Effect of opioids on Ca\(^\text{2+}/\text{cAMP}\) responsive element binding protein

Wiktor Bilecki and Ryszard Przewłocki

Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna St., 31-343 Cracow, Poland

Abstract. Ca\(^{2+}/\text{cAMP}\) response element binding protein (CREB) is an important factor linking the opioid-regulated secondary messenger systems to alterations in gene expression. Opioids regulate CREB level, its phosphorylation and binding to its corresponding response element in the promoters of several genes implicated in drug addiction. CREB mediates the action of opioids on the expression of several genes in brain regions responsible for drug-seeking behavior and manifestation of signs of dependence. Moreover, alterations in CREB level can affect the rewarding properties of morphine and regulate the self-administration of cocaine. At the cellular level CREB acts as convergence point for different cellular pathways. Opioids affect two different intracellular mediator systems: inhibitory - connected with cAMP, and stimulatory - involving calcium and the PKC pathway. Both can affect CREB but in different phases of opiate action. The presence of this biphasic mechanism can explain the phenomenon of the induction of some CRE-controlled genes after both acute and chronic morphine administration. Cellular studies also highlight the relevance of other ATF/CREB family members which can affect Ca\(^{2+}/\text{cAMP}\) response element (CRE) controlled transcription as well as other transcription factors which make the opioid induction longer lasting.

Key words: CREB(cAMP responsive element binding protein), opioid receptors, morphine, antisense, oligonucleotides, neuroblastoma, protein kinase, CRE binding activity
INTRODUCTION

Considerable interest has focused on molecular alterations which may contribute to pathophysiological states that occur following treatment with opiates. It is well known that opiates acting on opioid receptors (coupled to Gi/Go classes of the G proteins) acutely inhibit cyclic AMP (cAMP) formation, Ca$^{2+}$ conductance and activate potassium conductance, leading to hyperpolarization of the cell (Nestler 1992). The hyperpolarization resolves with time and cAMP levels are normalized and subsequently raised above baseline. The opiate-induced changes in the activation of protein kinase A lead to alterations in the phosphorylation of proteins relevant to opioid signaling and to changes in the expression of the CREB (calcium/cAMP response element binding protein), which may be important in the development and expression of opioid dependence (Nestler et al. 1993, Widnell et al. 1994, Maldonado et al. 1996). CREB exists as three alternatively spliced isoforms: CREB-α and CREB-δ which differ by a 14 amino acid segment, and CREB-β. The CREB-δ isoform comprises 80-90% of total CREB protein in the brain (Yamamoto et al. 1990). CREB-β is expressed ubiquitously and this isoform may compensate partly for the loss of the CREB-α and CREB-δ in CREB-knockout mice (Blendy et al. 1996, Glazewski et al. 1999). CREB activity is regulated by phosphorylation in response to diverse signals. Increases in calcium or cAMP result in phosphorylation of the serine residue within the kinase-inducible domain (KID) at position 133 (Ser$^{133}$) (Gonzalez and Montminy 1989). Recent data suggest that CREB phosphorylation alone does not alter the secondary structure of CREB; rather phosphorylation creates a high-affinity binding site for other proteins (Richards et al. 1996). In particular, phosphorylation of Ser$^{133}$ promotes interaction of CREB with the KIX domains of the coactivators CREB-binding protein (CBP) and P300 (Chrivita et al. 1993). The binding of CBP causes CREB to interact with the basal transcription factor TFIIB (Kwok et al. 1994) and TFIID (Horikoshi et al. 1988) and thereby initiates induction of CRE-dependent genes.

CREB-mediated transcription is thought to be critical for a variety of adaptive neuronal responses. It has been shown to be important for memory and learning in a number of systems (Frank and Greenberg 1994, Martin and Kandel 1996, Silva et al. 1998). Generation of mutant mice provided a molecular tool to study the relevance of CREB in vivo (Hummler et al. 1994). Mice completely lacking CREB are not viable (Rudolph et al. 1998). Mice lacking the CREB-α and CREB-δ isoforms are viable and show no impairment of development although they exhibit profound long-term memory deficits (Bourtchuladze et al. 1994, Kogan et al. 1997). In mice lacking the CREB-α and CREB-δ isoforms there is a strong reduction in naloxone-induced withdrawal behaviors and a small decrement in tolerance, but no alterations in pain sensitivity and the density of opiate receptors (Maldonado et al. 1996). However, there are several mechanisms compensating for this mutation (e.g. the increase in CREM, CREB-β and ATF-2). Moreover, this deficiency in CREB occurs throughout all tissues making precise evaluation of CREB function difficult.

