Reviews on Calcium Mediated Secondary Messengers in Chronic Opioids Exposure/Addiction

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ABSTRACT

Addiction and withdrawal are problems disturbing the health of the individual and also causes difficulties for society, raising the rates of divorce, unemployment and government spending on legal and medical systems. Opioids show an important pharmacological effect in the treatment of pain, with extremely addictive potential. Chronic opioid exposure is known to produce the complex behaviors of tolerance and dependence, a state exposed by opioid abstinence leading to withdrawal syndrome, as well as oxidative stress. Studies show that calcium mediated secondary messengers play a crucial role in the mechanism of addictive process and oxidative stress induced by chronic opioid usage. Calcium/calmodulin-dependent protein kinase II (CaMKII), is a major calcium regulated signal transducer that controls many neuronal systems and play important role in neuronal plasticity and can act as a key and direct promoting opioid tolerance and dependence and identifying such a direct mechanism may be useful for designing a pharmacology treatment for these conditions, recent studies, has been shown that calcium channels antagonist can be used in the treatment of withdrawal syndrome. Chronic opioid exposure associated with tolerance, dependence withdrawal syndrome and oxidative stress. Studies has shown that calcium mediated secondary messengers involved in the genesis of these conditions, better understanding of biological mechanisms underlie reduction in neuronal cell excitability could help in the identification of pharmacological targets for treatment.

INTRODUCTION

Opioids are highly effective for the treatment of pain (Williams et al., 2001). Chronic exposure to opioids eventually leads to drug addiction, which is believed to involve maladaptive changes in brain function (Pu et al., 2002). Although compounds of extremely high potency have been produced, the problem of tolerance to and dependence on these agonists persists. The adaptive changes in cellular and synaptic function induced by chronic morphine treatment (Williams et al., 2001). Tolerance describes the need for an increasing dose of opioid to achieve the same effect; this term was based originally on the tolerance that develops to many of the acute behavioral actions of opioids (e.g., analgesia and autonomic inhibition). Dependence describes an altered physiological state caused by repeated opioid exposure such that cessation of drug administration leads to a withdrawal syndrome characterized by serious physiological disturbances, emotional or motivational symptoms. This latter form of dependence is perhaps the strongest determinant of opioid addiction (Nestler et al., 1996; Nestler, 2002). Which can be defined as loss of control over drug use, or the compulsive seeking and taking of drugs despite adverse consequences (Nestler, 2001).

Globally opioid addiction and withdrawal are problems affecting the health of the individual and also causes problems for society, increasing the rates of divorce, unemployment and government expenditure on legal and medical systems (Saboory et al., 2012). In Mexico, according to the 2002 national addiction survey, opioids are among the drugs consumed without prescription, mainly in the urban zone by a large sector of the population, young and adult and are supply by physicians, friends, and stores with an increase in the use of these drugs with recreational purposes is currently in evidence, which produces resultant addictions (Guzman et al., 2006).
Over time, chronic opioid exposure causes adaptation in the brain reward pathways (Nestler, 2002).

**Role of Calcium**

McDonald and Lambert (2005); Smith et al. (2002) reported a reduced neuronal cell excitability resulting in reduced transmission of nerves impulses, inhibition of neurotransmitter release and pain stimulation following morphine administration. A recent study shows that calcium plays an important role in the genesis of morphine dependence and withdrawal (Seth et al., 2012). Calcium plays an important role in the transmission of pain signals in the central nervous system. At the presynaptic nerve terminal, voltage-gated calcium channels (VGCCs) open in response to action potentials to allow an influx of calcium ions. The influx is a graded process varying in a linear manner with the frequency of action potentials.

The influx, in turn, leads to release of various neurotransmitters that diffuse across the synaptic cleft to the postsynaptic membrane and binds to their specific receptors. Morphine is the drug of choice for treatment of chronic pain (McCarberg and Barkin, 2001).

