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# Neuroprotection and Pain Management

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## 1. Introduction

Pain, as a sub modality of somatic sensation, has been defined as a complex constellation of unpleasant sensory, emotional and cognitive experiences provoked by real or perceived tissue damage and manifested by certain autonomic, psychological, and behavioral reactions. The benefit of these unpleasant sensations, however, is underscored by extreme cases: patients lacking the ability to perceive pain due to hereditary neuropathies often maintain unrealized infections; self mutilate, and have curtailed life spans. Normally, nociception and the perception of pain are evoked only at pressures and temperatures extreme enough to potentially injured tissues and by toxic molecules and inflammatory mediators. As opposed to the relatively more objective nature of other senses, pain is highly individual and subjective and the translation of nociception into pain perception can be curtailed by stress or exacerbated by anticipation (Woolf).

Chronic pain is estimated to affect millions of people worldwide and is one of the most common reasons for physician visits (Scascighini et al. 2008). Inflammation may cause direct painful stimuli as well as sensitize nociceptors to stimulation (McMahon et al. 2005). Thus, there are multiple points along the pain pathway that represent opportunities for therapeutic intervention. Despite this, there are only a limited number of mechanisms through which current pain medications work. Major classes of analgesics include opioids, non-steroidal anti-inflammatory drugs, antidepressants, and anticonvulsants. Although these treatments provide relief, the effects are often incomplete and complicated by serious side effects and/or tolerance. Thus, therapeutics with novel mechanisms of actions are desperately needed (Finnerup et al. 2005).

What exactly, from a neurobiological perspective, is pain? Pain is actually three quite different things, although it is difficult to make the distinction; nociceptive pain, inflammatory pain and neuropathic pain. Nociceptive pain is not a clinical problem, except in the specific context of surgery and other clinical procedures that necessarily involve noxious stimuli, where it must be suppressed by local and general anesthetics or high-dose opioids (Woolf).

Nociception involves multiple steps from the peripheral receptor, the afferent nerve transmitting the impulse to the spinal cord, the signal processing in the dorsal horn, with inhibitory and facilitatory elements and finally transmission to higher cerebral centers where the peripheral nociceptive stimulus is perceived as pain (Arendt-Nielsen and Sumikura 2002).

The second kind of pain is also adaptive and protective. By heightening sensory sensitivity after unavoidable tissue damage, this pain assists in the healing of the injured body part by

creating a situation that discourages physical contact and movement. Pain hypersensitivity, or tenderness, reduces further risk of damage and promotes recovery, as after a surgical wound or in an inflamed joint, where normally innocuous stimuli now elicit pain. This pain is caused by activation of the immune system by tissue injury or infection, and is therefore called inflammatory pain.

Finally, there is the pain that is not protective, but maladaptive, resulting from abnormal functioning of the nervous system. This pathological pain, which is not a symptom of some disorder but rather a disease state of the nervous system, can occur after damage to the nervous system (neuropathic pain), but also in conditions in which there is no such damage or inflammation (dysfunctional pain) (Woolf).

The incidence of pain rises as people get older and women are more likely to be in pain than men. Pain management strategies include pain relieving medications, physical or occupational therapy and complementary therapies (such as acupuncture and massage).

Pharmacologic therapies are the foundation of chronic pain management. These therapies include nonopioids, opioids, and adjuvant analgesics, physical techniques physical measures, such as physical activity, physical and occupational therapy, orthotics, and assistive devices can serve as adjuncts to analgesics in the management of chronic pain (Paice and Ferrell).

On the other hand in recent years, we and others have focused on the relationship between neuroprotection and pain mechanism and management. Thus in this chapter we will review recent progress related to neuronal mechanism for using neuroprotective agents alone or in combination with antinociceptive drugs to reduce the pain. In addition we will focus on the effect of neuroprotective agents on prevention of tolerance to the analgesic effect of opiates.

## 2. Neuroprotection

Neuroprotection is the mechanism and strategies used to protect against neural injury or degeneration in the central nervous system (CNS). There is a wide range of neuroprotective products available or under investigation. Some products with neuroprotective effects are grouped into the following categories:

- Free radical scavengers
- Anti excitotoxic agents
- Anti apoptotic agents
- Anti inflammatory agents
- Neurotrophic factors

To better understand, we first discuss the mechanism by which neurotoxins induce toxicity.

## 3. Glutamate

Glutamate is a neurotransmitter with roles such as long-term potentiation and synaptic plasticity of the brain (Harris et al. 1984) and is also a excitotoxin whose neurotoxicity has been associated with numerous neurodegenerative diseases, such as Alzheimer disease (AD), (Kihara et al. 2002) vascular dementia, (Martinez et al. 1993) Parkinson disease (Greenamyre 2001) and amyotrophic lateral sclerosis (Cid et al. 2003).