More detailed studies involved analysis of CREB expression in brain regions implicated in opiate dependence and addiction. A role for CREB mediation of gene expression in response to opioids has been proposed (Nestler et al. 1993, 1996) based on the observation that administration of opioids affects components of the cAMP pathway. Support for this scheme comes from recent studies of rats dependent on cocaine in which upregulation of CREB-mediated transcription in the nucleus accumbens (NAc) counteracted the rewarding effects of cocaine and, conversely, overexpression of a dominant-negative mutant CREB in NAc increased the rewarding effects of cocaine (Carlezon et al. 1998).

CREB INVOLVEMENT IN THE ACTION OF OPIATES IN THE BRAIN

Nucleus accumbens

The brain region implicated in the reinforcing properties of opiates and possibly for the long-term motivational changes associated with opiate addiction is the NAc (Koob 1992). Morphine increases dopamine release in NAc (Di Chiara and North 1992). It has been proposed that morphine acts on inhibitory μ opioid receptors located on GABA interneurons (which are inhibitory as well) in substantia nigra pars reticulata (SNr) and ventral tegmental area (VTA) (Di Chiara and North 1992). This would disinhibit substantia nigra pars compacta (SNC) and VTA dopamine neurons, increase dopamine neuronal firing, and induce dopamine release in striatum and NAc (Bontempi and Sharp 1997). There are no data showing directly the effects of acute opiate administration on neural activity or cAMP system in the NAc. Total CREB protein level in the NAc was not af-
fected by acute opioids. However, acute morphine-μ-opioid receptors - induces c-Fos proteins and AP-1 binding in striatum and NAc (Liu et al. 1994). The c-Fos promoter is known to have the CRE and SRE elements, responsive to cAMP and Ca^{2+} and coactivation of both elements is required for Fos induction in single striatal cells (Robertson et al. 1995, Konradi et al. 1996). This is in agreement with the observation that CREB phosphorylation level rather than CREB levels is responsible for CREB activity (Faentzke et al. 1998). Functional relevance of CREB was demonstrated by the abolition of the ability of an acute exposure to cocaine to induce c-Fos by antisense oligonucleotide administration in this brain region. c-Fos induction after morphine was shown to be mediated by both dopamine and glutamate inputs to striatum/NAc from the SNc/VTA and cortex, respectively (Bontempi and Sharp 1997). Chronic morphine treatment also leads to a significant induction of some components of cAMP pathway (adenylyl cyclase level and PKA activity, c-Fos level, AP-1 DNA binding activity) but decreases other (G_{oi} levels) in the NAc (Terwilliger et al. 1991, Noble and Cox 1996). Given the observed upregulation of the PKA pathway in the NAc one should have expected CREB to be regulated by chronic morphine treatment as well. Indeed, it was, but levels of total CREB immunoreactivity were found to be decreased in the NAc (Widnell et al. 1996). This is a striking observation but the chronic morphine-reduced total CREB level could be considered as the compensatory mechanism to the increase in the CREB phosphorylation caused by upregulation of PKA pathway. Withdrawal from morphine elicited a dramatic increase in c-Fos and AP-1 binding (Nye and Nestler 1995) and given the decreased total CREB level this rapid induction apparently depended on increased CREB activity. It is another puzzle that c-Fos is induced in the NAc by acute and chronic morphine treatment as well as during morphine withdrawal. One possible explanation is that the observed c-Fos induction occurs within different neuronal populations, another possibility is that the induction is mediated via different mechanisms. The latter possibility could involve activation of CREB in each case: after acute or chronic morphine administration as well as during morphine withdrawal.

