose of morphine had no significant effect on the prodynorphin in the nucleus accumbens and striatum, but repeated treatment increased the prodynorphin mRNA level in both those structures. Furthermore, six intermittent injections of morphine every 2 h increased prodynorphin and kappa-opioid receptor mRNAs in the rats brain (Wang et al., 1999). Biochemical studies showed that opiate withdrawal increased expression of the prodynorphin gene, the levels of prodynorphin-derived peptides and their release in the nucleus accumbens (Trujillo et al., 1995; Przewlocka et al., 1996, 1997; Turchan et al., 1998; Tjon et al., 1997). Thus, the above results indicate that repeated morphine treatment leads to long-lasting up-regulation of the prodynorphin gene expression and possibly prodynorphin-dependent transmission in the nucleus accumbens and striatum. In contrast, the proenkephalin mRNA level was reduced in the nucleus accumbens and remained unchanged in the striatum. In line with that study, proenkephalin mRNA decreased in the rat striatum after chronic morphine exposure (Basheer and Tempel, 1993). Other drugs of abuse also alter the activity of prodynorphin neurons and the greatest exacerbation of changes was observed during drug withdrawal. The fact that different addictive drugs induce similar, in terms of their direction, effects on the activity of prodynorphin system with an accompanying decrease in the density of kappa-opioid receptors (Spangler et al., 1996; Przewlocka et al., 1997; Turchan et al., 1998) indicates an involvement of prodynorphin neurons within ventral tegmental area–nucleus accumbens system in common, neurochemical mechanisms of drug withdrawal and craving.

Further studies indicate that opioid peptides localized in the amygdala can be involved in the action of drugs of abuse. Numerous data have shown that proenkephalin neurons are abundant in the amygdala of rats and other species. The proenkephalin mRNA-containing nerve cell bodies have been reported to occur bilaterally in the central nucleus and the lateral/basolateral nucleus complex, but less prominently in other amygdaloid nuclei (Fallon and Leslie, 1986; Cassell and Gray, 1989; Honkaniemi et al., 1992; Song and Harlan, 1994). The greatest amount of prodynorphin mRNA and dynorphin in the rat amygdala has been observed in the central nucleus (Turchan et al., 2002; Ma et al., 2003). Thus, this structure seems to be particularly interesting, since both proenkephalin and prodynorphin, and opioid receptors are abundantly expressed therein. Previous study showed that levels of Met-enkephalin in the amygdala were decreased in morphine tolerant-dependent rats as well as during protracted naloxone-precipitated withdrawal (Gudehithlu et al., 1991). However, the levels of dynorphin(1–13) were increased in the amygdala in morphine abstinent rats (Rattan et al., 1992). In agreement, our recent study demonstrated that the chronic morphine administration resulted in an increase in prodynorphin mRNA level after prolonged withdrawal while proenkephalin mRNA slightly decreased within the amygdala (Przewlocka, in preparation). These results suggest that the increase in prodynorphin and the decrease in proenkephalin transcription upon morphine withdrawal may contribute to the state of altered homeostasis between the opioid peptide systems and may contribute to the emotional components of opioid abuse and the drug-induced craving.

6. Multiple opioid receptors

Three members of the opioid receptor family have been identified (Snyder and Pasternak, 2003) beginning with the delta-opioid receptor (Evans et al., 1992; Kieffer et al., 1992) and followed by cloning of mu-opioid receptor (Chen et al., 1993a,b; Fukuda et al., 1993) and kappa-opioid receptor (Li et al., 1993; Meng et al., 1993; Minami et al., 1993; Nishi et al., 1993). In addition to the well-established three types of opioid receptors, an orphan opioid-like nociceptin receptor (NOP), previously known as ORL1 or N/OFQ, was cloned a few years ago. This receptor has nearly 70% sequence homology with the opioid receptors.

The mu-opioid receptor binds morphine, and endomorphin may be its endogenous ligand although several opioid peptides, like β-endorphin or longer deriving from proenkephalin also bind to this receptor. β-Endorphin also binds to delta-opioid receptors. The enkephalins bind to the delta-opioid receptor with great affinity and, therefore, are considered to be endogenous delta-opioid receptor ligand. Dynorphins bind to kappa-opioid receptors. Summing up, a vast majority of opioid peptides do not bind exclusively to

one specific opioid receptor type, but have some affinity and may interact with other opioid receptors as well. Existence of several subtypes of the opioid receptors (mu_1, mu_2; $delta_1$, $delta_2$; $kappa_1$, $kappa_2$, $kappa_3$) has been suggested on the basis of functional studies. Types of opioid receptors cloned to date yielded a single receptor type, and the suggested subtypes are possibly alternative splicing products. In fact, molecular attempts to identify subtypes of opioid receptors have been undertaken and the existence of several variants of opioid receptors has been suggested (Koch et al., 1998; Uhl et al., 1999; Abbadie et al., 2000; Pasternak and Pan, 2000). In addition, oligomerization of various opioid receptors generates unique functional properties (Gomes et al., 2003). It has also recently been demonstrated that there is an association between delta- and mu-opioid receptors and that the occupancy of delta-opioid receptors (by delta-opioid receptor antagonists) enhances mu-opioid receptor binding and signaling activity (Gomes et al., 2004), which indicates functional interaction between the opioid receptors.

7. Effect of morphine on opioid receptors

Opioids may interact with opioid receptors causing alterations of receptor internalization (Burford et al., 1998; Koch et al., 1998), binding or postranslational modification and its biosynthesis (Buzas et al., 1996). Several authors have shown that after chronic treatment of rats with morphine or etorphine, mu-opioid receptors in some brain structures were down-regulated (Patel et al., 2002; Bhargava and Gulati, 1990; Tao et al., 1987, 1988; Bernstein and Welch, 1998) while the binding of delta-opioid receptors either remained unchanged or was down-regulated in the brain region (Tao et al., 1987, 1988; Bhargava and Gulati, 1990). In contrast, earlier studies with the mu-opioid receptor agonist [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO), the non-selective antagonist of opioid receptors naloxone or the mixed mu/delta agonist [D-Ala^2-D-Leu^5]-enkephalin (DADLE) showed an increase in their affinity or binding density in striatum of morphine dependent mice (Abdelhamid and Takemori, 1991). Our recent study in rats (Turchan et al., 1999) has shown that repeated administration of morphine decreased the density of delta-opioid receptors, and this effect was more pronounced in the nucleus accumbens than in the striatum. Apart from the specificity of the used ligands, differences in the results of the above studies may also be due to various patterns of drug administration, viz. subcutaneously implanted pellets (morphine) versus intermittent opiate administration. Furthermore, it cannot be excluded that short-term up-regulation of delta-opioid receptors may be followed by a long-term decrease in their density.

Opioid receptors were shown to be localized to the amygdalar nuclei (Mansour et al., 1994). It was observed that [^{3}H]naltrexone binding to the amygdala increased significantly in morphine tolerant rats when compared with the placebo-treated controls, although the binding was unaffected in morphine abstinent rats (Reddy et al., 1994). However, further study indicated that [^{3}H] [D-Ser^2,Thr^6]-leucine-enkephalin (DSTLE) binding to delta-opioid receptors and DAMGO binding to mu-opioid receptors in the amygdala and other brain regions of morphine tolerant-dependent and abstinent rats did not differ from their respective controls (Bhargava and Gulati, 1990; Bhargava et al., 1991). A lack of changes in the mu-opioid receptor binding as well as mu- and delta-opioid receptor mRNA levels in the rat brain was also observed (Buzas et al., 1996).

Our recent study (Przewlocka, in preparation) indicates that chronic morphine administration increased delta-opioid receptor expression in the rat amygdala, but it was without effect on mu- and kappa-opioid receptor mRNA levels. On the other hand, a decrease in mRNA coding for mu-, delta- and kappa-opioid receptors was seen in the amygdala after cessation of morphine administration. These dynamic alterations of delta-, mu- and kappa-opioid receptor expression may play a role in opioid dependence and withdrawal. A question arises about the nature of the functional significance of morphine-induced changes in the affinity or density of opioid receptors. Suppression of the naloxone-induced withdrawal syndrome in morphine-tolerant rats was also observed after selective delta-opioid receptor antagonists benzylidenenaltrexone and H-Tyr-Tic psi [CH2-NH]-Phe-Phe-OH (TIPPØ) (Fundytus et al., 1995). There is some evidence that at least two delta-opioid receptor subtypes, $delta_1$ and $delta_2$, are available (Menkens et al., 1992). A pharmacological study has shown that pretreatment with the selective antagonists of the $delta_1$-opioid receptor naltriben and naltrindole 5'-isothiocyanate suppressed the naloxone-induced withdrawal symptoms to the same extent as pretreatment with the $delta_2$ antagonist 7-benzylidenenaltrexone. This finding suggests that both $delta_1$ and $delta_2$ opioid receptors may play an important role in modulating the development of physical dependence on morphine (Suzuki et al., 1997). Furthermore, studies conducted on mice using antisense oligonucleotides suggested the involvement of $delta_2$ receptors in the development and/or expression of morphine tolerance (Sanchez-Blazquez et al., 1997). Recently, participation of delta-opioid receptors in the pathophysiology of heroin dependence in humans has also been indicated (Maye et al., 1997).

Several studies indicate that opioids may alter molecular characteristics of opioid receptors in culture cells. Acute opioid administration has little, if any, effect on opioid receptor binding parameters. On the other hand, chronic administration of opiates does cause some, but inconsistent changes in the opioid receptor binding. A majority of studies indicate that chronic morphine down-regulates the density of mu- and delta-opioid receptors in Neuro2A or C6 glial cells stably expressing either delta-or mu-opioid receptor (Chakrabarti et al., 1997; Yabaluri and Medzihradsky, 1997; Law and Loh, 1999). A lack of changes in the

receptor binding was seen in CHO cells stably transfected with mu-opioid receptor (Chen et al., 1996).

8. Opioid interaction with main neurotransmitter systems

8.1. Dopaminergic pathways

The principal neural effect of opiates consists in the stimulation dopaminergic neurons in the ventral tegmental area and an increase in the release of dopamine in the nucleus accumbens, which in result leads to a reward response (Herz, 1998; Xie et al., 1998; Piepponen et al., 1999). This effect can result from an indirect effect of opioids by modifying activity of certain populations of GABAergic interneurons that interact with dopaminergic ventral tegmental area neurons. The response to opioids of dopamine ventral tegmental area cells results from the presynaptic opioid inhibition of GABA release, which through disinhibition increased the dopaminergic neurons activity.

The dopamine D2 receptors appear to play a role in the motivational action of opioids. Although in D2 receptor knockout mice, the behavioral expression of morphine withdrawal was unchanged, a clear suppression of morphine rewarding properties was observed in a place-preference test (Maldonado et al., 1997). Morphine given acutely had no significant effect on D2 mRNA levels in the nucleus accumbens and striatum in mice. Repeated morphine had also no effect on the D2 mRNA level in the nucleus accumbens and striatum when measured after 2 h, but decreased 72 h after morphine withdrawal (Turchan et al., 1997). On the other hand, in rats, repeated morphine treatment caused a decrease in the dopamine D2 receptor mRNA levels (Georges et al., 1999). Thus, this study indicates that prolonged morphine treatment may alter D2 receptor-mediated dopaminergic transmission. On the other hand, the role of dopamine D1 receptor in both the rewarding and aversive effects of opioids has been suggested (Shippenberg et al., 1993). Furthermore, an involvement of D1 receptors in morphine-induced locomotor activity was demonstrated in D1-deficient mice (Becker et al., 2001). Thus, the above studies indicate that the mesolimbic dopamine system appears to be important in mediating both the acute rewarding and chronic opioid actions.

8.2. Noradrenergic pathways

The locus coeruleus is a small structure consisting mostly of brain noradrenergic neurons. The locus coeruleus cell bodies contain mu-opioid receptors and mu- and kappa-opioid receptors have also been found on presynaptic nerve endings in the locus coeruleus (Mansour et al., 1988). The mu-opioid receptors appear to mediate inhibitory action of both opiates and endogenous opioid peptides on adrenergic neurons (Aghajanian and Wang, 1987). It seems that some effects of chronic morphine are related to high activity of these neurons. This suggestion is substantiated by observations that strong behavioral withdrawal symptoms in rats addicted to morphine can be observed after opioid antagonists given directly into this structures (Bozarth, 1994; Maldonado et al., 1992). The locus coeruleus and also NTS adrenergic neurons appear to mediate somatic signs of opiate withdrawal. Chronic morphine treatment may led to a decrease in expression of inhibitory opioid peptides in these structures. These processes with the concurrently increased activity of glutamatergic neurons may lead to the enhancement of activity of noradrenergic system during drug dependence and withdrawal (Van Bockstael et al., 2000) and mediate accompanying somatic symptoms.