Glutamatergic synapses are the key excitatory synapses within the brain, and mechanisms of both hyperglutamatergic and hypoglutamatergic functioning have been implicated in the pathophysiology of CNS disorders (Olney et al. 1999).

Glutamatergic receptors include both ionotropic and metabotropic receptor subtypes. The ionotropic receptors include N-Methyl-D-Aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptors. Binding of glutamate to these receptors causes  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  entry into neurons, resulting in excitatory postsynaptic potentials and membrane depolarization. In addition, increased intracellular  $\text{Ca}^{2+}$  levels activate a number of signaling cascades (Berridge 1998). The NMDA receptor forms a channel allowing for ion influx, whereas the AMPA and kainate receptors open voltage-sensitive ion channels on the cell membrane. The NMDA receptor is voltage-gated and is blocked by magnesium and modulated by two coagonists, glycine and d-serine, as well as by several intracellular and extracellular mediators (Millan 2005). It has been proposed that NMDA receptor hypofunction may lead to excessive stimulation of other ionotropic receptors, causing a cascade of excitotoxic events including oxidative stress and apoptosis (Deutsch et al. 2001). Dysregulation of glutamatergic functioning has been observed across many components of the glutamate neurotransmission system.

The mechanism of glutamate-induced neuronal death has been extensively studied: glutamate induces neuronal death *via* stimulation of NMDA receptor through which  $\text{Ca}^{2+}$  enters the cell and activates  $\text{Ca}^{2+}$ -dependent nitric oxide (NO) synthase, resulting in excessive nitric oxide formation, production of radicals, mitochondrial dysfunction and cell death (Kaneko et al. 1997). It has been shown that glutamate induces neuronal death associated with necrosis and apoptosis. Necrosis is caused by catastrophic cell damage and is characterized by cell swelling, injury to cytoplasmic organelles and rapid collapse of internal homeostasis, leading to the lysis of membranes and the release of cellular contents, resulting in inflammation. On the other hand, apoptosis is a process characterized by cell shrinkage, membrane blebbing, nuclear pyknosis, chromatin condensation and genomic fragmentation (Kerr et al. 1972; Schulte-Hermann et al. 1992; Takada-Takatori et al. 2009).

In rodents, blocking of NMDA receptors is associated with increased release of glutamate within the cerebral cortex (Moghaddam et al. 1997), (Adams and Moghaddam 1998) and nucleus accumbens (Razoux et al. 2007). However, elevations in glutamate within the prefrontal cortex of rodents occurs during short-term administration of NMDA antagonists, whereas long-term administration over 7 consecutive days actually results in a trend for lower basal levels and lower dialysate levels of glutamate upon challenge (Zuo et al. 2006). Thus, excitotoxic events associated with NMDA antagonists may be reflected by initial increases in glutamatergic neurotransmission that are followed subsequently and chronically by lower levels.

#### **4. Apoptosis and N-Methyl-D-Aspartate antagonist-induced neurodegeneration**

As noted before glutamate can induce apoptosis via NMDA receptor activation. Apoptosis or programmed cell death is a process normally associated with the elimination of redundant neurons during neurodevelopment (Johnson et al. 1995). Apoptosis involves the regulation of a complex molecular cascade controlling the activation of a family of cysteine proteases known as caspase proteins (Glantz et al. 2006). Caspases are responsible for breaking down important structural and functional proteins, leading to cellular degradation and eventually death. Apoptosis results from a cascade of gene activation and involves genes that both promote (i.e., Bax) (Schlesinger et al. 1997), (Gross et al. 1998) and oppose

the process (i.e., Bcl-2) (Craig 1995), (Schlesinger et al. 1997), (Adams and Cory 1998). In a study we showed that there is a relation between glutamate increase and apoptosis promotion and increase in proapoptotic agent activity in both cerebral cortex and lumbar spinal cord of rat (Hassanzadeh et al.).

A vast array of stimuli can activate apoptosis in neurons (Sastry and Rao 2000). Many of these stimuli have been implicated in the pathophysiology of opioid-induced tolerance including glutamate excitotoxicity, increased calcium flux and mitochondria dysfunction and these mechanisms are discussed in detail later in this chapter.