**Locus coeruleus**

Opioid regulation of CREB was also thoroughly studied in the locus coeruleus - major noradrenergic nucleus in the brain implicated in controlling attention and activity of the autonomic nervous system. Locus coeruleus (LC) is also important in physical opiate dependence and withdrawal. Acutely, opiates inhibit LC neuronal firing in part via inhibition of adenylyl cyclase and cAMP-dependent protein phosphorylation (Aghajanian and Wang 1987, Duman et al. 1988, Alreja and Aghajanian 1991). Acute opiates also decrease the phosphorylation state of CREB without affecting its total protein level (Guitart et al. 1992). Consequently, levels of c-fos expression were decreased in LC from rats treated acutely with morphine. In contrast, chronic opiates up-regulate the cAMP system in these neurons, with increases observed in levels of G protein subunits, adenylyl cyclase, adenylyl cyclase activity, PKA and tyrosine hydroxylase levels (Duman et al. 1988, Lane-Ladd et al. 1997). The spontaneous firing rate of LC neurons in brain slices is also increased by previous chronic administration of morphine (Kogan et al. 1992). Chronic morphine administration was found to increase total levels of CREB immunoreactivity in LC. This CREB regulation in the LC was surprising because CREB expression, particularly in the brain, is generally considered to be constitutive. The increase in CREB immunoreactivity was associated with an equivalent increase in CRE binding (Widnell et al. 1994). However, in the chronic morphine-treated state, despite the up-regulated cAMP system, CREB phosphorylation was reduced down to control levels probably due to the inhibition of the system by persistent exposure to opiates (Guitart et al. 1992). Consequently, c-Fos levels in rats treated chronically with morphine were decreased along with CREB phosphorylation. It is interesting to note that among adenylyl cyclase only types VIII and I are upregulated by chronic morphine, whereas several other types are not affected (Lane-Ladd et al. 1997). These forms of adenylyl cyclase are activated by Ca^{2+} and are only mildly activated by G_{ois} (Cooper et al. 1995). After precipitation of the opioid withdrawal the up-regulated cAMP system contributes to the dramatic increase of LC firing rates, an increase in CREB phosphorylation and a two- to three-fold increase in levels of mRNA and protein for c-fos (Hayward et al. 1990, Holmes et al. 1995).

**Functional relevance of CREB in drug addiction: an antisense oligonucleotide approach**

Due to the lack of specific inhibitors it has proven difficult to determine the role of a constitutive transcription
factor in vivo. The use of an antisense oligonucleotide strategy to specifically decrease levels of CREB surmounts this problem to some degree. Functional consequences of the morphine-induced CREB in LC were studied by injections of antisense oligonucleotides against CREB mRNA. In drug-naive rats CREB antisense oligonucleotide infusions reduced CREB immunoreactivity in the injected LC compared with the uninjected control side (Lane-Ladd et al. 1997). Such infusions also elicited reduction in basal levels of types VIII and I of adenylyl cyclase and G_{ai}. Thus the application of CREB antisense oligonucleotides reduced basal level of some, but not all, components of the PKA pathway. This pathway is up-regulated in the LC following chronic morphine treatment (see above). However, of these genes CREB was shown to act at a CRE within promoter of tyrosine hydroxylase gene only (Lazaroff et al. 1995). Promoters of the other regulated genes have not yet been isolated (type VIII adenylyl cyclase) or lack functional CREs (PKA, type I adenylyl cyclase). Nevertheless, CREB antisense oligonucleotide blocked the morphine-induced upregulation of type VIII adenylyl cyclase and tyrosine hydroxylase and also completely prevented the morphine-induced increase in spontaneous firing rates of LC neurons. On the other hand type I adenylyl cyclase and PKA upregulation were not affected by CREB antisense oligonucleotide in morphine-treated animals despite significant reduction of their basal levels in naive animals.

Antisense oligonucleotide infusions also significantly attenuated the signs of certain withdrawal behaviors (teeth chattering, wet dog shakes, ptosis, irritability, vacuous chewing) but not others (piloerector, lacrimation, salivation, diarrhea, stereotypy, weight loss). Interestingly, greater attenuation of these behaviors was seen 15-30 min. after precipitation of withdrawal than during the first quarter when the withdrawal syndrome is the most pronounced (Lane-Ladd et al. 1997). This suggests that CREB may be responsible for a secondary neuroadaptation to sustained activity of cAMP pathway in LC during withdrawal.

In NAc antisense oligonucleotides against CREB down-regulated CREB protein levels to an extent similar to that of chronic morphine. Also G_{ai} and levels of the catalytic subunit of PKA were reduced by administration of antisense oligonucleotides into the NAc. Although the antisense-induced reduction of G_{ai} resembled the action of chronic morphine on CREB, their effects on the PKA were in opposition. This is important because PKA phosphorylates CREB on Ser^{133} (Gonzalez and Montminy 1989).