8.3. Glutamatergic pathways

Several findings suggest that the glutamatergic system may play a role in the opioid effects and indicate that, in particular, the *N*-methyl-D-aspartate (NMDA) receptor plays a central role in neuroplastic changes after opiate abuse. Recent study showed that withdrawal from morphine increased glutamate transmission in the brain (Sepulveda et al., 2004). An early study demonstrated supersensitivity of cortical neurons to glutamate after chronic morphine treatment in rats (Satoh et al., 1976). Further, Trujillo and Akil (1991) reported that NMDA receptor antagonist, (5*R*, 10*S*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801), inhibited morphine tolerance and dependence in mice and the same effect was also reported in rats (Zhu and Barr, 2001). In addition, pretreatment with an antisense oligonucleotide to the *N*-methyl-D-aspartate receptor subunit 1 (NMDAR1) attenuated some signs of morphine withdrawal (Zhu and Ho, 1998). Chronic antagonism of group I metabotropic glutamate receptor reduced morphine withdrawal signs in rats (Fundytus and Coderre, 1994). Finally, chronic morphine augmented metabotropic glutamate receptor-induced inhibition of NMDA-mediated neurotransmission in the nucleus accumbens (Martin et al., 1999). Recent studies have indicated that expression of various glutamate receptors changes following the rewarding brain stimulation (Carlezon et al., 2001) as well as morphine withdrawal (Jang et al., 2000). Increase of [^{3}H] AMPA binding to alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor was also demonstrated during morphine withdrawal (Jang et al., 2000). Furthermore, acute or chronic morphine administration increased NMDAR1 mRNA level in the central and basolateral nuclei of the amygdala (Turchan et al., 2003). Behavioral studies have revealed that the blockade of NMDA receptors in the amygdala prevents fear conditioning (Campeau et al., 1992; Fanselow and Kim, 1994) which shows that NMDA-dependent form of synaptic plasticity in this region might contribute to the formation of conditioned emotional responses to opiates. Thus, the studies suggest possible link

between the expression of NMDAR1 and changes in the activity of the limbic system neurons and morphine-induced behavior. Hence, the changes in glutamatergic transmission in the nucleus accumbens and amygdala may underlie neuroadaptations that lead to opiate dependence (Siggins et al., 2003).

8.4. Peptidergic systems

One of the important problems, which needs to be to remembered when discussing physiological aspects of dependence, is the interactions of opioids with anti-opioid peptidergic systems. The activity of some peptidergic systems, which may act in an opposite way to opioids, is increased under chronic opiate treatments, of which cholecystokinin, thyreoliberin and the neuropeptide FF and α-MSH have to be mentioned. These peptides may reduce the development of morphine tolerance (cholecystokinin, thyreoliberin, α-MSH) and weaken (thyreoliberin) symptoms of withdrawal (Szekely et al., 1979; Bertolini et al., 1981; Ramarao and Bhargava, 1990; Rothman et al., 1993; Pu et al., 1994; Stanfa et al., 1994). Several early studies showed that morphine influences activity of corticotropin-releasing factor (CRF) neurons. CRF system seems to participate in hormonal alterations induced by opiates and in anxiety and aversive effects of withdrawal. In fact acute morphine enhances release of CRF in the hypothalamus (Buckingham, 1982; Nikolarakis et al., 1989) while after chronic administration, tolerance to this effect develops (Pechnick, 1993). Such chronic morphine administration caused CRF decrease in the hypothalamus (Milanes et al., 1997; Laorden et al., 2003). It was shown that the CRF in the limbic system was activated during drug withdrawal (Koob and Le Moal, 1997). Furthermore, it was demonstrated that morphine increased the CRF level in the bed nucleus of the stria terminalis. These data clearly evidenced the impact of opioids on the function of several peptydergic systems. On the other hand, it is likely that endogenous opioid peptides may participate in the regulation of their activity in the brain structures after drug abuse.

9. Molecular basis of opioid addiction

For understanding of opioid addiction, it is important to characterize opioid-induced molecular and cellular adaptations in the specific neuronal network involved in the changes of behaviors associated with opioid addiction. Recent studies indicate that exposure to opiates leads to short- and long-term adaptive changes in the intracellular signaling pathways. Acute activation of opioid receptors inhibits cAMP signaling as well as alters voltage gated Ca^{2+} channel function and activates K^{+} inwardly rectifying channel G protein receptor coupling, signal transduction pathways and transcription factors. Chronic opioid treatment gradually desensitizes the inhibitory effects and augments the acute effects by altering opioid receptor binding, G protein functions, transcription factors adaptation and finally, expression of their target genes (Nestler, 1992).

9.1. G-proteins

It is well known that opioid receptors are coupled to Gi/Go classes of the G proteins. Several studies indicate that opiates may alter molecular characteristics of opioid receptors by influencing their interaction with G proteins. Opioids may alter functioning of G proteins by influencing their availability, efficacy of coupling, and by modulating their concentration and subcellular localization. Chronic morphine treatment was shown to change subcellular localization of Gi or Gs in COS and A 431 cells transfected with mu- and kappa-opioid receptors (Ammer and Schulz, 1997; Bayewitch et al., 2000). Furthermore, Gs protein coupling and concentration of stimulatory Gsα protein were increased in SH-SYS5 cultured cells (Ammer and Schulz, 1996). Interestingly, in the rat locus coeruleus, chronic morphine increased the abundance of the Go and Gi-subunits, while in the rat nucleus accumbens decreased the levels of Gi (Nestler and Aghajanian, 1997). Similar treatment results in attenuation of activation of G-proteins via mu-opioid receptor. This was evidenced by GTPγS binding, which reflects GTPase activity. The GTPγS binding decreased following chronic morphine treatment in the rat brain (Sim et al., 1996) and in SH-SYS5 cultured cells (Elliott et al., 1997). Morphine treatment induces changes in some G-protein mRNA levels, which may result in G-proteins availability and affect opioid signal transduction. Acute treatments resulted in an increase in Go mRNA and a significant decrease in Gαi1 and Gαi2 mRNAs while chronic morphine administration increased Gα1 and Gαβ i1 and Giα2 mRNAs levels in the rat prefrontal cortex (Kaewsuk et al., 2001). Chronic morphine elevated the Go and attenuated the Gs protein biosynthesis in all regions of the hippocampal formation (Przewlocka et al., 1994). Furthermore, the level of Gαβ s mRNA was significantly decreased in several areas of the brain during the butorphanol withdrawal while level of Gαi mRNA was significantly decreased only in the cerebral cortex of butorphanol tolerant rats (Kim et al., 2003).

On the other hand, an increase in the expression of Gαs in the paraventricular nucleus of hypothalamus and Gαo mRNA in the claustrum and endopiriform nucleus of rats chronically treated with morphine was reported (Parolaro et al., 1993; Rubino et al., 1995). The G protein expression changed further during opiate withdrawal. Thus, the studies indicate that chronic morphine treatment alters G protein concentration and expression in discrete brain regions, resulting in adaptive changes in multiple intracellular signaling that could account partly for opiate tolerance and dependence.

9.2. Cyclic AMP

Opiates, acting on opioid receptors via G_i/G_o classes of the G proteins, inhibit cyclic AMP (cAMP) formation (Sharma et al., 1975; Collier and Francis, 1975; Brandt et al., 1976). This results in inhibition of neuronal excitability and synaptic transmission mediated via cAMP and represents a classic cellular mechanism of opioid action. With continuing opioid exposure, activity of cAMP signaling gradually recovers and increases (up-regulation) above control level. Thus, prolonged exposure to opioids leads to tolerance of cAMP transmission at the single cell level. Abrupt removal of opioids after chronic exposure to these drugs induces subsequent adaptation that, at the cellular level, is manifested by an increase in cAMP levels and was suggested to represent a cellular model of tolerance and dependence (Nestler, 1993). Essentially similar regulation of the cAMP pathways was also demonstrated in vivo in certain brain structures. Opiates given acutely slightly inhibited the adenylyl cyclase (Terwilliger et al., 1991; Van Vliet et al., 1991), while this inhibition was gradually reversed during opioid tolerance and greatly increased during withdrawal in the locus coeruleus (Duman et al., 1988) and other brain regions (Terwilliger et al., 1991; Mamiya et al., 2001). Thus, opioid withdrawal activates the intracellular cAMP signaling pathway. The increase in cAMP levels results in the activation of cAMP-dependent protein kinase (PKA) alterations in the phosphorylation of proteins relevant to opioid signaling and enhancement of transmitter release. The opiate-induced changes further lead to changes in the expression of the Ca^{2+}/cAMP response element binding protein (CREB), which may be important to the development and expression of opioid dependence (Nestler, 1993; Widnell et al., 1994; Maldonado et al., 1996).

9.3. Ca^{2+}

Activation of opioid receptors leads to inhibition of Ca^{2+} current and enhancement of potassium conductance resulting in suppression of cellular activity. On the other hand, opioids also exhibit excitatory activity in the brain caused by both diminution of inhibitory transmission as well as direct excitatory activities. Direct excitatory opioid action was demonstrated at the cellular levels. Opioids, via Gi/o $\gamma\beta$ subunit, stimulate inositol lipid hydrolysis (Jin et al., 1994), and production of IP_3 and diacyloglicerol, which in consequence may lead to mobilization of intracellular Ca^{2+} stores and an increase in intracellular Ca^{2+} concentration in rat periaqueductal gray neurons (Connor and Christie, 1998) and in NG-108-15, SH-SY5Y and ND8-47 cells (Jin et al., 1994; Tang et al., 1996; Connor and Henderson, 1996; Smart and Lambert, 1996a,b). Activation of mu-opioid receptor increased intracellular Ca^{2+} oscillations in neurons in vitro (Przewlocki et al., 1999) and in primary afferent neurons (Tang et al., 1996). Thus, acute opioid exposure can cause an increase in intracellular Ca^{2+} and may further activate the mitogen-activated protein (MAP) kinase (Fukuda et al., 1996), which may also be activated by alternative PKA pathway (Impey et al., 1998; Punch et al., 1997) during opioid withdrawal.

9.4. MAP kinases

The most abundant members of mitogen-activated protein kinase (MAPK) family in neurons are extracellular signal-regulated kinases (ERK) ERK1 and ERK2 (Ortiz et al., 1995). The MAPK/ERK pathway is a signaling cascade, which plays a crucial role in several cellular regulatory processes (Alberola-Ila and Hernandez-Hoyos, 2003; Impey et al., 1999; Mazzucchelli et al., 2002). The MAPK/ERK pathway can integrate signals from second messenger systems, such as Ca^{2+} and diacylglycerol (DAG), and control activity-dependent regulation of neuronal functions (Grewal et al., 1999) neuronal differentiation and survival (Fukunaga and Miyamoto, 1998; Anderson and Tolkovsky, 1999). Recent studies suggested an involvement of ERK signaling in action of morphine. The acute (Narita et al., 2002; Valjent et al., 2004; Muller and Unterwald, 2004) and chronic (Berhow et al., 1995; Ma et al., 2001; Narita et al., 2002) administration of morphine produced an increase in phosphorylated-ERK immunoreactivity in the brain and in cultured cells in vitro (Ma et al., 2001; Belcheva et al., 2002; Bilecki et al., 2004). These acute effects were mediated by opioid receptors since they were blocked by a mu-opioid receptor antagonist, naloxone. Activation of the ERK cascade has been suggested to trigger initial events leading to phosphorylation and desensitization of the mu-opioid receptor (Schmidt et al., 2000). On the other hand, a contrasting evidence has also been presented which indicates that opioid receptor internalization is not an obligatory result of ERK activation by Kramer and Simon (2000). The effects of opioids on cell ERK1/2 phosphorylation following acute and chronic treatment as well as during the withdrawal have been examined on human neuroblastoma cell line SH SY5Y (Bilecki et al., in preparation), which express endogenous mu-opioid receptor and develop cellular tolerance and dependence to morphine after prolonged treatment (Wang and Sadee, 2000). We found recently that, whereas acute activation of mu-opioid receptors rapidly activated ERK1/2, the prolonged stimulation of mu-opioid receptors was associated with a slight decrease in ERK1/2 phosphorylation. Interestingly, a decrease in ERK activation after chronic morphine treatment has recently been shown in the nucleus accumbens (Eitan et al., 2003; Muller and Unterwald, 2004). Moreover, precipitation of withdrawal with antagonist of opioid receptors profoundly potentiated the decrease in ERK1/2 phosphorylation. These observation raises the question how chronic opioid action can affect ERK phosphorylation at the cellular level and which mechanisms are involved in the regulation of ERK activity following acute and chronic morphine treatment. Using the

inhibitors of PKA, protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent kinase II (CAMKII), we showed that the PKC and CAMKII but not PKA appeared to be responsible for the induction of ERK1/2 phosphorylation following acute opioid exposure in SH-SY5Y cells (Bilecki et al., in preparation). On the other hand in the brain, ERK/MAPK can be activated by chronic morphine administration (Berhow et al., 1995; Ma et al., 2001; Narita et al., 2002) and the effect appears to be mediated by PKA. Taken together PKC and CAMKII may be important during acute opioid treatment while PKA-dependent activation of ERK/MAPK by opioids may be evoked by an up-regulation of adenylyl cyclase activity with chronic treatment. At the end, both acute and chronic opioids phosphorylates ERK/MAPK and activate transcription factors such as Ca^{2+}/cAMP response element binding protein (CREB).