It binds to m-opioid receptor (MOR) on the pre-and postsynaptic membranes. However, binding of morphine to MOR leads to inhibition of neurons concerned with transmission of pain. MOR does so by blocking VGCCs, opening inwardly rectifying potassium channels and inhibiting activity of adenylyl cyclase (North 1993). More recent observations have extended the actions of opioids to include the activation of protein kinase C (PKC), the release of calcium from extracellular stores, the activation of the mitogen-activated protein kinase (MAPK) cascade, and the realization that receptor trafficking plays an important role in receptor function (Belcheva et al., 2005).

**Ca\(^{2+}\) Mediated Second Messenger**

Calcium is a universal second messenger used to regulate a wide range of cellular processes. This role in signaling has to be conducted against the rigid homeostatic mechanisms that ensure that the resting level of Ca\(^{2+}\) is kept low (i.e. between 20 and 100 nmol l-1) in order to avoid the cytotoxic effects of a prolonged elevation of Ca\(^{2+}\). Cells have evolved a sophisticated signaling system based on the generation of brief pulses of Ca\(^{2+}\) which enables this ion to be used as a messenger, thus avoiding its toxic effects (Berridge, 1997).

These second messengers can provide further pathways for opioid control of neuronal activity. Opioid effects on intracellular Ca\(^{2+}\) are of particular interest because of the widespread role of intracellular Ca\(^{2+}\) in a variety of cellular processes such as protein phosphorylation, membrane excitability, synaptic transmission, synaptic plasticity, genomic expression, cell growth and differentiation, and neurotoxicity (Siesjo, 1994; Ghosh and Greenberg, 1998). The chronic administration of morphine and related opioid drugs results in tolerance and dependence which limits the clinical utility of these agents. Neuronal plasticity is probably responsible in large part for tolerance and dependence.

**Opioid and CaMKII activity**

Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) plays a crucial role in the neuroplastic events underlying memory formation and other phenomena (Liang et al., 2004). CaMKII is an enzyme expressed in many tissues but is found in principally high abundance in the nervous system (Hanson and Schulman, 1992). CaMKII is a major calcium regulated signal transducer that regulates many neuronal systems including receptor-gated ion channels, calcium-dependent ion currents, and the synthesis and release of neurotransmitters (Schulman, 1993). Ca\(^{2+}\) regulation has further expanded the spectrum of opioid-induced biological effects on all three cloned opioid receptors (Piros et al., 1996). Many of Ca\(^{2+}\)-mediated events occur when the released Ca\(^{2+}\) binds to and activates the regulatory protein calmodulin, which may activate calcium-calmodulin-dependent protein kinases, or may act directly on other effector proteins. Besides calmodulin, there are many other Ca\(^{2+}\)-binding proteins that mediate the biological effects of Ca\(^{2+}\). In neurons, concomitant increases in cytosolic and mitochondrial calcium are important for the synchronization of neuronal synaptic transmission with mitochondrial energy metabolism (Ivannikov et al., 2013). A study conducted by Przewlocki et al. (1999) suggested that activation of opioid receptors can enhance several components of neuronal Ca\(^{2+}\) signaling pathways significantly and, as a consequence, enhance intracellular Ca\(^{2+}\) signals, the results provide evidence of a novel neuronal mechanism of opioid action on CNS neuronal networks that may contribute to both short and long-term effects of opioids. Learning and memory are proposed to be essentially involved in opioid addiction, it is interesting to hypothesize that selective inflection of those genes that play key roles in learning and memory processes could affect the development of opioid tolerance and dependence. Calcium/calmodulin-dependent protein kinase II (CaMKII) belongs to this group of genes, because it is expressed predominantly in cerebral cortex and hippocampus (Erondu and Kennedy, 1985) and is vital in certain types of learning and memory, inhibition or disruption of this kinase impairs spatial learning and memory tasks in rats (Fan et al., 1999). Interestingly, study has shown that morphine treatment increases the expression of CaMKII in rat hippocampus but not in other brain regions (Lou et al., 1999). A study conducted by Liang et al (2004) suggested that involving enhanced spinal expression of CaMKII after chronic exposure of mice to morphine would seem to fit well with an overall scheme in which CaMKII participates in the neuroplastic changes underlying tolerance to the analgesic effects of morphine. Studies has shown that CaMKII are essential for the development of opioid tolerance and dependence (Fan et al., 1999; Lou et al., 1999), and suggested that CaMKII is important in neuronal development and formation of long-term potentiation (Bortolotto and Collingridge, 1998), so inhibition of this kinase prevents the neuronal changes induced by chronic opioid treatment and the subsequent tolerance and dependence (Fan et al., 1999). However, intracellular Ca\(^{2+}\), calmodulin, and CaMKII can all be regulated by opioids. Cytosolic free Ca\(^{2+}\) was increased after the treatment with opioids (Fields and Sarne, 1997;
Quillan et al., 2002). Likewise, chronic treatments with opioids have been found to increase calmodulin activity (Nehmad et al., 1982).