## 5. Neuroactive steroids are neuroprotective

Neuroactive steroids are endogenous neuromodulators synthesized either within the brain (neurosteroids) or in the periphery by the adrenal glands and gonads. In addition to the classic effect of steroids on gene transcription via binding to intracellular steroid receptors, neuroactive steroids can alter neuronal excitability via nongenomic effects by acting at inhibitory GABA<sub>A</sub> receptors and/or excitatory NMDA receptors, among others (Shulman and Tibbo 2005), (Marx et al. 2006). There is also evidence for a potential role of these neurosteroids in controlling GABA and glutamate release. Neuroactive steroids have also been implicated in neuroprotection, myelination, and modulation of the stress response. A number of neuroactive steroids are present in human postmortem brain at physiologically relevant nanomolar concentrations and serve as allosteric modulators of the GABA<sub>A</sub> receptor (Marx et al. 2006). Neuroactive steroids that are effective modulators of GABA<sub>A</sub> and/or T-type Ca<sup>2+</sup> channels are promising tools for studying the role of these channels in peripheral pain perception. They appear to be very effective in alleviating peripheral Nociception in rat models of acute and chronic pain (Jevtovic-Todorovic et al. 2009).

## 6. Acetyl Choline Receptors (AChRs) and neuroprotection

Agonists and antagonist selective for AChR subtypes have been used in experimental and clinical research. Some of those compounds are potential candidates for the treatment of neurodegenerative disease such as Alzheimer's disease, Parkinson's disease and others. A growing list of *in vivo* and *in vitro* research suggest that AChRs modulators are gaining importance as clinically relevant neuroprotective drugs (Mudo et al. 2007).

The inhibition of  $\alpha 7$  AChRs decreases the GABAergic tone causing increased ACh release into the synaptic cleft (Giorgetti et al. 2000), which then activates the  $\alpha 4\beta 2$  AChRs located post-synaptically. The selective  $\alpha 7$  inhibitor methyllycaconitine (Ivy Carroll et al. 2007) mimics, at least in part, the neuroprotective effect of 4R (Ferchmin et al. 2003). Other *in vivo* and *in vitro* studies confirm that  $\alpha 7$  inhibition can be neuroprotective (de Fiebre and de Fiebre 2005), (Laudenbach et al. 2002), (Martin et al. 2004).

Protection of neurons from neuronal damage and cell death in neurodegenerative disease is a major challenge in neuroscience research. Donepezil, galantamine and tacrine are acetylcholinesterase inhibitors used for the treatment of Alzheimer's disease, and were believed to be symptomatic drugs whose therapeutic effects are achieved by slowing the hydrolysis of acetylcholine at synaptic termini. However, recent accumulated evidence strongly suggests that these acetylcholinesterase inhibitors also possess neuroprotective properties whose mechanism is independent of acetylcholinesterase inhibition. It has been

shown that acetylcholinesterase inhibitors protect neurons from glutamate-induced neurotoxicity in the primary culture of rat cortical neurons.

The long-standing belief was that acetylcholinesterase inhibitors are symptomatic agents that ameliorate cholinergic deficits by slowing the hydrolysis of acetylcholinesterase at synaptic nerve termini; however, recent studies have shown that acetylcholinesterase inhibitors have other pharmacological properties, for example, neuroprotection against toxic insults, such as glutamate and up-regulation of nicotinic receptors (Akaike 2006), (Takada-Takatori et al. 2009).

Several reports have indicated that activation of cholinergic neurons in the central nervous system produces antinociception and analgesia in a variety of animals, including humans (Harte et al. 2004) provide evidence supporting the involvement of the intralaminar thalamus in muscarinic induced antinociception. Pharmacological experiments have shown that the microinjection of acetylcholine or carbachol into specific brainstem nuclei can produce antinociception and can be reversed by muscarinic receptor antagonists (Brodie and Proudfit 1984), (Yaksh et al. 1985). Meanwhile some other types of receptors or drugs produce analgesia by mediation of ACh. Sumatriptan (5-HT<sub>1</sub>agonist) is able to induce antinociception by increasing cholinergic neurotransmission (Ghelardini et al. 1997). D2 antagonist prochlorperazine exerts an antinociceptive effect mediated by a central cholinergic mechanism (Ghelardini et al. 2004), (Yang et al. 2008). In addition, more recently we showed that an acetylcholinesterase inhibitor, donepezil, could prevent tolerance to the analgesic effect of morphine (unpublished data).

## 7. Cannabinoids, pain and neuroprotection

Pain severely impairs quality of life. Currently available treatments, generally opioids and anti-inflammatory drugs, are not always effective for certain painful conditions. The discovery of the cannabinoid receptors in the 1990s led to the characterization of the endogenous cannabinoid system in terms of its components and numerous basic physiologic functions. Cannabinoid<sub>1</sub> (CB<sub>1</sub>) receptors are present in nervous system areas involved in modulating nociception and evidence supports a role of the endocannabinoids in pain modulation. Cannabinoids have antinociceptive mechanisms different from that of other drugs currently in use, which thus opens a new line of promising treatment to mitigate pain that fails to respond to the pharmacologic treatments available, especially for neuropathic and inflammatory pains (Manzanares et al. 2006).