9.5. CREB

CREB is a transcription factor that binds to cAMP-responsive elements (CRE) in the promoter region of target genes and modulates their expression. PKA, Ca^{2+}/calmodulin-dependent kinases or MAPK can phosphorylate CREB at Ser^{133} and induce its transcriptional activity (Meyer and Habener, 1993; Sheng et al., 1991).

CREB has been associated with opioid abuse (Shaw-Lutchman et al., 2002). Indeed, CREB have been identified to be activated by opioids in rodents brain (Guitart et al., 1992; Nye and Nestler, 1996; Widnell et al., 1996; Gutstein et al., 1998) and in cell cultures (Bilecki et al., 2004). It was found that acute morphine administration decreased, whereas withdrawal increased, the extent of CREB phosphorylation in the locus coeruleus (Guitart et al., 1992). On the other hand, chronic morphine treatment increased the levels of CREB protein while phosphorylated CREB was not affected or even decreased (Widnell et al., 1994; Guitart et al., 1992). In the nucleus accumbens, chronic, but not acute, morphine treatment was found to decrease CREB protein immunoreactivity (Widnell et al., 1996). CREB, is a constitutively expressed transcription factor activated through the cAMP pathway. Therefore, it was supposed that the alterations in CREB activity following morphine treatment were caused by the cAMP pathway activation (Widnell et al., 1994; Coven et al., 1998). Acute exposure of various cultured cells to mu-opioid receptor agonists induced an inhibition of adenylate cyclase activity (Crain and Shen, 1990; Chakrabarti et al., 1995, 1997). Prolonged exposure led to tolerance while removal of opioids after chronic exposure induced increase in cAMP levels (Chakrabarti et al., 1995; Avidor-Reiss et al., 1995). However, despite the up-regulated cAMP system during chronic morphine treatment in Neuro2A MOR cells (Chakrabarti et al., 1995; Chakrabarti et al., 1997), CREB phosphorylation was reduced down to control levels (Bilecki et al., 2004). Furthermore, studies with the cultured CATHa cells (that have many properties of locus coeruleus neurons) showed that up-regulation of the cAMP pathway was accompanied by a decrease in CREB immunoreactivity and CRE binding (Widnell et al., 1994). These results are in opposition to the increased CREB level in the locus coeruleus during chronic morphine treatment.

Activation of protein kinase C was reported to cause desensitization of opioid-induced K^+ and Ca^{2+}-activated Cl^- currents (Mestek et al., 1995; Ueda et al., 1995). In addition, chronic activation of the mu-opioid receptors in Neuro2a MOR cells resulting in the loss of the response to agonists has been attributed to PKC activation (El Kouhen et al., 1999). Thus, it is noteworthy that an opioid-induced increase in protein kinase C activity has been linked to the development of tolerance to opioids (Narita et al., 1994). Interestingly, protein kinase C plays an important role in the control of neurite outgrowth, which requires the CREB/ATF1 family of transcription factors (Shimomura et al., 1998). In NG108-15, neuroblastoma×glioma cells and in Neuro2a MOR cultured cells, acute morphine administration, via delta- and mu-opioid receptors, respectively, stimulated CREB phosphorylation (Bilecki and Przewlocki, 2000; Bilecki et al., 2004). This stimulation did not involve the cAMP pathway, but required Ca^{2+}, calmodulin and activation of protein kinase C (PKC) (Bilecki and Przewlocki, 2000). Thus, the induction of CREB by acute opioids may involve the activation of PKC.

Chronic exposure to opiates gradually increases cAMP-dependent protein kinase (PKA) activity in the brain (Terwilliger et al., 1991) and, therefore, opioid withdrawal increases the extent of CREB phosphorylation (Guitart et al., 1992). Furthermore, in Neuro2a MOR cells, opioid withdrawal also elevated phosphorylated CREB (Bilecki et al., 2004). The activation of CREB was antagonized by PKA inhibitor indicating that the up-regulation of the cAMP pathway is in fact responsible for CREB phosphorylation during withdrawal. Thus, it is likely that PKA signaling may mediate CREB activation during opioid withdrawal while PKC pathway appears to be responsible for CREB phosphorylation following acute morphine treatment.

Furthermore, we demonstrated recently that the alterations in CREB phosphorylation following opioid treatment were accompanied by changes in CRE DNA binding activity (Bilecki et al., 2004). CREB binds CREs independently of its state of dimerization (Waeber and Habene, 1991; Richards et al., 1996), and morphine does not affect total CREB protein level, which suggests involvement of other factors, possibly activating transcription factor (ATF); ATF-2 or ATF-2:c-Jun dimers. The binding of these factors to various CRE motifs is similar to, or stronger than that of CREB, and the strength of their DNA-binding in vitro correlates with their capacity for transactivation (Benbrook and Jones, 1990). It is worth noticing that, upon the prolonged morphine-treatment, CRE DNA binding activity, like CREB phosphorylation, was also reduced down to control levels. Precipitation of morphine withdrawal in vitro in Neuro2a MOR cells led to

an increase in CREB phosphorylation as well as CRE DNA binding activity.

The activation of CREB as well as an increase in CRE DNA binding activity during withdrawal, along with the lack of changes after chronic treatment might suggest that CREB may form the cellular basis of tolerance and dependence (Bilecki and Przewlocki, 2000; Bilecki et al., 2004; Shaw-Lutchman et al., 2002; Chartoff et al., 2003).

CREB phosphorylation at Ser^{133} is necessary for transcriptional activation, but under some conditions, this phosphorylation is inadequate to drive transcription (Lee et al., 1990). Therefore, functional significance of the alterations in CREB phosphorylation and CRE DNA binding activity, i.e. their linkage to the regulation of gene expression, needed further confirmation, whether opioids enhance CRE-dependent transcriptional activation. Acute treatment of Neuro2a MOR cells transiently transfected with 4xGluCRET8Luc which expresses basal luciferase activity, with mu-opioid receptor selective ligand, DAMGO enhanced CRE-dependent transcriptional activation and this effect coincided with the increased phosphorylation of CREB as well as its binding to DNA (Bilecki et al., 2004). It thus suggests that acute effect of opioids on CREB phosphorylation/CRE DNA binding may result in alterations in gene expression. After chronic morphine treatment, CRE-dependent transcriptional activation was normalized as it did with CREB phosphorylation and DNA binding. Furthermore, precipitation of opiate withdrawal in Neuro2a MOR cells led to a small but substantial increase in CRE-dependent transcriptional activation. Regional and cellular mapping of CRE-mediated transcription has recently revealed an increased CRE-dependent β-galactosidase expression during chronic morphine treatment and morphine withdrawal both in the locus coeruleus and nucleus accumbens (Shaw-Lutchman et al., 2002). Experiments with the use of CRE-LacZ reporter mice showed that morphine activated CRE-mediated transcription in the nucleus accumbens shell (Barrot et al., 2002). These studies suggest that CREB in the shell of nucleus accumbens has an important role in the brain's reward pathways, and local manipulations of CREB activity may affect morphine reward. Furthermore, Shaw-Lutchman et al. (2002) performed regional and cellular mapping of the β-galactosidase expression and demonstrated decrease-following acute morphine and increase of β-galactosidase expression during naltrexone-precipitated morphine withdrawal in the extended amygdala. Thus, the studies indicate that CRE-dependent expression may contribute to neuronal plasticity upon acute and chronic morphine alterations of brain homeostasis.

9.6. Activator protein AP-1

Phosphorylated CREB activates the transcription of c-fos and in result transcriptionally active dimers of Fos/Jun protein family which constitutes another transcription factor, activator protein 1 (AP-1). Acute administration of morphine causes c-Fos activation in some brain structures. Induction of c-fos mRNA by morphine was seen in dorsomedial caudate-putamen, several thalamic nuclei, central grey, and nucleus tractus solitarius (Gutstein et al., 1998). Acute morphine treatment induced Fos proteins and AP-1 binding activity in the striatum and nucleus accumbens, the brain region implicated in the reinforcing properties of opiates (Liu et al., 1994). Furthermore, chronic morphine treatment and the drug withdrawal increased levels of the several Fos, Fras and of AP-1 binding activity in rat striatum and nucleus accumbens (Couceyro and Douglass, 1995; Nye and Nestler, 1996). These observation indicated that acute morphine administration alters the gene expression and AP-1 binding activity in several areas known to involved in central effects of opioids. We have recently found that acute opioid administration induced the AP-1 DNA binding activity in cultured Neuro2A MOR cells, that was accompanied by the increase in the AP-1-dependent transcriptional activation (Bilecki et al., 2004). The increase in AP-1 binding activity and AP-1-dependent transcription was also evident during withdrawal.

In summary, these data provide evidence for a direct involvement of CREB and AP-1 transcription factors in the regulation of gene transcription in response to opioids. There is now compelling evidence that both acute and chronic opioid administration appear to activate different intracellular pathways which may result in alteration of gene expression. It is important to notice that short-lasting acute opioid signal can be transformed into long-lasting alterations at the level of gene transcription that evokes a cascade of genomic changes which may participate in the mechanisms of development of opioid addiction. The changes in brain CREB activity appear to be involved in opioid effects, in particular tolerance and dependence (Nestler, 1993; Widnell et al., 1994 Maldonado et al., 1996). Thus, CREB and activator protein AP-1 act as an integration point for both the acute and chronic opioid action. Moreover, the strength and duration of their activation act in concert with downstream transcriptional responses.

10. Regulation of gene expression by opioids

Acute and chronic morphine administration leads to dynamic changes in the immediate-early gene expression in the nucleus accumbens, prefrontal cortex and in several additional forebrain regions, including portions of the extended amygdala. The altered expression of transcription factors resulted in adaptive changes in the expression of membrane receptors, channels, intracellular signaling proteins and plethora of target genes within the mesolimbic system (Przewlocka et al., 1994; Turchan et al., 1997; Nestler, 2000; Kaewsuk et al., 2001; Ammon et al., 2003).

10.1. Striatum

Both acute and chronic morphine administration and withdrawal were found to change abundance of several genes in the nucleus accumbens and striatum. Treatment of rats with morphine for 5 days was associated with a reduction in the levels of striatal mRNA for the voltage-sensitive K^+ channel suggesting the altered K^+ conductance (Mackler and Eberwine, 1994). Furthermore, the expression of glutamate transporter (GLT-1) mRNA was significantly decreased in the striatum of morphine-dependent rats, and significantly increased after the naloxone-precipitated withdrawal (Ozawa et al., 2001). In the striatum, a profound increase following chronic morphine treatment was observed in the levels of synapsin IIa mRNA, a neuron-specific phosphoprotein involved in the regulation of neurotransmitter release (Matus-Leibovitch et al., 1997). Finally, chronic morphine treatment changed the expression of opioid peptide and their receptor genes in the striatum and nucleus accumbens in rodents (see above).