The above data indicate that although antisense oligonucleotides against CREB reduced the CREB protein level in both NAc and LC, their specific action on claimed CREB targets is rather unclear. Firstly, antisense oligonucleotides against CREB decreased the basal but not morphine-induced levels of adenylyl cyclase type I and catalytic and regulatory subunits of PKA whose gene promoters lack functional CRE elements. Secondly, antisense oligonucleotides against CREB reduced the level of G_{ai} in NAc but not in LC, and the reason for this is unclear. Lastly, in the unphosphorylated state, CREB can bind DNA but does not activate transcription. The mutation of CREB molecule by a Ser^{133}→Ala^{133} substitution functions as a potent dominant-negative repressor of CREB-dependent gene expression both in vitro and in vivo (Faentzke et al. 1998). CREB activity is regulated by phosphorylation, and unphosphorylated CREB under certain circumstances can even act as a repressor of gene transcription (Vallejo 1995). The effects of CREB antisense oligonucleotides on the factors regulated by chronic morphine treatment are gathered in Table I.

Functional relevance of CREB antisense oligonucleotides administration was demonstrated by the abolition of the ability of acute exposure to cocaine to induce c-Fos in the NAc and LC. Furthermore, intra-NAc injections of CREB antisense oligonucleotides produced a transient decrease in cocaine self-administration. Similarly, overexpression of CREB in this region decreases while, conversely, overexpression of a dominant-negative mutant of CREB increases the rewarding effects of cocaine. CREB regulates dynorphin expression in the NAc. Altered transcription of dynorphin likely contributes to the CREB level regulation of the reinforcing properties of cocaine (Carlezon et al. 1998). However, this conception remains in disagreement with the reported reduction of CREB level in NAc after chronic morphine treatment with concurrent elevation of prodynorphin, its peptide α-neoendorphin and c-fos (i.e. CREB regulated genes) (Nye and Nestler 1995, Przewlocka et al. 1996, Nylander et al. 1997). One possible explanation is that changes in CREB level can not be simply attributed to its phosphorylation, although phosphorylation is crucial for controlling other genes.
and can be independent from CREB level. Another likely explanation is that CREB as well as CRE link opioid receptors through general ATF/CRE transcription factors to alteration in target gene expression.

**CELLULAR STUDIES**

Activation of CREB in the brain depends largely on the innervation of the structures studied (Liu and Graybiel 1998). NAc receives inputs from several limbic structures including the amygdala, the hippocampus and the limbic prefrontal cortex. These limbic-associated circuits as well as the presence of different neuronal populations make the evaluation of the relevance of CREB very complicated. Moreover, studies on CREB phosphorylation in the brain require the development of special techniques which do not directly measure CREB phosphorylation (Guitart et al. 1992). The direct effect of opioids on the regulation of CREB and its phosphorylation has been studied in NG108-15 neuroblastoma x glioma cells (Bilecki et al. 2000). This cell line was established as a cellular model for studying opioid effects and several phenomena of opioid action in the brain. The molecular mechanism of tolerance, dependence, and withdrawal on the basis of the action of opioids on cAMP pathway components was first shown in these cells. Acute morphine, via δ-opioid receptors, exerts a stimulatory effect on CREB phosphorylation but not total CREB level in these cells. This stimulation required Ca\(^{2+}\)/calmodulin and activation of protein kinase C but did not involve the morphine-suppressed cAMP pathway. Increased phosphorylated CREB level was accompanied by induction in c-Fos levels, an effect blocked by Ca\(^{2+}\)/calmodulin protein kinase C inhibitors, suggesting that the CREB mediates the observed effect (Fig. 1). Moreover, opioid-stimulated c-Fos levels remained elevated for longer time periods (up to 6 h) than phosphorylated CREB levels (up to 3 h). This suggests that c-Fos may make the acute opioid-stimulation even longer-lasting. Prolonged (up to 5 days) treatment with morphine reduced phosphorylated CREB and c-Fos levels down to control levels. In contrast, precipitated withdrawal induced profoundly phosphorylated CREB and c-Fos levels in these cells. This indicates that opioids