Cannabis extracts and synthetic cannabinoids are still widely considered illegal substances. The Cannabis sativa plant has been exploited for medicinal, agricultural and spiritual purposes in diverse cultures over thousands of years. Cannabis has been used recreationally for its psychotropic properties, while effects such as stimulation of appetite, analgesia and anti-emesis have lead to the medicinal application of cannabis. Indeed, reports of medicinal efficacy of cannabis can be traced back as far as 2700 BC, and even at that time reports also suggested a neuroprotective effect of the cultivar (Scotter et al.).

Preclinical and clinical studies have suggested that they may result useful to treat diverse diseases, including those related with acute or chronic pain. The discovery of cannabinoid receptors, their endogenous ligands, and the machinery for the synthesis, transport, and degradation of these retrograde messengers, has equipped us with neurochemical tools for novel drug design. Agonist-activated cannabinoid receptors, modulate nociceptive thresholds, inhibit release of pro-inflammatory molecules, and display synergistic effects

with other systems that influence analgesia, especially the endogenous opioid system. Cannabinoid receptor agonists have shown therapeutic value against inflammatory and neuropathic pains, conditions that are often refractory to therapy. Although the psychoactive effects of these substances have limited clinical progress to study cannabinoid actions in pain mechanisms, preclinical research is progressing rapidly.

There has been anecdotal and preliminary scientific evidence of cannabis affording symptomatic relief in diverse neurodegenerative disorders. These include multiple sclerosis, Huntington's, Parkinson's and Alzheimer's diseases, and amyotrophic lateral sclerosis. This evidence implied that hypofunction or dysregulation of the endocannabinoid system may be responsible for some of the symptomatology of these diseases.

In Huntington's disease, Alzheimer's disease, as well as in ALS, pathologic changes in endocannabinoid levels and CB2 expression are induced by the inflammatory environment. CB1 activation has been shown to be effective in limiting cell death following excitotoxic lesions, while CB2 is involved in dampening inflammatory immune cell response to disease. These two targets may therefore work together to provide both neuroprotection to acute injury and immune suppression during more chronic responses (Scotter et al.).

During the last two decades, a large number of research papers have demonstrated the efficacy of cannabinoids and modulators of the endocannabinoid system in suppressing neuropathic pain in animal models. Cannabinoids suppress hyperalgesia and allodynia (i.e. mechanical allodynia, mechanical hyperalgesia, thermal hyperalgesia and, where evaluated, cold allodynia), induced by diverse neuropathic pain states through CB1 and CB2-specific mechanisms (Rahn and Hohmann 2009).

On the other hand, responses to cannabinoid (CB) receptor activation include opening of potassium channels, inhibition of calcium currents, and stimulation of various protein kinases (Deadwyler et al. 1995; Gomez del Pulgar et al. 2000; Galve-Roperh et al. 2002; Karanian et al. 2005b; Molina-Holgado et al. 2005; Karanian et al. 2007). Some of the many such signaling elements activated by endocannabinoids play important roles in neuronal maintenance (Bahr et al. 2006; Galve-Roperh et al. 2008). CB receptor transmission elicits modulatory effects on calcium channels, resulting in reduced neurotransmitter (e.g., GABA, glutamate) release (Hajos et al. 2000; Kreitzer and Regehr 2001; Wilson et al. 2001). One particular mitogen-activated protein kinase, extracellular signal-regulated kinase (ERK), is involved in cannabinergic signaling, as are focal adhesion kinase (FAK) and phosphatidylinositol 3'-kinase (PI3K). These signaling elements appear to play key roles in the neuroprotective nature of the endocannabinoid system, and the associated signaling pathways are disrupted by blocking CB receptor activation (Hwang et al.; Wallace et al. 2003; Khaspekov et al. 2004; Karanian et al. 2005a; Karanian et al. 2005b).

Together, these studies indicate that the neuroprotectant cannabinoids have antinociceptive properties.