10.2. Amygdala

Little is known about the effects of morphine on gene expression in the amygdala. (Pu et al., 1994) found that chronic morphine treatment was associated with the increased cholecystokinin mRNA hybridization signals in each subnucleus of the rat amygdala. Furthermore, an increase of the synapsin I mRNA levels in the amygdala was found in the morphine-treated rats (Matus-Leibovitch et al., 1995). Chronic exposure to morphine modified the expression of corticotropin releasing factor (McNally and Akil, 2002; Maj et al., 2003) as well as NMDAR1 in the central and basolateral nuclei (Turchan et al., 2003). Finally, acute morphine administration decreased the level of MC4-R mRNA in the rat amygdala (Starowicz et al., 2003).

10.3. Cortex

In the morphine-dependent rats, naloxone-precipitated withdrawal induced a marked increase in the expression of α2a-adrenoceptor mRNA in the cerebral cortex (Busquets et al., 1997). Some studies indicated that morphine administration altered expression of certain G-proteins in the cortex. Morphine given chronically increased Gββ 1 and Gαβ i1 and Gαβ i2 mRNAs levels in the rat prefrontal cortex (Kaewsuk et al., 2001), which may influence the opioid signaling. Furthermore, acute morphine increased G protein-coupled receptor kinase (GRK) mRNA levels in the cerebral cortex (Fan et al., 2002). On the contrary, chronic morphine treatment resulted in down-regulation of GRK2 expression in the cerebral cortex (Fan et al., 2002). GRK may cause desensitization of opioid signaling, which plays an important role in opioid tolerance. Thus, morphine not only induces a negative feedback regulation of its signals through the activation of GRK, but also via controlling the levels of GRK gene expression. Furthermore, chronic morphine treatment and withdrawal induce up-regulation of c-Jun N-terminal kinase 3 gene expression in the rat frontal cortex (Fan et al., 2003). Finally, expression of glial cell-derived neurotrophic factor (GDNF) and its receptors in the frontal cortex was increased significantly after naloxone-precipitated morphine withdrawal (Zhou et al., 2000).

10.4. Cellular models

Several studies analyzed the alteration of morphine-dependent gene expression in cellular models of opioid tolerance and dependence. In PC12 cells stably expressing cloned mu-opioid receptor, opioid agonists increased expression of RGS4, a G-protein signaling protein which has been shown to modulate the function of some heterotrimeric G-proteins by stimulating the GTPase activity of G-protein α subunits (Nakagawa et al., 2001). The increase in calmodulin III gene expression by mu-opioid receptor stimulation was further demonstrated in PC12 cells (Niu et al., 2000). Furthermore, endothelial cells obtained from human vascular tissues treated with morphine exhibited a down-regulation of estrogen receptor-β (Cadet et al., 2002). Treatment of primary normal human astrocyte cultures with morphine suppressed IL-8 and macrophage-inflammatory protein-1β gene expression, whereas expression of their receptor genes, CCR3 and CCR5, was simultaneously enhanced (Mahajan et al., 2002). Methadone, a regimen for the treatment of opioid dependence, was found to induce the expression of CCR5, a co-receptor of human immunodeficiency virus (HIV)/simian form of HIV entry, on human CEM x174 lymphocytes (Suzuki et al., 2002).

Taking together, opioid-induced alterations in gene expression, in particular within the brain reward network, may underlie the persistence of opioid addiction and addictive behavior (Nestler and Aghajanian, 1997). Identification of groups of genes which orchestrated the dysregulation of the brain reward system is now possible by use of gene chips microarrays.

11. Gene expression profiling

11.1. Striatum

Loguinov et al. (2001) using Affymetrix microarrays (GMS 417; Affymetrix) analyzed a gene expression profile in the striatum of mice after a single injection of morphine. It was found that two groups of genes encoding proteins involved in mitochondrial respiration and cytoskeleton-related proteins were transiently down-regulated.

More recently, another study was concerned with the long-lasting changes in gene expression after intermittent exposure to morphine in the rat (Spijker et al., 2004). The authors studied the expression profiles of 159 genes in the rat nucleus accumbens during 2-week exposure to the same daily dose of morphine (10 mg/kg s.c.) and after 3 weeks of drug abstinence. This study showed profound changes in gene expression occurring not only during chronic exposure, but also long after cessation of morphine treatment. The authors believe that stage-specific expression of genes may have a role in the reorganization of neuronal networks and the resulting behavioral state that is apparent upon long-term abstinence. Furthermore, Jacobs et al. (2004) compared the genomic response in the rat nucleus accumbens shell after long-term withdrawal from active and passive heroin administration. They investigated long-term changes in gene expression in the rat nucleus accumbens using a heroin SA paradigm and found several genes with altered expression following prolonged heroin self-administration. Interestingly, the authors found several genes to be down-regulated by heroin and indicated few putative genes, such as GDNF or ARPP-21, which changed differentially upon heroin self- and forced-administration.

11.2. Cortex

Recent study demonstrated that chronic morphine administration elicited profound increases in expression of genes encoding heat shock proteins (hsp70, hsp 27, hsp 40, hsp105, GRP78), while the precipitated withdrawal induced the expression of several transcription factors (krox20, CREM, NGFI-B, IkB) in the prefrontal cortex of rats subjected to non-contingent morphine injections (Ammon et al., 2003). Persistent changes in gene expression upon withdrawal were seen in few genes involved in synaptic activity, such as Arc and splice variant of Homer 1, Ania-3 as well as rPer2, a protein involved in regulation of circadian rhythms.

11.3. Amygdala

Morphine and other drugs of abuse activate immediate-early gene expression in several additional forebrain regions, including portions of the extended amygdala. The amygdala has been implicated in a range of brain functions involved in addictive or associated behaviors, in particular heroin-induced reinstatement of extinguished heroin seeking behavior (Fuchs and See, 2002). However, little is known about neuroadaptive changes in specific gene expression in this brain region. In our present study (Rodriguez Parkitna et al., 2004, in press) using Affymetrix gene arrays, we found several mRNAs whose abundance was regulated in the rat amygdala by chronic morphine administration. The expression of gamma-aminobutyric acid $(GABA)_A$ γ1 receptor subunit was decreased by morphine self-administration. The γ1 subunit is the least abundant of three variants of γ1 containing receptors, which appear to be insensitive to benzodiazepines (Mehta and Ticku, 1999). This decrease was evident in self-administering rats while the increase was observed in yoked morphine-treated rats after a 30-day morphine withdrawal. Interestingly, $GABA_A$ receptor γ1 subunit expression was down-regulated by acute treatment, but profoundly up-regulated by chronic morphine and further increased after 2 days of withdrawal from chronic intermittent morphine administration. In line with these observations, in the chronic intermittent ethanol model of alcohol dependence, an increase in γ1 mRNA abundance was observed in the hippocampus (Cagetti et al., 2003), while similar alterations in the cerebral cortex (Devaud et al., 1995; Devaud et al., 1997) were previously reported. Thus, the changes in abundance of the $GABA_A$ receptor γ1 subunit determining functional properties of GABA receptor might be relevant to the liability of morphine as well as ethanol to produce dependence and withdrawal syndrome. Furthermore, the increase in $GABA_A$ γ1 subunit was also detected in the locus coeruleus after morphine withdrawal in the rat (Heikkila et al., 2001). Interestingly, recent study demonstrated that the functional conductance properties of the rat ventral tegmental area cells containing $GABA_A$ receptor switch from an inhibitory to an excitatory signaling mode after chronic opiate exposure and subsequent withdrawal (Laviolette et al., 2004).

The increase in mRNA abundance in the amygdala after morphine administration was observed particularly among cellular-stress related genes (hsp70, hsp27 and crystalline) (Rodriguez Parkitna et al., 2004). Changes in abundances of the heat shock proteins mRNAs suggest that morphine administration activates the response to cellular stress or may reflect cellular reaction to proapoptotic effect of morphine (Hu et al., 2002; Mao et al., 2002). Few genes, noggin, *N*-cadherin, upstream binding factor-2 (UBF2) and PKC γ, demonstrated by the arrays to be down-regulated by morphine, appear to be involved in neurogenesis. The role of these changes more likely consists in the regulation of plasticity rather than neurogenesis. UBF2 was shown to act as a transcription factor for the RNA polymerase II and to be associated with the Wnt/β-catenin pathway like *N*-cadherin and PKC kinases (Grueneberg et al., 2003). Therefore, the results may suggest the involvement of the Wnt pathway in the development of addiction. This is further substantiated by previously reported changes in mRNA abundances of two main proteins involved in Wnt signaling, β-catenin in the hippocampus upon cocaine administration (Freeman et al., 2001a) and glycogen synthase kinase 3 in the nucleus accumbens shell after heroin self-administration (Jacobs et al., 2002). Another interesting gene which was down-regulated upon morphine administration was NF-1 I/A which encodes a variant of the CAAT-box binding protein, a component of the basic transcription complex (Rodriguez). Yet after the 30-day recovery period, there

was a clear increase in its mRNA level in the rats that underwent the self-administration procedure. Interestingly, the NF I/A was reported to be up-regulated in the rat forebrain (Yuferov et al., 2003) and nucleus accumbens (Freeman et al., 2001b) after administration of cocaine. Further experiments are needed to establish the role of the above-mentioned genes in the molecular action of morphine.

Research of the last years has brought large progress in our understanding of adaptive changes produced by opiates. The repeated administration of opioids can induce prolonged changes in brain functions. Several points are of key significance to these changes. Firstly, the protracted exposure to addictive drugs leads to adaptations in the structure of dopaminergic neurons in the reward system. Secondly, there are adaptations in the intracellular steps of the cascade downstream of the opioid receptors including G proteins, protein kinases, and phosphorylation and activation of different cellular proteins. They lead to alterations in the expression of transcription factors and target genes, determining the plasticity and alterations of the cells in the nervous system. These long-lasting changes in gene expression can underlie opioid tolerance and dependence, and, consequently, opioid abuse. Identification of the candidate genes should at least partly determine susceptibility to the addiction and recognize targets for future therapy.

Acknowledgement

This paper was supported by KBN, Warsaw, grant no. 6 P05A 107 20 and PBZ-KBN-033/P05/2000.

References

Abbadie, C., Pan, Y., Drake, C.T., Pasternak, G.W., 2000. Comparative immunohistochemical distributions of carboxy terminus epitopes from the mu-opioid receptor splice variants MOR-1D, MOR-1 and MOR-1C in the mouse and rat CNS. Neuroscience 100 (1), 141–153.

Abdelhamid, E.E., Takemori, A.E., 1991. Characteristics of μ and β opioid binding sites in the striatal slices of morphinetolerant and-dependent mice. Eur. J. Pharmacol. 198, 157–163.

Aghajanian, G.K., Wang, Y.Y., 1987. Common alpha-2 and opiate effector mechanisms in locus coeruleus: intracellular studies in brain slices. Neuropharmacology 26, 789–800.

Alberola-Ila, J., Hernandez-Hoyos, G., 2003. The Ras/MAPK cascade and the control of positive selection. Immunol. Rev. 191, 79–96.

Ammer, H., Schulz, R., 1996. Morphine dependence in human neuroblastoma SH-SY5Y cells is associated with adaptive changes in both the quantity and functional interaction of PGE1 receptors and stimulatory G proteins. Brain Res. 707 (2), 235–244.

Ammer, H., Schulz, R., 1997. Enhanced stimulatory adenylyl cyclase signaling during opioid dependence is associated with a reduction in palmitoylated Gs alpha. Mol. Pharmacol. 52 (6), 993–999.

Ammon, S., Mayer, P., Riechert, U., Tischmeyer, H., Hollt, V., 2003. Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal. Brain Res. Mol. Brain. Res. 12 (1–2), 113–125.

Anderson, C.N., Tolkovsky, A.M., 1999. Role for MAPK/ERK in sympathetic neuron survival: protection against a p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside. J. Neurosci. 19 (2), 664–673.

Avidor-Reiss, T., Bayewitch, M., Levy, R., Matus-Leibovitch, N., Nevo, I., Vogel, Z., 1995. Adenylylcyclase supersensitization in mu-opioid receptor-transfected Chinese hamster ovary cells following chronic opioid treatment. J. Biol. Chem. 270 (50), 29732–29738.

Barrot, M., Olivier, J.D., Perrotti, L.I., DiLeone, R.J., Berton, O., Eisch, A.J., Impey, S., Storm, D.R., Neve, R.L., Yin, J.C., Zachariou, V., Nestler, E.J., 2002. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc. Natl. Acad. Sci. U. S. A. 99 (17), 11435–11440.

Basheer, R., Tempel, A., 1993. Morphine-induced reciprocal alterations in G alpha s and opioid peptide mRNA levels in discrete brain regions. J. Neurosci. Res. 36 (5), 551–557.