Tang et al. (2006) strongly supported the hypothesis that CaMKII can act as a key and direct factor in promoting opioid tolerance and dependence. Identifying such a direct mechanism may be useful for designing pharmacological treatments for these conditions. CaMKII activity was increased after the treatment with morphine; the effect exhibited a temporal correction with the development of opioid tolerance and dependence. In mice treated with morphine (100 mg/kg s.c.), morphine tolerance and dependence developed in 2 to 6 h. An acute supraspinal administration of KN93 [2-[N-(2-hydroxyethyl)]-N-(4-methoxybenzenesulfonyl)amino-N-(4-chlorocinnamyl)-N methylbenzylamine], a CaMKII inhibitor, was able to dose-dependently reverse the already-established antinociceptive tolerance to morphine (p < 0.001 for 15–30 nmol; not significant for 5 nmol). KN92 [2-[N-(4-methoxybenzenesulfonyl)] amino-N-(4-chlorocinnamyl)-N-methylbenzylamine] (30 nmol i.c.v.), a kinase-inactive analog of KN93, did not affect opioid tolerance, study conducted by Fan et al (1999) supported the above result. Previous study proposed that CREB play an important role in the development of opioid tolerance and dependence (Nestler, 2001). CaMKII affects a number of downstream effectors, a key CaMKII downstream effector, activation of CREB (pCREB) in mice that have been treated with morphine. As expected, chronic treatment with morphine increased the levels of pCREB (Tang et al., 2006). Another study conducted by Bileckiet et al. (2005), has shown that chronic morphine treatment and withdrawal decrease the level of phosphorylation of extracellular signal-regulated kinase (ERK) due to participation of up-regulation of PKC and CaMKII pathways which seems to be engaged in the ERK inhibition, their results provide evidence that both opioid administration and opioid withdrawal, affecting similar intracellular pathways, can exert different effects on ERK activity. Study by Gray et al. (1999), suggested that their data are consistent with a model in which systemic amphetamine, by reversing the dopamine transporter, increases dopamine release and activates dopamine receptors on ventral striatal output neurons, resulting in transsynaptic, calcium-dependent glutamate and dopamine release in vivo. It is the secondary, calcium-dependent release that appears to be regulated by k-opioid receptor inhibition. By decreasing calcium influx, stimulation of k-opioid receptors, residing on dopaminergic and glutamatergic terminals in the ventral striatum, would eliminate the calcium-dependent component of amphetamine-induced dopamine and glutamate release, contributing to inhibition of amphetamine stimulated behaviors. Pierce et al. (1998) suggested that that neuronal calcium, acting via calcium-dependent kinases, promotes the expression of behavioral sensitization to opioids.