## **8. Neuroprotection and tolerance to the analgesic effect**

### **8.1 Opioid tolerance**

Many types of neuronal cells and brain nuclei have the property of changing, acutely or chronically, their regular behavior by the action of pharmacological agents, such as psychoactive drugs. Acute changes, those that cease in a short time, would not be important to the chronic altered behavior if the cell recovered its original drug-free state, but it is observed that some adaptation occurs that impairs such a recovery. In fact, the disturbed

cell under the influence of a drug tries to compensate for its acute effects by promoting changes in the opposite direction, transiently restoring its homeostasis. However, when the acute action of the drug is finished, the cell is imbalanced by its own reactive response (Sharma SK et al. 1975). As a consequence, the phenomenon of tolerance develops, that is, the need for an increased dose of the drug to produce the same effect (McQuay 1999). After tolerance is established, the withdrawal of the drug may produce physical or psychological symptoms opposed to the acute pharmacological actions of the drug itself. Opioid drugs are used clinically as unsurpassed analgesic agents but are also illegally abused on the street to induce a sense of well-being and euphoria. Tolerance to opioids, defined as a loss of effect following repeated treatments such that a higher dose is required for equivalent effect, limits the analgesic efficacy of these drugs and contributes to the social problems surrounding recreational opioid abuse.

In order to safely use morphine in clinic, we need to know how morphine tolerance and dependence are developed and what kinds of medicines could inhibit or prevent such mechanisms. In line with this, various approaches have been attempted to clarify the mechanisms underlying morphine tolerance and dependence. Here we summarize various proposed hypotheses and introduce our new approaches in this area.

## 8.2 Mechanisms for acute morphine tolerance

Prolonged and repeated exposures to opioid agonists reduce the responsiveness of G protein coupled opioid receptors. This reduction in receptor function is hypothesized to contribute to opioid tolerance, dependence, and addiction in humans (Nestler 1992). Substantial experimental evidence has divided this reduced function into separate but correlated receptor traffickings, 1) desensitization, 2) internalization, 3) sequestration/recycling, 4) down regulation (Law et al. 2000). The molecular events underlying opioid tolerance are currently discussed in relation to all these receptor trafficking mechanisms. According to current understanding, opioid receptors are desensitized on the cell surface through a phosphorylation process in the C-terminal (Afify et al. 1998) and/or third intracellular loop. On the other hand, receptor internalization or receptor disappearance from the cell surface, is now believed to contribute to resensitization through dephosphorylation during endosomal stages (Krueger et al. 1997; Zhang et al. 1997). Down-regulation is a loss of receptor protein in cells through increased degradation or decreased synthesis of the receptor. Little is known, however, regarding the regulation of this mechanism and involvement in opioid tolerance. Thus, much research has been done on the molecular basis of events in receptor phosphorylation in the membranes and internalization. Recent studies revealed that cAMP-dependent protein kinase A (PKA) (Harada et al. 1990), protein kinase C (PKC) (Ueda et al. 1995),  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinases (Koch et al. 1997), G protein-coupled receptor kinases (GRKs) (Zhang et al. 1998), and mitogen-activated protein kinase (Polakiewicz et al. 1998) have roles in opioid receptor phosphorylation. PKC and GRK mechanisms are likely candidates for opioid desensitization and internalization (Ueda et al. 1995; Zhang et al. 1998).

## 8.3 PKC hypothesis

A number of reports have demonstrated that PKC is involved in the opioid tolerance or desensitization. Most of recent reports have demonstrated that PKC activators or inhibitors modulate opioid signaling in cells expressing opioid receptors. A series of reports have demonstrated the involvement of PKC in opioid tolerance by correlating both in vitro and in vivo studies.

#### 8.4 Mechanisms for chronic morphine tolerance and dependence

Clear difference between acute morphine tolerance and chronic one has not been demonstrated for a long time. In algogenic-induced nociceptive flexion (ANF) test in mice the peripheral morphine analgesia developed the acute tolerance by 4 h pretreatment with morphine (Ueda et al. 2001). However, the peripheral analgesia had no change in mice that were given morphine for 5 days, a treatment which caused a marked chronic tolerance to systemic morphine analgesia (Ueda and Inoue 1999). Thus, it is evident that acute morphine tolerance mediates distinct mechanisms from the chronic one, and chronic tolerance is likely mediated through a complicated neuronal network present in the central nervous system.

#### 8.5 cAMP hypothesis

Since the report by Sharma et al. (1975), it has been accepted that cAMP may play a key role in the morphine tolerance and dependence. According to this so-called cAMP hypothesis, a morphine-induced decrease in cAMP production is getting disappeared during long-period exposure to morphine (Sharma SK et al. 1975). As the naloxone application causes an abrupt increase in cAMP production, some unidentified mechanisms are supposed to mediate an increase in cAMP production through specific gene expressions during chronic morphine treatment. A candidate could be a cAMP-responsive element binding protein (CREB), which is involved in the gene expression of adenylyl cyclase. In vivo study using knockout mice demonstrates that CREB plays roles in the development of morphine dependence (Maldonado et al. 1996). Although several compounds possessing the antagonistic activity are reported to inhibit morphine tolerance and dependence, they have serious side effects at the same time (Trujillo and Akil 1991; Mao et al. 1992; Trujillo 1995; Mao 1999; Habibi-Asl and Hassanzadeh 2004; Habibi-Asl 2005; Asl et al. 2008).