Bayewitch, M.L., Nevo, I., Avidor-Reiss, T., Levy, R., Simonds, W.F., Vogel, Z., 2000. Alterations in detergent solubility of heterotrimeric G proteins after chronic activation of G(i/o)-coupled receptors: changes in detergent solubility are in correlation with onset of adenylyl cyclase superactivation. Mol. Pharmacol. 57 (4), 820–825.

Becker, A., Grecksch, G., Kraus, J., Peters, B., Schroeder, H., Schulz, S., Hollt, V., 2001. Loss of locomotor sensitisation in response to morphine in D1 receptor deficient mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 363 (5), 562–568.

Belcheva, M.M., Haas, P.D., Tan, Y., Heaton, V.M., Coscia, C.J., 2002. The fibroblast growth factor receptor is at the site of convergence between mu-opioid receptor and growth factor signaling pathways in rat C6 glioma cells. J. Pharmacol. Exp. Ther. 303 (3), 909–918.

Benbrook, D.M., Jones, N.C., 1990. Heterodimer formation between CREB and JUN proteins. Oncogene 5 (3), 295–302.

Berhow, M.T., Russell, D.S., Terwilliger, R.Z., Beitner-Johnson, D., Self, D.W., Lindsay, R.M., Nestler, E.J., 1995. Influence of neurotrophic factors on morphine- and cocaine-induced biochemical changes in the mesolimbic dopamine system. Neuroscience 68 (4), 969–979.

Bernstein, M.A., Welch, S.P., 1998. Mu-Opioid receptor down-regulation and cAMP-dependent protein kinase phosphorylation in a mouse model of chronic morphine tolerance. Brain Res. Mol. Brain Res. 55 (2), 237–242.

Bertolini, A., Poggioli, R., Fratta, W., 1981. Withdrawal symptoms in morphine-dependent rats intracerebroventricularly injected with ACTH1-24 and with beta-MSH. Life Sci. 29 (3), 249–252.

Bhargava, H.N., Gulati, A., 1990. Down-regulation of brain and spinal cord m-opiate receptors in morphine tolerant dependent rats. Eur. J. Pharmacol. 190, 305–311.

Bhargava, H.N., Gulati, A., Rahmani, N.H., 1991. Differences in the binding of [^{3}H][D-Ser2,Thr6]leucine-enkephalin and [^{3}H][D-Pen2,D-Pen5]enkephalin to brain membranes of morphine tolerant-dependent rats. Eur. J. Pharmacol. 202 (3), 403–408.

Bilecki, W., Przewlocki, R., 2000. Effect of opioids on Ca2+/cAMP responsive element binding protein. Acta Neurobiol. Exp. (Wars) 60 (4), 557–567.

Bilecki, W., Wawrzczak-Bargiela, A., Przewlocki, R., 2004. Activation of AP-1 and CRE-dependent gene expression. J. Neurochem. (in press).

Bozarth, M.A., 1994. Physical dependence produced by central morphine infusions: an anatomical mapping study. Neurosci. Biobehav. Rev. 18, 373–383.

Brandt, M., Fischer, K., Moroder, L., Wunsch, E., Hamprecht, B., 1976. Enkephalin evokes biochemical correlates of opiate tolerance and dependence in neuroblastoma×glioma hybrid cells. FEBS Lett. 68 (1), 38–40.

Bronstein, D.M., Trujillo, K.A., Akil, H., 1988. Effects of morphine on endogenous opioid biosynthesis. NIDA Res. Monogr. 90, 256–265.

Bronstein, D.M., Przewłocki, R., Akil, H., 1990. Effects of morphine treatment on proopiomelanocortin systems in rat brain. Brain Res. 519, 102–111.

Buckingham, J.C., 1982. Secretion of corticotrophin and its hypothalamic releasing factor in response to morphine and opioid peptides. Neuroendocrinology 35 (2), 111–116.

Burford, N.T., Tolbert, L.M., Sadee, W., 1998. Specific G protein activation and mu-opioid receptor internalization caused by morphine, DAMGO and endomorphin 1. Eur. J. Pharmacol. 342, 123–126.

Busquets, X., Ventayol, P., Garcia-Sevilla, J.A., 1997. Naloxone-precipitated withdrawal in morphine-dependent rats increases the expression of alpha 2a-adrenoceptor mRNA in brain. Brain Res. Mol. Brain Res. 45 (1), 154–158.

Buzas, B., Rosenberger, J., Cox, B.M., 1996. Mu and delta opioid receptor gene expression after chronic treatment with opioid agonist. NeuroReport 7, 1505–1508.

Cadet, P., Mantione, K., Bilfinger, T.V., Stefano, G.B., 2002. Morphine down regulates human vascular tissue estrogen receptor expression determined by real-time RT-PCR. Neuroendocrinol. Lett. 23 (2), 95–100.

Cagetti, E., Liang, J., Spigelman, I., Olsen, R.W., 2003. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABAA receptors. Mol. Pharmacol. 63 (1), 53–64.

Campeau, S., Miserendino, M.J., Davis, M., 1992. Intra-amygdalar infusion of the *N*-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. Behav. Neurosci. 106, 569–574.

Carlezon Jr., W.A., Todtenkopf, M.S., McPhie, D.L., Pimentel, P., Pliakas, A.M., Stellar, J.R., Trzcinska, M., 2001. Repeated exposure to rewarding brain stimulation downregulates GluR1 expression in the ventral tegmental area. Neuropsychopharmacology 25, 234–241.

Cassell, M.D., Gray, T.S., 1989. Morphology of peptide-immunoreactive neurons in the rat central nucleus of the amygdala. J. Comp. Neurol. 281 (2), 320–333.

Chakrabarti, S., Law, P.Y., Loh, H.H., 1995. Neuroblastoma Neuro2A cells stably expressing a cloned mu-opioid receptor: a specific cellular model to study acute and chronic effects of morphine. Brain Res. Mol. Brain Res. 30 (2), 269–278.

Chakrabarti, S., Yang, W., Law, P.Y., Loh, H.H., 1997. The mu-opioid receptor down-regulates differently from the delta-opioid receptor: requirement of a high affinity receptor/G protein complex formation. Mol. Pharmacol. 52 (1), 105–113.

Chartoff, E.H., Papadopoulou, M., Konradi, C., Carlezon Jr., W.A., 2003. Dopamine-dependent increases in phosphorylation of cAMP response element binding protein (CREB) during precipitated morphine withdrawal in primary cultures of rat striatum. J. Neurochem. 87 (1), 107–118.

Chen, Y., Liu, J., Yu, L., 1996. Functional coupling of a mu opioid receptor to G proteins and adenylyl cyclase: modulation by chronic morphine treatment. Addict. Biol. 1 (1), 49–59.

Chen, Y., Mestek, A., Liu, J., Yu, L., 1993a. Molecular cloning of a rat kappa opioid receptor reveals sequence similarities to the mu and delta opioid receptors. Biochem. J. 295 (Pt 3), 625–628.

Chen, Y., Mestek, A., Liu, J., Hurley, J.A., Yu, L., 1993b. Molecular cloning and functional expression of a mu-opioid receptor from rat brain. Mol. Pharmacol. 44 (1), 8–12.

Collier, H.O., Francis, D.L., 1975. Morphine abstinence is associated with increased brain cyclic AMP. Nature 255 (5504), 159–162.

Connor, M., Christie, M.J., 1998. Modulation of Ca2+ channel currents of acutely dissociated rat periaqueductal grey neurons. J. Physiol. 509 (Pt 1), 47–58.

Connor, M., Henderson, G., 1996. Delta- and mu-opioid receptor mobilization of intracellular calcium in SH-SY5Y human neuroblastoma cells. Br. J. Pharmacol. 117 (2), 333–340.

Couceyro, P., Douglass, J., 1995. Precipitated morphine withdrawal stimulates multiple activator protein-1 signaling pathways in rat brain. Mol. Pharmacol. 47 (1), 29–39.

Coven, E., Ni, Y., Widnell, K.L., Chen, J., Walker, W.H., Habener, J.F., Nestler, E.J., 1998. Cell type-specific regulation of CREB gene expression: mutational analysis of CREB promoter activity. J. Neurochem. 71 (5), 1865–1874.

Crain, S.M., Shen, K.F., 1990. Opioids can evoke direct receptor-mediated excitatory effects on sensory neurons. Trends Pharmacol. Sci. 11 (2), 77–81.

Davidson, C., Stamford, J.A., 1993. Neurochemical evidence of functional A10 dopamine terminals innervating the ventromedial axis of the neostriatum in vitro voltammetric data in rat brain slices. Brain Res. 615, 229–239.

Devaud, L.L., Smith, F.D., Grayson, D.R., Morrow, A.L., 1995. Chronic ethanol consumption differentially alters the expression of gamma-aminobutyric acidA receptor subunit mRNAs in rat cerebral cortex: competitive, quantitative reverse transcriptase-polymerase chain reaction analysis. Mol. Pharmacol. 48 (5), 861–868.

Devaud, L.L., Fritschy, J.M., Sieghart, W., Morrow, A.L., 1997. Bidirectional alterations of GABA(A) receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. J. Neurochem. 69 (1), 126–130.

Di Chiara, G., 1998. A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. J. Psychopharmacol. 12 (1), 54–67.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Duman, R.S., Tallman, J.F., Nestler, E.J., 1988. Acute and chronic opiate-regulation of adenylate cyclase in brain: specific effects in locus coeruleus. J. Pharmacol. Exp. Ther. 246 (3), 1033–1039.

Eitan, S., Bryant, C.D., Saliminejad, N., Yang, Y.C., Vojdani, E., Keith Jr., D., Polakiewicz, R., Evans, C.J., 2003. Brain region-specific mechanisms for acute morphine-induced mitogen-activated protein kinase modulation and distinct patterns of activation during analgesic tolerance and locomotor sensitization. J. Neurosci. 23 (23), 8360–8369.

El Kouhen, R., Kouhen, O.M., Law, P.Y., Loh, H.H., 1999. The absence of a direct correlation between the loss of [D-Ala2, MePhe4,Gly5-ol]Enkephalin inhibition of adenylyl cyclase activity and agonist-induced mu-opioid receptor phosphorylation. J. Biol. Chem. 274 (14), 9207–9215.

Elliott, J., Guo, L., Traynor, J.R., 1997. Tolerance to mu-opioid agonists in human neuroblastoma SH-SY5Y cells as determined by changes in guanosine-5′-*O*-(3-[35*S*]-thio)triphosphate binding. Br. J. Pharmacol. 121 (7), 1422–1428.

Evans, C.J., Keith Jr., D.E., Morrison, H., Magendzo, K., Edwards, R.H., 1992. Cloning of a delta opioid receptor by functional expression. Science 258 (5090), 1952–1955.

Fallon, J.H., Leslie, F.M., 1986. Distribution of dynorphin and enkephalin peptides in the rat brain. J. Comp. Neurol. 249 (3), 293–336.

Fan, X., Zhang, J., Zhang, X., Yue, W., Ma, L., 2002. Acute and chronic morphine treatments and morphine withdrawal differentially regulate GRK2 and GRK5 gene expression in rat brain. Neuropharmacology 43 (5), 809–816.

Fan, X.L., Zhang, J.S., Zhang, X.Q., Ma, L., 2003. Chronic morphine treatment and withdrawal induce up-regulation of c-Jun N-terminal kinase 3 gene expression in rat brain. Neuroscience 122 (4), 997–1002.

Fannselow, M.S., Kim, J.J., 1994. Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. Behav. Neurosci. 108, 210–212.

Freeman, W.M., Brebner, K., Lynch, W.J., Robertson, D.J., Roberts, D.C., Vrana, K.E., 2001. Cocaine-responsive gene expression changes in rat hippocampus. Neuroscience 108 (3), 371–380.

Freeman, W.M., Nader, M.A., Nader, S.H., Robertson, D.J., Gioia, L., Mitchell, S.M., Daunais, J.B., Porrino, L.J., Friedman, D.P., Vrana, K.E., 2001. Chronic cocaine-mediated changes in non-human primate nucleus accumbens gene expression. J. Neurochem. 77 (2), 542–549.

Fuchs, R.A., See, R.E., 2002. Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. Psychopharmacology (Berl.) 160 (4), 425–433.

Fukuda, K., Kato, S., Mori, K., Nishi, M., Takeshima, H., 1993. Primary structures and expression from cDNAs of rat opioid receptor delta- and mu-subtypes. FEBS Lett. 327 (3), 311–314.