<table>
<thead>
<tr>
<th>Adenylyl cyclase</th>
<th>Protein kinase A subunits</th>
<th>Tyrosine hydroxylase</th>
<th>G(_i)</th>
<th>FIRING</th>
<th>CREB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic morphine in NAc</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
</tr>
<tr>
<td>Chronic morphine in LC</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
<td>††</td>
</tr>
<tr>
<td>CREB antisense oligonucleotides in NAc</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
</tr>
<tr>
<td>CREB antisense oligonucleotides in LC</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adenylyl cyclase</th>
<th>Protein kinase A subunits</th>
<th>Tyrosine hydroxylase</th>
<th>G(_i)</th>
<th>FIRING</th>
<th>CREB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic morphine in NAc</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
</tr>
<tr>
<td>Chronic morphine in LC</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
</tr>
<tr>
<td>CREB antisense oligonucleotides in NAc</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
</tr>
<tr>
<td>CREB antisense oligonucleotides in LC</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
<td>†↑</td>
</tr>
</tbody>
</table>

Table I

The effect of CREB antisense oligonucleotide on the levels of factors regulated by chronic morphine treatment in the nucleus accumbens (Nac) and locus coeruleus (LC)
could affect two different intracellular mediator systems: inhibitory - connected with cAMP, and stimulatory - involving calcium and the PKC pathway, and affect CREB in different phases of opiate action. Similar to growth factor signals, Ca\(^{2+}\) can also activate the mitogen activated protein kinases (MAPKs) pathway (Finkbeiner and Greenberg 1996) which has been recently shown to mediate CREB phosphorylation in response to Ca\(^{2+}\) influx and PKC activation in oligodendrocyte progenitor cells (Pende et al. 1997). We have found that in NG108-15 cells the mitogen-activated protein kinases (MAPKs) pathway is activated on stimulation of \(\delta\)-opioid receptors. This increase of phosphorylated MAPKs levels was short-lasting (5-20 min.) and similar to CREB phosphorylation involved PKC and neither cAMP nor cGMP de-
dependent protein kinase pathway. Therefore the MAPKs could also contribute to the induction of CREB phosphorylation following opioid treatment as well as to the induction of c-Fos synthesis (Fig. 2). Moreover their stimulation by acute opioids confirms the existence of an activatory intracellular system coupled to δ-opioid receptors and involving Ca$^{2+}$. Recent data from cultured native CNS neurons also show that the activation of opioids receptors can augment several components of neuronal Ca$^{2+}$ signaling pathways and, as a consequence, enhance intracellular Ca$^{2+}$ signals (Przewłocki et al. 1999).

Due to the fact that morphine analgesia and dependence are connected predominantly with μ receptors, a model containing μ receptors (Neuro2a cells, transfected with the cloned μ receptor Neuro2a MOR) was also studied. Morphine, acting via μ-opioid receptors, acutely induced CREB phosphorylation in Neuro2a MOR cells but this effect was shorter (15-45 min.) and weaker than stimulation observed in NG108-15 cells. Acute administration of opiates (morphine, endomorphin-1, DAMGO) also increased binding to consensus CRE and AP-1 elements in Neuro2a MOR cells. Total CREB protein level was not affected significantly by morphine and because CREB binds CREs irrespective of its state of dimerization (Waeber and Habener 1991, Richards et al. 1996) this suggests involvement other factors - possibly ATF-2: 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). A concomitantly increase in CRE DNA binding activity was observed 40-60 min. after morphine treatment, indicating a

**Fig. 3.** Schematic representation of the mechanisms involved in chronic opiate action on CREB phosphorylation at the cellular level. A, chronic opiate treatment leads to a compensatory upregulation of the cAMP pathway, however CREB phosphorylation remains unchanged possibly due to the activation of nuclear protein phosphatases. B, withdrawal profoundly activates cAMP pathway and induces Ca$^{2+}$ influx which in turn promote CREB phosphorylation and activation of target genes (e.g. c-Fos).
secondary adaptation, possibly dependent on newly synthesized factors. In contrast, increase in AP-1 DNA binding activity displayed a fast onset reaching its maximum at 15 min and declined slowly over several hours to the basal level. Opioid activation of μ-opioid receptors induced also transient (5-10 min) phosphorylation of MAPKs pathway which may not only contribute to CREB phosphorylation but also lead to c-fos induction and thus contribute to increase in AP-1 DNA binding activity (Fig. 1).