#### 8.6 Anti-opioid hypothesis

In addition to mechanisms at the single cellular level, the plasticity through neuronal networks would be involved in the development of morphine tolerance and dependence, as above-mentioned. One of approaches to cut in the mechanisms is based on the view that enhanced anti-opioid neuronal activity during chronic morphine treatments might suppress the acute morphine actions. The candidates include cholecystokinin (Mitchell et al. 2000), neuropeptide FF (Lake et al. 1992), nociceptin (Ueda et al. 2000) and glutamate, as an NMDA receptor ligand (Ueda et al. 2000; Mao and Mayer 2001). Among them the nociceptin (N/OFQ) system has been extensively characterized to be involved in the development of morphine tolerance and dependence. NMDA receptor has been long supposed to play important roles in the development of morphine tolerance and dependence (Trujillo and Akil 1991). Although several compounds possessing the antagonistic activity are reported to inhibit morphine tolerance and dependence, they have serious side effects at the same time (Trujillo and Akil 1991; Mao et al. 1992; Trujillo 1995; Mao 1999; Habibi-Asl and Hassanzadeh 2004; Habibi-Asl 2005; Asl et al. 2008).

#### 8.7 Apoptosis hypothesis

Apoptosis, or programmed cell death, is an active process of normal cell death during development and also occurs as a consequence of the cytotoxic effect of various neurotoxins (e.g., MPTP/MPP<sup>+</sup>, MDMA, ethanol and cocaine) (Sastry and Rao 2000). Among the drugs of abuse, cocaine has been shown to cause a direct cytotoxic effect on the foetal rat heart, and to induce apoptosis in foetal rat myocardial cells in a dose-dependent manner (Xiao et al. 2000).

The induction of apoptosis in neurons has been demonstrated to share the same basic mechanisms with all other cell types (Sastry and Rao 2000). In vitro studies also indicate that exposure to  $\mu$ - and/or  $\kappa$ -opioid receptor agonists of neuronal cultures from embryonic chick brain (Goswami et al. 1998) and specific cell lines (Dawson et al. 1997; Singhal et al. 1998; Singhal et al. 1999) increases their vulnerability to death by apoptotic mechanisms. The molecular mechanisms of apoptosis (i.e., the detailed cascade of events from the cell surface to final changes in the nucleus) have not been established yet, but various key proteins are involved in the regulation of programmed cell death (Sastry and Rao 2000). Some members of the Bcl-2 family of proteins, such as Bcl-2 and Bcl-xL, suppresses apoptosis, while the expression of other, such as the homologues Bax and Bak, are pro-apoptotic (Adams and Cory 1998). Specifically, the Bcl-2 oncoprotein, localized mainly to the mitochondrial membranes, has been shown to play an important role in protecting neurons from apoptotic cell death (Hockenbery et al. 1990), probably by preventing the release of cytochrome c (induced by Bax) and the subsequent activation of specific proteases termed caspases, the proteolytic enzymes which are crucial for the execution of nuclear fragmentation and apoptosis (Adams and Cory 1998; Sastry and Rao 2000). In fact, Bax mRNA and Bax protein are increased in the substantia nigra of MPTP-treated mice (degeneration of dopamine neurons by apoptosis) (Hassouna et al. 1996), and the release of cytochrome c from the mitochondria and the subsequent activation of caspases-3/9 was shown to play a key role in cocaine-induced apoptosis in foetal rat myocardial cells (Xiao et al. 2000). The results of our studies demonstrated that chronic morphine administration in rat, induced apoptosis; decrease in Bcl-2 and increase in caspase3 activity in both cerebral cortex and lumbar spinal cord in rat (Hassanzadeh et al.). Another key element involved in the regulation of apoptosis is the Fas glycoprotein (also known as CD95 or Apo1), a cell surface receptor that belongs to the tumor necrosis factor receptor family (death receptors) and that is expressed abundantly in various tissues (Nagata 1999). In contrast to Bcl-2 mitochondrial protein, the Fas receptor triggers cell apoptosis when it binds to its ligand, Fas, and Fas-mediated death bypasses the usual long sequence of signaling enzymes and immediately activates a pre-existing caspase cascade (Nagata 1999). In the context of the induction of aberrant apoptosis in opioid addiction, it was of great interest the in vitro study demonstrating the ability of morphine to increase, through a naloxone-sensitive mechanism, the expression (mRNA) of the pro-apoptotic receptor Fas in mouse splenocytes and in human blood lymphocytes (Yin et al. 1999). A relevant consequence of the morphine-induced potentiation of apoptosis in lymphocytes (Singhal et al. 1999; Yin et al. 1999) is the reduction of the immune response (and the increase in recurrent infections) observed in heroin addicts (Govitrapong et al. 1998).