Fukuda, M., Gotoh, I., Gotoh, Y., Nishida, E., 1996. Cytoplasmic localization of mitogen-activated protein kinase kinase directed by its NH2-terminal, leucine-rich short amino acid sequence, which acts as a nuclear export signal. J. Biol. Chem. 271 (33), 20024–20028.

Fukunaga, K., Miyamoto, E., 1998. Role of MAP kinase in neurons. Mol. Neurobiol. 16 (1), 79–95.

Fundytus, M.E., Coderre, T.J., 1994. Effect of activity at metabotropic, as well as ionotropic (NMDA), glutamate receptors on morphine dependence. Br. J. Pharmacol. 113 (4), 1215–1220.

Fundytus, M.E., Schiller, P.W., Shapiro, M., Weltrowska, G., Coderre, T.J., 1995. Attenuation of morphine tolerance and dependence with the highly selective delta-opioid receptor antagonist TIPP[psi]. Eur. J. Pharmacol. 286 (1), 105–108.

Georges, F., Stinus, L., Bloch, B., Le Moine, C., 1999. Chronic morphine exposure and spontaneous withdrawal are associated with modifications of dopamine receptor and neuropeptide gene expression in the rat striatum. Eur. J. Neurosci. 11 (2), 481–490.

Gianoulakis, C., 2004. Endogenous opioids and addiction to alcohol and other drugs of abuse. Curr. Top. Med. Chem. 4 (1), 39–50.

Gomes, I., Filipovska, J., Devi, L.A., 2003. Opioid receptor oligomerization. Detection and functional characterization of interacting receptors. Methods Mol. Med. 84, 157–183.

Gomes, I., Gupta, A., Filipovska, J., Szeto, H.H., Pintar, J.E., Devi, L.A., 2004. A role for heterodimerization of mu and delta opiate receptors in enhancing morphine analgesia. Proc. Natl. Acad. Sci. U. S. A. 101 (14), 5135–5139.

Grewal, S.S., York, R.D., Stork, P.J., 1999. Extracellular-signal-regulated kinase signalling in neurons. Curr. Opin. Neurobiol. 9 (5), 544–553.

Grueneberg, D.A., Pablo, L., Hu, K.Q., August, P., Weng, Z., Papkoff, J., 2003. A functional screen in human cells identifies UBF2 as an RNA polymerase II transcription factor that enhances the beta-catenin signaling pathway. Mol. Cell. Biol. 23 (11), 3936–3950.

Gudehithlu, K.P., Tejwani, G.A., Bhargava, H.N., 1991. Beta-endorphin and methionine-enkephalin levels in discrete brain regions, spinal cord, pituitary gland and plasma of morphine tolerant-dependent and abstinent rats. Brain Res. 553 (2), 284–290.

Guitart, X., Thompson, M.A., Mirante, C.K., Greenberg, M.E., Nestler, E.J., 1992. Regulation of cyclic AMP response element-binding protein (CREB) phosphorylation by acute and chronic morphine in the rat locus coeruleus. J. Neurochem. 58 (3), 1168–1171.

Gutstein, H.B., Thome, J.L., Fine, J.L., Watson, S.J., Akil, H., 1998. Pattern of c-fos mRNA induction in rat brain by acute morphine. Can. J. Physiol. Pharm. 76 (3), 294–303.

Heikkila, A.T., Echenko, O., Uusi-Oukari, M., Sinkkonen, S.T., Korpi, E.R., 2001. Morphine withdrawal increases expression of GABA(A) receptor epsilon subunit mRNA in locus coeruleus neurons. NeuroReport 12 (13), 2981–2985.

Herz, A., 1997. Endogenous opioid systems and alcohol addiction. Psychopharmacology (Berl.) 129 (2), 99–111.

Herz, A., 1998. Opioid reward mechanisms: a key role in drug abuse? Can. J. Physiol. Pharm. 76 (3), 252–258.

Honkaniemi, J., Pelto-Huikko, M., Rechardt, L., Isola, J., Lammi, A., Fuxe, K., Gustafsson, J.A., Wikstrom, A.C., Hokfelt, T., 1992. Colocalization of peptide and glucocorticoid receptor immunoreactivities in rat central amygdaloid nucleus. Neuroendocrinology 55 (4), 451–459.

Hu, S., Sheng, W.S., Lokensgard, J.R., Peterson, P.K., 2002. Morphine induces apoptosis of human microglia and neurons. Neuropharmacology 42 (6), 829–836.

Hyytia, P., Koob, G.F., 1995. $GABA_A$ receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. Eur. J. Pharmacol. 283, 151–159.

Impey, S., Obrietan, K., Storm, D.R., 1999. Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. Neuron 23 (1), 11–14.

Impey, S., Obrietan, K., Wong, S.T., Poser, S., Yano, S., Wayman, G., Deloulme, J.C., Chan, G., Storm, D.R., 1998. Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21 (4), 869–883.

Jacobs, E.H., Spijker, S., Verhoog, C.W., Kamprath, K., de Vries, T.J., Smit, A.B., Schoffelmeer, A.N., 2002. Active heroin administration induces specific genomic responses in the nucleus accumbens shell. FASEB J. 16 (14), 1961–1963.

Jacobs, E.H., de Vries, T.J., Smit, A.B., Schoffelmeer, A.N., 2004. Gene transcripts selectively down-regulated in the shell of the nucleus accumbens long after heroin self-administration are up-regulated in the core independent of response contingency. FASEB 18 (1), 200–202.

Jang, C.G., Rockhold, R.W., Ho, I.K., 2000. An autoradiographic study of [^{3}H]AMPA receptor binding and in situ hybridization of AMPA sensitive glutamate receptor A (GluR-A) subunits following morphine withdrawal in the rat brain. Brain Res. Bull. 52 (3), 217–221.

Jin, W., Lee, N.M., Loh, H.H., Thayer, S.A., 1994. Opioids mobilize calcium from inositol 1,4,5-trisphosphate-sensitive stores in NG108-15 cells. J. Neurosci. 14, 1920–1929.

Kaewsuk, S., Hutamekalin, P., Ketterman, A.J., Khotchabhakdi, N., Govitrapong, P., Casalotti, S.O., 2001. Morphine induces short-lived changes in G-protein gene expression in rat prefrontal cortex. Eur. J. Pharmacol. 411 (1–2), 11–16.

Kieffer, B.L., Befort, K., Gaveriaux-Ruff, C., Hirth, C.G., 1992. The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. Proc. Natl. Acad. Sci. U. S. A. 89 (24), 12048–12052.

Kim, D.S., Lim, H.K., Jang, S., Oh, S., 2003. Changes of the level of G protein alpha-subunit mRNA by tolerance to and withdrawal from butorphanol. Neurochem. Res. 28 (12), 1771–1778.

Koch, T., Schulz, S., Schroder, H., Wolf, R., Raulf, E., Hollt, V., 1998. Carboxyl-terminal splicing of the rat mu opioid receptor modulates agonist-mediated internalization and receptor resensitization. J. Biol. Chem. 273 (13), 13652–13657.

Koob, G.F., 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13, 177–184.

Koob, G.F., Le Moal, M., 1997. Drug abuse: hedonic homeostatic dysregulation. Science 278 (5335), 52–58.

Koob, G.F., Nestler, E.J., 1997. The neurobiology of drug addiction. J. Neuropsychiatry Clin. Neurosci. 9 (3), 482–497.

Koob, G.F., Swerdlow, N.R., 1988. The functional output of the mesolimbic dopamine system. Ann. N.Y. Acad. Sci. 537, 216–227.

Koob, G.F., Roberts, A.J., Schulteis, G., Parsons, L.H., Heyser, C.J., Hyytia, P., Merlo-Pich, E., Weiss, F., 1998. Neurocircuitry targets in ethanol reward and dependence. Alcohol Clin. Exp. Res. 22 (1), 3–9.

Kornetsky, C., Porrino, L.J., 1992. Brain mechanisms of drug-induced reinforcement. Res. Publ. - Assoc. Res. Nerv. Ment. Dis. 70, 59–77.

Kramer, H.K., Simon, E.J., 2000. Mu and delta-opioid receptor agonists induce mitogen-activated protein kinase (MAPK) activation in the absence of receptor internalization. Neuropharmacology 39 (10), 1707–1719.

Laorden, M.L., Milanes, M.V., Angel, E., Tankosic, P., Burlet, A., 2003. Quantitative analysis of corticotropin-releasing factor and arginine vasopressin mRNA in the hypothalamus during chronic morphine treatment in rats: an in situ hybridization study. J. Neuroendocrinol. 15 (6), 586–591.

Laviolette, S.R., Gallegos, R.A., Henriksen, S.J., van der Kooy, D., 2004. Opiate state controls bi-directional reward signaling via GABAA receptors in the ventral tegmental area. Nat. Neurosci. 7 (2), 160–169.

Law, P.Y., Loh, H.H., 1999. Regulation of opioid receptor activities. J. Pharmacol. Exp. Ther. 289 (2), 607–624.

Lee, C.Q., Yun, Y.D., Hoeffle, rJ.P., Habener, J.F., 1990. Cyclic-AMP-responsive transcriptional activation of CREB-327 involves interdependent phosphorylated subdomains. EMBO J. 9 (13), 4455–4465.

Li, S., Zhu, J., Chen, C., Chen, Y.W., Deriel, J.K, Ashby, B., Liu-Chen, L.Y., 1993. Molecular cloning and expression of a rat kappa opioid receptor. Biochem. J. 295 (Pt 3), 629–633.

Liu, J., Nickolenko, J., Sharp, F.R., 1994. Morphine induces c-fos and junB in striatum and nucleus accumbens via D1 and *N*-methyl-D-aspartate receptors. Proc. Natl. Acad. Sci. U. S. A. 91 (18), 8537–8541.

Loguinov, A.V., Anderson, L.M., Crosby, G.J., Yukhananov, R.Y., 2001. Gene expression following acute morphine administration. Physiol. Genomics 6 (3), 169–181.

Ma, J., Ye, N., Lange, N., Cohen, B.M., 2003. Dynorphinergic GABA neurons are a target of both typical and atypical antipsychotic drugs in the nucleus accumbens shell, central amygdaloid nucleus and thalamic central medial nucleus. Neuroscience 121 (4), 991–998.

Ma, W., Zheng, W.H., Powell, K., Jhamandas, K., Quirion, R., 2001. Chronic morphine exposure increases the phosphorylation of MAP kinases and the transcription factor CREB in dorsal root ganglion neurons: an in vitro and in vivo study. Eur. J. Neurosci. 14 (7), 1091–1094.

Mackler, S.A., Eberwine, J.H., 1994. Cellular adaptation to opiates alters ion-channel mRNA levels. Proc. Natl. Acad. Sci. U. S. A. 91 (1), 385–389.

Mahajan, S.D., Schwartz, S.A., Shanahan, T.C., Chawda, R.P., Nair, M.P., 2002. Morphine regulates gene expression of alpha- and beta-chemokines and their receptors on astroglial cells via the opioid mu receptor. J. Immunol. 169 (7), 3589–3599.

Maj, M., Turchan, J., Smialowska, M., Przewlocka, B., 2003. Morphine and cocaine influence on CRF biosynthesis in the rat central nucleus of amygdala. Neuropeptides 37 (2), 105–110.

Maldonado, R., Blendy, J.A., Tzavara, E., Gass, P., Roques, B.P., Hanoune, J., Schutz, G., 1996. Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. Science 273 (5275), 657–659.

Maldonado, R., Negus, S., Koob, G.F., 1992. Precipitation of morphine withdrawal syndrome in rats by administration of mu-, delta- and kappa-selective opioid antagonists. Neuropharmacology 31 (12), 1231–1241.

Maldonado, R., Saiardi, A., Valverde, O., Samad, T.A., Roques, B.P., Borrelli, E., 1997. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. Nature 388 (6642), 586–589.

Mamiya, T., Noda, Y., Ren, X., Hamdy, M., Furukawa, S., Kameyama, T., Yamada, K., Nabeshima, T., 2001. Involvement of cyclic AMP systems in morphine physical dependence in mice: prevention of development of morphine dependence by rolipram, a phosphodiesterase 4 inhibitor. Br. J. Pharmacol. 132 (5), 1111–1117.

Mansour, A., Fox, C.A., Burke, S., Meng, F., Thompson, R.C., Akil, H., Watson, S.J., 1994. Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. J. Comp. Neurol. 350 (3), 412–438.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., Watson, S.J., 1988. Anatomy of CNS opioid receptors. Trends Neurosci. 11 (7), 308–314.