Role of Ca\(^{2+}\) in Opioid Induced Oxidative Stress

The oxidative stress (OS) is defined as a state where oxidative forces exceed the antioxidant system due to loss of balance between them, it is not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptive phenomena and regulation of intracellular signal transduction (Yoshikawa and Naito, 2002). It is characterized by excessive formation of free radicals or reactive oxygen species (ROS) and reactive nitrogen species (RNS) that exceeds the capacity of antioxidant mechanisms to eliminate these radicals. ROS derived from oxygen include radical species such as superoxide (O\(_2^−\)) and hydroxyl radical (HO\(_\cdot\)), along with non-radical species such as hydrogen peroxide (H\(_2\)O\(_2\)) (Yung et al., 2006). The risks to health caused by addictive drugs in relation to oxidative stress and free radicals, since the processes of peroxidation of lipoperoxidation are severely damaged in these individuals due to a loss in the balance between oxidation and antioxidation (Zhou et al., 2001). However, Morphine caused activation of NADPH oxidase and production of superoxide, which contribute to macrophage injury, are apparently mediated by activation of μ-opioid receptors and subsequent activation of the phospholipase D pathway and increase in intracellular Ca\(^{2+}\) concentration (Bhat et al., 2004). The increase of cytosolic content of calcium through a combination of effects on calcium pumps, exchangers, channels and binding proteins (Zima and Blatter, 2006), affects the multiple aspects of calcium homeostasis and calcium dependent signaling (Tang et al., 2013). Ca\(^{2+}\) is one of the most potent, specific and tightly controlled cellular regulators, and virtually every calcium control mechanism in the cell is both sensitive to oxidative stress and able to modulate it (Ermak and Davies, 2002; Bogeskiet al., 2011). Reactive oxygen and nitrogen species can be used as messengers in normal cell functions. However, at oxidative stress levels they can disrupt normal physiological pathways and cause cell death. Such a switch is largely mediated through Ca\(^{2+}\) signaling. OS causes Ca\(^{2+}\) influx into the cytoplasm from the extracellular environment and from the endoplasmic reticulum or sarcoplasmic reticulum (ER/SR) through the cell membrane and the ER/SR channels, respectively. Rising Ca\(^{2+}\) concentration in the cytoplasm causes Ca\(^{2+}\) influx into mitochondria and nuclei. In mitochondria Ca\(^{2+}\) accelerates and disrupts normal metabolism leading to cell death. In nuclei Ca\(^{2+}\) modulates gene transcription and nucleases that control cell apoptosis (Ermak and Davies, 2002). However, Guzman et al. (2006) revealed that Glutathione (GSH) is the main regulator of the redox balance and contributes to the protection of tissues exposed to oxidizing agent, and shown decrease glutathione levels in both weaned and adult animals and suggested that opioids like morphine unprotected the brain from oxidative stress. Sumathi et al. (2011) stated that the glutathione level in the brain was lowered in morphine treated rats than in the control animals. Heroin has been shown to elevate dopamine (DA) level, and It well known that increase in DA oxidative metabolism lead to increased ROS formation and thus, ROS have been frequently associated with neuronal cell death due to damage to carbohydrates, amino acids, phospholipids, and nucleic acids. Also decrease in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in mice brain (Xu et al., 2006). Zhou et al. (2011) revealed that OS and apoptosis in the CNS,
mechanisms for morphine induced neurotoxicity, the study determined the activities of SOD, CAT and GPx in the C6 cells, and observed the decreased activities of SOD, CAT, and GPx in C6 cells after morphine treatment. Studies have been established that glutamate actions mediated through N-methyl-D-aspartate (NMDA) subtype glutamate receptors has been implicated in the development of tolerance and dependence (Bajot et al., 2006; Murray et al., 2007), and glutamate actions mediated through NMDA receptors result from the subsequent activation of nitric oxide synthase (NOS) and formation of NO, which implicated in morphine tolerance and dependence, thus morphine-induced NO overproduction and oxidative stress (Abdel-Zaher et al., 2010). Similar studies has shown that chronic opioids abuse resulted in the decrease level of antioxidant and anti-oxidases status (Zhou et al., 2000; Sumathi et al., 2011). Furthermore, chronic morphine exposure can be connected with some pathological consequences including neurotoxicity and neuronal dysfunction, hepatotoxicity, kidney dysfunction, oxidative stress and apoptosis. However attention has been paid to morphine than other opioids in connection with oxidative stress. It was believed that there are two conceivable ways how this drug participate in the development of OS that can either promote formation of free radicals or reduced activity of different components of antioxidant systems in target cells (Skrabalova et al., 2013). Samarghandian et al (2014), study the effect of long-term treatment of morphine on enzymes, oxidative stress indices and antioxidant status in male rat liver. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and liver malondialdehyde (MDA) level as well as activities of superoxide dismutase (SOD), glutathione-s-transferase (GST) and catalase (CAT) were measured. Serum levels of ALT, AST and LDH were significantly higher in the morphine group compared with the control group and the activities of SOD, GST and CAT were significantly lower in the morphine group compared with the control group (P < 0.01). Similar study conducted by Zhang et al (2004) indicated that there was serious oxidative stress and damage, and the exogenous antioxidants were able to prevent oxidative damage of biomolecules and the hepatotoxicity in morphine-administered mice. Therefore, strategies of blocking oxidative stress may be useful in the development of therapy for chronic morphine exposure.