On the other hand, over a decade, the NMDA receptor (NMDAR), a subgroup of glutamate receptors, has been implicated in the development of opioid tolerance (Trujillo and Akil 1991; Mao et al. 1994). Activation of NMDARs can lead to neurotoxicity under many circumstances (Rothman and Olney 1986; Moncada et al. 1992; Catania et al. 1993). For instance, peripheral nerve injury has been shown to activate spinal cord NMDARs, which results in not only intractable neuropathic pain but also neuronal cell death by means of apoptosis (Mao et al. 1997; Whiteside and Munglani 2001). Furthermore, cross talk between the cellular mechanisms of opioid tolerance and neuropathic pain has been proposed, suggesting that a common cellular mechanism may be involved in both neuropathic pain and opioid tolerance (Mayer et al. 1999). Thus, it is possible that the cellular process leading to the development of opioid tolerance may also cause neurotoxic changes in response to prolonged opioid administration. More recently, we examined the hypothesis that neurotoxicity in the form of apoptotic cell

death would be induced in association with the development of morphine tolerance. In confirmation of Mao et al. findings, we demonstrated that chronic opioid injection leads to apoptosis in the CNS which was in association with the development of tolerance to the analgesic effect (Habibi-Asl et al. 2009a). Figure1 shows the possible mechanisms of opioid-induced neuronal apoptosis and its association with opioid tolerance.