Mao, J., Sung, B., Ji, R.R., Lim, G., 2002. Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. J. Neurosci. 22 (17), 7650–7661.

Martin, G., Ahmed, S.H., Blank, T., Spiess, J., Koob, G.F., Siggins, G.R., 1999. Chronic morphine treatment alters NMDA receptor-mediated synaptic transmission in the nucleus accumbens. J. Neurosci. 19, 9081–9089.

Matus-Leibovitch, N., Ezra-Macabee, V., Saya, D., Attali, B., Avidor-Reiss, T., Barg, J., Vogel, Z., 1995. Increased expression of synapsin I mRNA in defined areas of the rat central nervous system following chronic morphine treatment. Brain Res. Mol. Brain Res. 34 (2), 221–230.

Matus-Leibovitch, N., Nevo, I., Vogel, Z., 1997. Differential distribution of synapsin IIa and IIb mRNAs in various brain structures and the effect of chronic morphine administration on the regional expression of these isoforms. Brain Res. Mol. Brain Res. 45 (2), 301–316.

Mazzucchelli, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W., Welzl, H., Wolfer, D.P., Pages, G., Valverde, O., Marowsky, A., Porrazzo, A., Orban, P.C., 2002. Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. Neuron 34 (5), 807–820.

Maye, P., Rochlitz, H., Rauch, E., Rommelspacher, H., Hasse, H.E., Schmidt, S., Hollt, V., 1997. Association between a delta opioid receptor gene polymorphism and heroin dependence in man. NeuroReport 8, 2547–2550.

McNally, G.P., Akil, H., 2002. Role of corticotropin-releasing hormone in the amygdala and bed nucleus of the stria terminalis in the behavioral, pain modulatory, and endocrine consequences of opiate withdrawal. Neuroscience 112 (3), 605–617.

Mehta, A.K., Ticku, M.K., 1999. An update on GABAA receptors. Brain Res. Brain Res. Rev. 29 (2–3), 196–217.

Meng, F., Xie, G.X., Thompson, R.C., Mansour, A., Goldstein, A., Watson, S.J., Akil, H., 1993. Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc. Natl. Acad. Sci. U. S. A. 90 (21), 9954–9958.

Menkens, K., Bilsky, E.J., Wild, K.D., Portoghese, P.S., Reid, L.D., Porreca, F., 1992. Cocaine place preference is blocked by the delta-opioid receptor antagonist, naltrindole. Eur. J. Pharmacol. 219, 345–346.

Mestek, A., Hurley, J.H., Bye, L.S., Campbell, A.D., Chen, Y., Tian, M., Liu, J., Schulman, H., Yu, L., 1995. The human mu opioid receptor: modulation of functional desensitization by calcium/calmodulin-dependent protein kinase and protein kinase C. J. Neurosci. 15 (3Pt 2), 2396–2406.

Meyer, T.E., Habener, J.F., 1993. Cyclic adenosine 3′ ,5′ -monophosphate response element binding protein (CREB) and related transcription-activating deoxyribonucleic acid-binding proteins. Endocr. Rev. 14 (3), 269–290.

Milanes, M.V., Laorden, M.L., Chapleur-Chateau, M., Burlet, A., 1997. Differential regulation of corticotropin-releasing factor and vasopressin in discrete brain regions after morphine administration: correlations with hypothalamic noradrenergic activity and pituitary–adrenal response. Naunyn-Schmiedeberg's Arch. Pharmacol. 356 (5), 603–610.

Minami, M., Toya, T., Katao, Y., Maekawa, K., Nakamura, S., Onogi, T., Kaneko, S., Satoh, M., 1993. Cloning and expression of a cDNA for the rat kappa-opioid receptor. FEBS Lett. 329 (3), 291–295.

Mocchetti, I., Ritter, A., Costa, E., 1989. Down-regulation of proopiomelanocortin synthesis and beta-endorphin utilization in hypothalamus of morphine-tolerant rats. J. Mol. Neurosci. 1 (1), 33–38.

Muller, D.L., Unterwald, E.M., 2004. In vivo regulation of ERK and Akt phosphorylation by acute and chronic morphine. J. Pharmacol. Exp. Ther. 310 (2), 774–782.

Nakagawa, T., Minami, M., Satoh, M., 2001. Up-regulation of RGS4 mRNA by opioid receptor agonists in PC12 cells expressing cloned mu receptor. Eur. J. Pharmacol. 433 (1), 29–36.

Narita, M., Makimura, M., Feng, Y., Hoskins, B., Ho, I.K., 1994. Influence of chronic morphine treatment on protein kinase C activity: comparison with butorphanol and implication for opioid tolerance. Brain Res. 650 (1), 175–179.

Narita, M., Ioka, M., Suzuki, M., Narita, M., Suzuki, T., 2002. Effect of repeated administration of morphine on the activity of extracellular signal regulated kinase in the mouse brain. Neurosci. Lett. 324 (2), 97–100.

Nestler, E.J., 1992. Molecular mechanisms of drug addiction. J. Neurosci. 12 (7), 2439–2450.

Nestler, E.J., 1993. Cellular responses to chronic treatment with drugs of abuse. Crit. Rev. Neurobiol. 7 (1), 23–39.

Nestler, E.J., 2000. Genes and addiction. Nat. Genet. 26 (3), 277–281.

Nestler, E.J., 2001. Psychogenomics: opportunities for understanding addiction. J. Neurosci. 21 (21), 8324–8327.

Nestler, E.J., Aghajanian, G.K., 1997. Molecular and cellular basis of addiction. Science 278 (5335), 58–63.

Nikolarakis, K.E., Pfeiffer, A., Stalla, G.K., Herz, A., 1989. Facilitation of ACTH secretion by morphine is mediated by activation of CRF releasing neurons and sympathetic neuronal pathways. Brain Res. 498 (2), 385–388.

Niu, S., Kuo, C.H., Gan, Y., Nishikawa, E., Sadakata, T., Ichikawa, H., Miki, N., 2000. Increase of calmodulin III gene expression by mu-opioid receptor stimulation in PC12 cells. Jpn. J. Pharmacol. 84 (4), 412–417.

Nishi, M., Takeshima, H., Fukuda, K., Kato, S., Mori, K., 1993. cDNA cloning and pharmacological characterization of an opioid receptor with high affinities for kappa-subtype-selective ligands. FEBS Lett. 330 (1), 77–80.

Nye, H.E., Nestler, E.J., 1996. Induction of chronic Fos-related antigens in rat brain by chronic morphine administration. Mol. Pharmacol. 49 (4), 636–645.

Ortiz, J., Harris, H.W., Guitart, X., Terwilliger, R.Z., Haycock, J.W., Nestler, E.J., 1995. Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. J. Neurosci. 15 (2), 1285–1297.

Ozawa, T., Nakagawa, T., Shige, K., Minami, M., Satoh, M., 2001. Changes in the expression of glial glutamate transporters in the rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal. Brain Res. 905 (1–2), 254–258.

Parolaro, D., Rubino, T., Gori, E., Massi, P., Bendotti, C., Patrini, G., Marcozzi, C., Parenti, M., 1993. In situ hybridization reveals specific increases in G alpha s and G alpha o mRNA in discrete brain regions of morphine-tolerant rats. Eur. J. Pharmacol. 244 (3), 211–222.

Pasternak, G.W., Pan, Y.X., 2000. Antisense mapping: assessing functional significance of genes and splice variants. Methods Enzymol. 314, 51–60.

Patel, M.B., Patel, C.N., Rajashekara, V., Yoburn, B.C., 2002. Opioid agonists differentially regulate mu-opioid receptors and trafficking proteins in vivo. Mol. Pharmacol. 62 (6), 1464–1470.

Pechnick, R.N., 1993. Effects of opioids on the hypothalamo-pituitary-adrenal axis. Annu. Rev. Pharmacol. Toxicol. 33, 353–382.

Piepponen, T.P., Honkanen, A., Kivastik, T., Zharkovsky, A., Turtia, A., Mikkola, J.A., Ahtee, L., 1999. Involvement of opioid mu1-receptors in opioid-induced acceleration of striatal and limbic dopaminergic transmission. Pharmacol. Biochem. Behav. 63 (2), 245–252.

Przewlocka, B., Lason, W., Przewlocki, R., 1994. The effect of chronic morphine and cocaine on Gs and Go protein messenger RNA levels in the rat hippocampus. Neuroscience 63, 1111–1116.

Przewlocka, B., Turchan, J., Lason, W., Przewlocki, R., 1996. The effect of single and repeated morphine administration on the prodynorphin system activity in the nucleus accumbens and striatum of the rat. Neuroscience 70 (3), 749–754.

Przewlocka, B., Turchan, J., Lason, W., Przewlocki, R., 1997. Ethanol withdrawal enhances prodynorphin system activity in the rat nucleus accumbens. Neurosci. Lett. 238, 13–16.

Przewlocki, R., Parsons, K.L., Sweeney, D.D., Trotter, C., Netzeband, J.G., Siggins, G.R., Gruol, D.L., 1999. Opioid enhancement of calcium oscillations and burst events involving NMDA receptors and L-type calcium channels in cultured hippocampal neurons. J. Neurosci. 19 (22), 9705–9715.

Pu, S., Zhuang, H., Lu, Z., Wu, X., Ha, J., 1994. Cholecystokinin gene expression in rat amygdaloid neurons: normal distribution and effect of morphine tolerance. Brain Res. Mol. Brain Res. 21 (3–4), 183–189.

Punch, L.J., Self, D.W., Nestler, E.J., Taylor, J.R., 1997. Opposite modulation of opiate withdrawal behaviors on microinfusion of a protein kinase A inhibitor versus activator into the locus coeruleus or periaqueductal gray. J. Neurosci. 17 (21), 8520–8527.

Ramarao, T., Bhargava, H.N., 1990. Effect of thyrotropin releasing hormone on the development of tolerance to the analgesic and hyperthermic actions of morphine in the rat. Neuropeptides 15, 213–217.

Rattan, A.K., Koo, K.L., Tejwani, G.A., Bhargava, H.N., 1992. The effect of morphine tolerance dependence and abstinence on immunoreactive dynorphin (1–13) levels in discrete brain regions, spinal cord, pituitary gland and peripheral tissues of the rat. Brain Res. 584 (1–2), 207–212.

Reddy, P.L., Veeranna, Matwyshyn, G.A., Thorat, S.N., Bhargava, H.N., 1994. Effect of morphine tolerance and abstinence on the binding of [^{3}H]naltrexone to discrete brain regions and spinal cord of the rat. Gen. Pharmacol. 25 (2), 355–361.

Richards, J.P., Bachinger, H.P., Goodman, R.H., Brennan, R.G., 1996. Analysis of the structural properties of cAMP-responsive element-binding protein (CREB) and phosphorylated CREB. J. Biol. Chem. 271 (23), 13716–13723.

Rodriguez Parkitna, J.M., Bilecki, W., Mierzejewski, P., Stefanski, R., Ligeza, A., Bargiela, A., Ziolkowska, B., Kostowski, W., Przewlocki, R., 2004. Differential gene expression in rat amygdala after morphine self-administration. J. Neurochem. (in press).

Rothman, R.B., Brady, L.S., Xu, H., Long, J.B., 1993. Chronic intracerebroventricular infusion of the antiopioid peptide, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH2 (NPFF), downregulates mu opioid binding sites in rat brain. Peptides 14 (6), 1271–1277.

Rubino, T., Parenti, M., Patrini, G., Massi, P., Parolaro, D., 1995. Morphine withdrawal syndrome and G protein expression: a study of the time course in the rat central nervous system. Eur. J. Neurosci. 7 (11), 2334–2340.

Samson, H.H., Harris, R.A., 1992. Neurobiology of alcohol abuse. Trends Pharmacol. Sci. 13 (5), 206–211.

Sanchez-Blazquez, P., Garcia-Espana, A., Garzon, J., 1997. Antisense oligodeoxynucleotides to opioid mu and delta receptors reduced morphine dependence in mice: role of delta-2 opioid receptors. J. Pharmacol. Exp. Ther. 280, 1423–1431.

Satoh, M., Zieglgansberger, W., Herz, A., 1976. Actions of opiates upon single unit activity in the cortex of naive and tolerant rats. Brain Res. 115 (1), 99–110.