Potential Role of Calcium Antagonist in the Treatment of Addiction

The voltage-dependent calcium channels have been the subjects of intensive research after chronic opioid exposure for the last few years (Little, 1995). A recent study has shown that calcium plays an important role in the genesis of morphine dependence and withdrawal, and suggests that calcium channel blockers could be useful in the management of opioid withdrawal (Seth et al., 2012). Yamamoto et al (1978) reveal that a rise and fall of calcium level toward normal value in both chronic and acute morphine treatment during withdrawal syndrome, which suggest that different calcium related mechanism are involve in both types of dependence. The original classification showed the L-subtypes of channel to be selectively blocked by dihydropyridine calcium antagonists, which are used therapeutically as hypotensive and antiarrhythmic agents (Tsien et al; 1988). Considerable evidence has been published indicating that dihydropyridine-sensitive calcium channels play a role in physical dependence on morphine and other addictive drugs (Little 1995; Verma et al., 2001; Rabbani et al., 2003; Rabbani et al., 2004). Antkiewicz (1999) revealed that number of dihydropyridine sensitive binding sites in the CNS represent voltage-sensitive calcium channels, which increase in rats showing signs of morphine withdrawal. Acute and chronic administration of L-type calcium channel blockers such as nimodipine, nifedipine and nicardipine were found to protect against naloxone-precipitated morphine withdrawal in mice and rats (Rabbani et al., 2003; and Rabbani et al., 2004).

CONCLUSION

Derivatives from the opium plant have been described as analgesics and used for pain control since 3500 bc. It was not until 1806 that a pure opioid substance was isolated. This substance was called “morphine,” named after the Greek god Morpheus. Since that time the opium plant has yielded other derivatives, and synthetic analogues of morphine have been produced for medicinal use. The use of opioid medications has fluctuated because of a variety of factors, including but not limited to production, availability, governmental regulation, and physician and societal attitudes. Over the last 20 years the prescribing pattern of opioids has escalated significantly for a number of reasons. The increased trend in prescription writing has been accompanied by a concordant rise in the incidence of diversion and abuse, as well as an increase in the incidence of complications, including overdose and death. Over the past decade, evidence for a sustained benefit of opioids in alleviating chronic pain has remained weak and inadequate, although evidence of risk associated with use of the drugs has clearly escalated.

This change in which evidence of the efficacy of opioids has not changed whereas risk has increased should have a significant impact on treatment decisions based on risk-benefit analysis. Studies have shown that calcium mediated secondary messengers involved in the genesis of these conditions, better understanding of biological mechanisms underlie reduction in neuronal cell excitability could help in the identification of pharmacological targets for treatment.

REFERENCES


Bajo M, Crawford EF, Roberto M, Madamba SG, and Siggins GR. Chronic morphine treatment alters expression of N-methyl-D-