Prolonged, up to 5 days morphine treatment normalized back to basal the levels of c-Fos, AP-1 and CRE DNA binding activity and slightly decreased the levels of phosphorylated CREB and MAPKs. The attenuation of the opioid effects on the above factors may underlie the molecular basis for tolerance (Fig. 3A). Washing out morphine or application of the opioid antagonists induced withdrawal in both NG108-15 and Neuro2a MOR cells. Withdrawal from morphine elicited increase in c-Fos and phosphorylated CREB levels and induced CRE and AP-1 DNA binding activity with higher potency but with kinetics similar to acute opioid action in these cells (Fig. 3B). On the other hand, decreased phosphorylated MAPKs levels caused by chronic morphine were further lowered during withdrawal. Interestingly, the kinetics of the withdrawal-elicited decrease in the phosphorylation of MAPKs and the withdrawal-elicited increase in the CREB phosphorylation were similar suggesting the involvement of one common mechanism in these two opposite effects. The upregulation of the cAMP pathway during withdrawal is the most likely and obvious mechanism because the induction of PKA pathways is frequently a negative regulator of the MAPKs cascade (Neary 1997) and forskolin almost completely blocked the phosphorylation of MAPKs in these cells. Meaningfully, chronic morphine treatment and withdrawal increase the phosphorylation of the stress-activated protein kinases (SAPKs) also known as Jun N-terminal kinases. Their major targets are transcription factors c-Jun and ATF-2 which can form heterodimers and bind to CRE elements. In addition the phosphorylation of c-Jun in the AP-1 complex by SAPKs can increase AP-1 DNA binding activity (Fig. 4).

The impact of opioids on the CRE- and AP-1-regulated expression of target genes was also investigated in Neuro2a MOR cells transiently transfected with luciferase genes under control of CRE and AP-1 elements. Acute administration of μ opioid agonist DAMGO increased both CRE and AP-1 controlled expression of re-
porter gene, an effect totally attenuated after chronic DAMGO treatment. The application of opioid antagonist naloxone precipitated withdrawal in Neuro2a MOR cells and again induced CRE- and AP-1-controlled expression of the reporter gene. The above findings clearly indicate that on the cellular level the main mediators of opioid action on gene expression are Ca\(^{2+}\) and cAMP. Depending on the time of the exposure to opioids or opioid-withdrawal, both pathways are able to induce CREB phosphorylation, CRE DNA binding activity and CRE-controlled expression of reporter genes, as well as other transcription factors and the genes influenced by them. This cascade of events creates the basis for general and long-lasting cellular adaptations to opioid action. Interestingly, cells treated with morphine and subsequently cultured for several days in the absence of any opioids maintain some sort of tolerance to morphine action on the level of CREB phosphorylation, highlighting the role of CREB in this process. Recent studies of CREB activation in organotypic cultures of striatum have shown that dynamics of CREB phosphorylation is brain region-dependent, raising an intriguing possibility that different levels of CREB activation might serve to accommodate different neuronal information processing in these different brain regions (Liu and Graybiel 1998). The data derived from cell cultures may also explain the in vivo phenomenon of opioid-induced expression of genes like c-fos or dynorphin whether during acute or chronic treatment as well as during withdrawal.

**SUMMARY**

CREB is one of the most important factors linking the opioid-regulated secondary messenger systems to alterations in gene expression. Opioids regulate CREB level, its phosphorylation, and binding to promoters of several genes implicated in drug addiction. CREB controls expression of addiction-related genes in brain regions responsible for drug-seeking behavior and manifestation of signs of dependence. In cultures of cells expressing \(\mu\) and \(\delta\)-opioid receptors, opioids affect CREB and via CREB several genes implicated in development of tolerance and addiction. On the other hand, our understanding of this process is affected by the sole presence of the CREB acting at its CRE site on DNA. Our recent finding highlights that not only CREB but also other putative factors affecting CRE DNA binding activity could be crucial in transcriptional adaptation to opioids. Recent data provide evidence that regulation of gene expression contributes importantly to development of tolerance and addiction. However, detailed knowledge of the molecular steps by which opioids induce adaptations within different cells is yet to be discovered. Knowledge of the molecular mechanisms of these processes would provide a basis for development of new strategies for treatment of addiction.

**REFERENCES**