Schmidt, H., Schulz, S., Klutzny, M., Koch, T., Handel, M., Hollt, V., 2000. Involvement of mitogen-activated protein kinase in agonist-induced phosphorylation of the mu-opioid receptor in HEK 293 cells. J. Neurochem. 74 (1), 414–422.

Sepulveda, J., Oliva, P., Contreras, E., 2004. Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine. Eur. J. Pharmacol. 483 (2–3), 249–258.

Sharma, S.K., Nirenberg, M., Klee, W.A., 1975. Morphine receptors as regulators of adenylate cyclase activity. Proc. Natl. Acad. Sci. U. S. A. 72 (2), 590–594.

Shaw-Lutchman, T.Z., Barrot, M., Wallace, T., Gilden, L., Zachariou, V., Impey, S., Duman, R.S., Storm, D., Nestler, E.J., 2002. Regional and cellular mapping of cAMP response element-mediated transcription during naltrexone-precipitated morphine withdrawal. J. Neurosci. 22 (9), 3663–3672.

Sheng, M., Thompson, M.A., Greenberg, M.E., 1991. CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252 (5011), 1427–1430.

Shimomura, A., Okamoto, Y., Hirata, Y., Kobayashi, M., Kawakami, K., Kiuchi, K., Wakabayashi, T., Hagiwara, M., 1998. Dominant negative ATF1 blocks cyclic AMP-induced neurite outgrowth in PC12D cells. J. Neurochem. 70 (3), 1029–1034.

Shippenberg, T.S., Bals-Kubik, R., Herz, A., 1993. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. J. Pharmacol. Exp. Ther. 265 (1), 53–59.

Sim, L.J., Selley, D.E., Xiao, R., Childers, S.R., 1996. Differences in G-protein activation by mu- and delta-opioid, and cannabinoid, receptors in rat striatum. Eur. J. Pharmacol. 307 (1), 97–105.

Siggins, G.R, Martin, G., Roberto, M., Nie, Z., Madamba, S., De Lecea, L., 2003. Glutamatergic transmission in opiate and alcohol dependence. Ann. N.Y. Acad. Sci. 1003, 196–211.

Smart, D., Lambert, D.G., 1996a. The stimulatory effects of opioids and their possible role in the development of tolerance. Trends Pharmacol. Sci. 17 (7), 264–269.

Smart, D., Lambert, D.G., 1996b. Delta-Opioids stimulate inositol 1,4,5-trisphosphate formation, and so mobilize Ca2+ from intracellular stores, in undifferentiated NG108-15 cells. J. Neurochem. 66 (4), 1462–1467.

Snyder, S.H., Pasternak, G.W., 2003. Historical review: opioid receptors. Trends Pharmacol. Sci. 24 (4), 198–205.

Song, D.D., Harlan, R.E., 1994. The development of enkephalin and substance P neurons in the basal ganglia: insights into neostriatal compartments and the extended amygdala. Brain Res. Dev. Brain Res. 83 (2), 247–261.

Spangler, R., Ho, A., Zhou, Y., Maggos, C.E., Yuferov, V., Kreek, M.J., 1996. Regulation of kappa opioid receptor mRNA in the rat brain binge pattern cocaine administration and correlation with preprodynorphin mRNA. Mol. Brain Res. 19, 323–327.

Spijker, S., Houtzager, S.W., De Gunst, M.C., De Boer, W.P., Schoffelmeer, A.N., Smit, A.B., 2004. Morphine exposure and abstinence define specific stages of gene expression in the rat nucleus accumbens. FASEB J. 18 (7), 848–850.

Stanfa, L., Dickenson, A., Xu, X-J., Wiesenfeld-Hallin, Z., 1994. Cholecystokinin and morphine analgesia: variations on a theme. TIPS 15, 65–66.

Szekely, J.I., Miglecz, E., Dunai-Kovacs, Z., Tarnawa, I., Ronai, A.Z., Graf, L., Bajusz, S., 1979. Attenuation of morphine tolerance and dependence by alpha-melanocyte stimulating hormone(alpha-MSH). Life Sci. 24 (21), 1931–1938.

Starowicz, K., Sieja, A., Bilecki, W., Obara, I., Przewlocka, B., 2003. The effect of morphine on MC4 and CRF receptor mRNAs in the rat amygdala and attenuation of tolerance after their blockade. Brain Res. 990 (1–2), 113–119.

Suzuki, T., Tsuji, M., Mori, T., Misawa, M., Nagase, H., 1997. Involvement of α1 and β2 opioid receptor subtypes in the development of physical dependence on morphine in mice. Pharmacol. Biochem. Behav. 57, 293–299.

Suzuki, S., Carlos, M.P., Chuang, L.F., Torres, J.V., Doi, R.H., Chuang, R.Y., 2002. Methadone induces CCR5 and promotes AIDS virus infection. FEBS Lett. 519 (1-3), 173–177.

Tang, T., Stevens, B.A., Cox, B.M., 1996. Opioid regulation of intracellular free calcium in cultured mouse dorsal root ganglion neurons. J. Neurosci. Res. 44 (4), 338–343.

Tao, P.-L., Law, P.-Y., Loh, H.H., 1987. Decrease in delta and mu opioid receptor binding capacity in rat brain after chronic etorphine treatment. J. Pharmacol. Exp. Ther. 240, 809–816.

Tao, P.L., Chang, L.R., Law, P.Y., Loh, H.H., 1988. Decrease in delta-opioid receptor density in rat brain after chronic [D-Ala2,D-Leu5]enkephalin treatment. Brain Res. 462, 313–320.

Terwilliger, R.Z., Beitner-Johnson, D., Sevarino, K.A., Crain, S.M., Nestler, E.J., 1991. A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. Brain Res. 548 (1–2), 100–110.

Tjon, G.H., Voorn, P., Vanderschuren, L.J., de Vries, T.J., Michiels, N.H., Jonker, A.J., Klop, H., Nestby, P., Mulder, A.H., Schoffelmeer, A.N., 1997. Delayed occurrence of enhanced striatal preprodynorphin gene expression in behaviorally sensitized rats: differential long-term effects of intermittent and chronic morphine administration. Neuroscience 76 (1), 67–76.

Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251 (4989), 85–87.

Trujillo, K.A., Bronstein, D.M., Sanchez, I.O., Akil, H., 1995. Effects of chronic opiate and opioid antagonist treatment on striatal opioid peptides. Brain Res. 698 (1–2), 69–78.

Turchan, J., Lason, W., Budziszewska, B., Przewlocka, B., 1997. Effects of single and repeated morphine administration on the prodynorphin, proenkephalin and dopamine D2 receptor gene expression in the mouse brain. Neuropeptides 31 (1), 24–28.

Turchan, J., Przewlocka, B., Lason, W., Przewlocki, R., 1998. Effects of repeated psychostimulant administration on the prodynorphin system activity and kappa opioid receptor density in the rat brain. Neuroscience 85, 1051–1059.

Turchan, J., Przewlocka, B., Toth, G., Lason, W., Borsodi, A., Przewlocki, R., 1999. The effect of repeated administration of morphine, cocaine and ethanol on mu and delta opioid receptor density in the nucleus accumbens and striatum of the rat. Neuroscience 91, 971–977.

Turchan, J., Maj, M., Przewlocka, B., Przewlocki, R., 2002. Effect of cocaine and amphetamine on biosynthesis of proenkephalin and prodynorphin in some regions of the rat limbic system. Pol. J. Pharmacol. 54 (4), 367–372.

Turchan, J., Maj, M., Przewlocka, B., 2003. The effect of drugs of abuse on NMDAR1 receptor expression in the rat limbic system. Drug Alcohol Depend. 72 (2), 193–196.

Ueda, H., Miyamae, T., Fukushima, N., Takeshima, H., Fukuda, K., Sasaki, Y., Misu, Y., 1995. Opioid mu- and kappa-receptor mediate phospholipase C activation through Gi1 in *Xenopus* oocytes. Brain Res. Mol. Brain Res. 32, 166–170.

Uhl, G.R., Ryan, J.P., Schwartz, J.P., 1988. Morphine alters preproenkephalin gene expression. Brain Res. 459 (2), 391–397.

Uhl, G.R., Sora, I., Wang, Z., 1999. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc. Natl. Acad. Sci. U. S. A. 96 (14), 7752–7755.

Valjent, E., Pages, C., Herve, D., Girault, J.A., Caboche, J., 2004. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. Eur. J. Neurosci. 19 (7), 1826–1836.

Van Bockstael, E.J., Peoples, J., Menko, A.S., McHugh, K., Drolet, G., 2000. Decreases in endogenous opioid peptides in the rat medullocoerulear pathway after chronic morphine treatment. J. Neurosci. 20 (23), 8659–8666.

Van Vliet, B.J., Ruuls, S.R., Drukarch, B., Mulder, A.H., Schoffelmeer, A.N., 1991. Beta-adrenoceptor-sensitive adenylate cyclase is inhibited by activation of mu-opioid receptors in rat striatal neurons. Eur. J. Pharmacol. 195 (2), 295–300.

Van Ree, J.M., Niesink, R.J., Van Wolfswinkel, L., Ramsey, N.F., Kornet, M.M., Van Furth, W.R., Vanderschuren, L.J., Gerrits, M.A., Van den Berg, C.L., 2000. Endogenous opioids and reward. Eur. J. Pharmacol. 405 (1–3), 89–101.

Waeber, G., Habene, J.F., 1991. Nuclear translocation and DNA recognition signals colocalized within the bZIP domain of cyclic adenosine 3′ ,5′ -monophosphate response element-binding protein CREB. Mol. Endocrinol. 5 (10), 1431–1438.

Wang, Z., Sadee, W., 2000. Dissociation of morphine-induced cyclic AMP-upregulation and mu-opioid receptor desensitization. Proc. West Pharmacol. Soc. 43, 9–10.

Wang, X.M., Zhou, Y., Spangler, R., Ho, A., Han, J.S., Kreek, M.J., 1999. Acute intermittent morphine increases preprodynorphin and kappa opioid receptor mRNA levels in the rat brain. Brain Res. Mol. Brain Res. 66 (1–2), 184–187.

Widnell, K.L., Russell, D.S., Nestler, E.J., 1994. Regulation of expression of cAMP response element-binding protein in the locus coeruleus in vivo and in a locus coeruleus-like cell line in vitro. Proc. Natl. Acad. Sci. U. S. A. 91 (23), 10947–10951.

Widnell, K.L., Chen, J.S., Iredale, P.A., Walke, W.H., Duman, R.S., Habener, J.F., Nestler, E.J., 1996. Transcriptional regulation of CREB (cyclic AMP response element-binding protein) expression in CATH.a cells. J. Neurochem. 66 (4), 1770–1773.

Wise, R.A., Bozarth, M.A., 1987. A psychomotor stimulant theory of addiction. Psychol. Rev. 94 (4), 469–492.

Wood, P.L., Rao, T.S., 1991. Morphine stimulation of mesolimbic and mesocortical but not nigrostriatal dopamine release in the rat as reflected by changes in 3-methoxytyramine levels. Neuropharmacology 30 (4), 399–401.

Xie, G.X., Fuller, S.A., Stein, E.A., 1998. Dopamine release in the nucleus accumbens during heroin self-administration is modulated by kappa

opioid receptors: an in vivo fast-cyclic voltammetry study. J. Pharmacol. Exp. Ther. 284 (1), 151–161.

Yabaluri, N., Medzihradsky, F., 1997. Down-regulation of mu-opioid receptor by full but not partial agonists is independent of G protein coupling. Mol. Pharmacol. 52 (5), 896–902.

Yuferov, V., Kroslak, T., Laforge, K.S., Zhou, Y., Ho, A., Kreek, M.J., 2003. Differential gene expression in the rat caudate putamen after "binge" cocaine administration: advantage of triplicate microarray analysis. Synapse 48 (4), 157–169.

Zhou, W., Liu, H., Xie, X., 2000. The expression of glial cell derived neurotrophic factor and its receptor GDNFR-alpha and GDNFR-beta mRNA in spinal cord, brainstem and frontal cortex during morphine withdrawal in rats. Zhonghua Yixue Zazhi 80 (2), 135–139.

Zhu, H., Barr, G.A., 2001. Inhibition of morphine withdrawal by the NMDA receptor antagonist MK-801 in rat is age-dependent. Synapse 40 (4), 282–293.

Zhu, H., Ho, I.K., 1998. NMDA-R1 antisense oligonucleotide attenuates withdrawal signs from morphine. Eur. J. Pharmacol. 352 (2–3), 151–156.