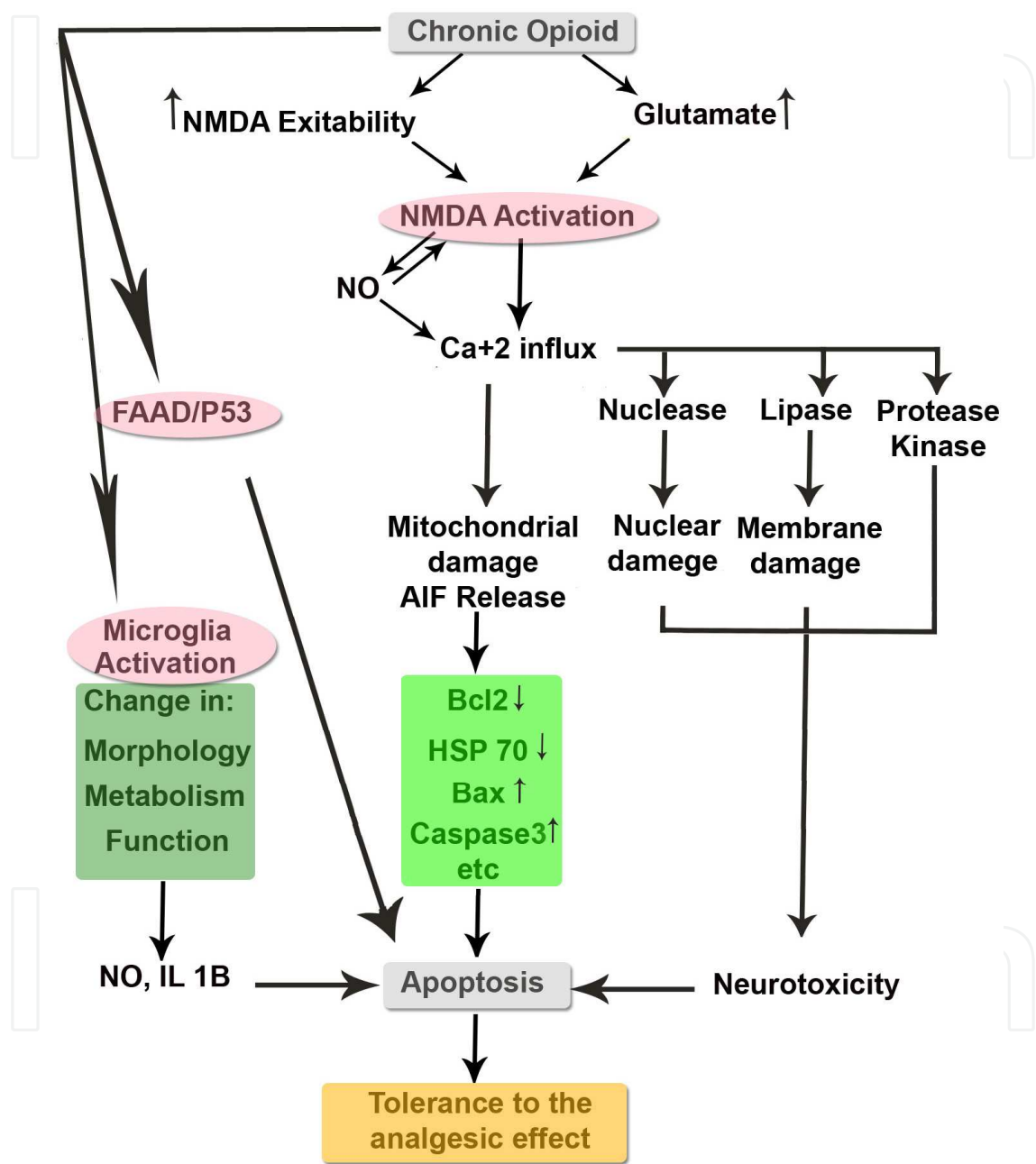


Fig. 1. Schematic diagram illustrating the possible mechanisms of opioid-induced neuronal apoptosis and tolerance. The results of before studies suggest that chronic opioid administration may induce NMDAR, microglia, FAAD/P53,... activation resulting in intracellular positive apoptosis regulators induction. The resultant apoptosis contributes to the cellular mechanism of opioid tolerance. NMDA: N-Methyl-D-Aspartate, NO: Nitric Oxide, AIF: Apoptosis-Inducing Factor, FADD: Fas-Associated Death Domain,

Opioid tolerance manifests as a loss of agonist potency and as a shift of the dose-response curve to the right. During the past decades, many studies have focused on excitatory amino acid receptors to investigate the role which they play in the development of tolerance to the antinociceptive action of opiates. This idea was suggested by Trujillo and Akil who reported that the NMDA receptor antagonist, MK801 (dizocilpine), inhibited the development of tolerance to the antinociceptive effect of morphine and morphine physical dependence (Trujillo and Akil 1991).

Using behavioral studies, we and others have shown that a variety of NMDA receptor antagonists have the ability to inhibit the development of opiate tolerance and dependence (Trujillo and Akil 1991; Trujillo 1995; Habibi-Asl and Hassanzadeh 2004; Asl et al. 2008; Habibi-Asl et al. 2009b). There are also several lines of evidence which suggest that activation of NMDARs leads to removing the magnesium blockade (Begon et al. 2001) in the calcium channel and toxic calcium influx, which activates numerous enzymes, including neuronal nitric oxide (NO) synthase (NOS). In our unpublished data we observed that nitric oxide donors such as nitroglycerin or nicorandil increased the tolerance to the analgesic effect of morphine. On the other hand the nitric oxide synthase inhibitor, N-Nitro-L-Arginine Methyl Ester (LNAME) could prevent the tolerance. It has been demonstrated that Magnesium (Mg)-deficient rats develop a mechanical hyperalgesia which is reversed by a N-Methyl-D-Aspartate (NMDA) receptor antagonist (Begon et al. 2001). Our study in agreement with those studies showed that systemic administration of magnesium sulfate could attenuate morphine tolerance to the analgesic effect (Habibi-Asl 2005; Habibi-Asl et al. 2009b). Also we showed that selenium with similar mechanism appeared to have a weaker effect than magnesium (Charkhpour M et al. 2009).

Our recently published finding, indicated that riluzole (2- amino-6-[trifluoromethoxy] benzothiazole), an antiglutamatergic agent, decreases the development of tolerance, shifting the first day of established tolerance from the 8th day in the control group to the 13th day (Habibi-Asl et al. 2009a). Riluzole interferes with responses mediated by excitatory amino acids, even though it does not interact with any known binding sites on the NMDA, kainate or AMPA glutamate receptors (Debono et al. 1993). The neuroprotective effect of riluzole, which has been shown both in vivo and in vitro, is believed to be beneficial in various neurodegenerative diseases and amelioration of trauma and stroke (Doble 1999; Albo et al. 2004).

The results indicated that there was a significant shift to the right in the dose-response curve as well as an increase in the antinociceptive 50% effective dose (ED<sub>50</sub>) of morphine for animals who received morphine also compared with those that received morphine and riluzole. On the other hand, co-administration of riluzole delayed the onset of morphine-induced apoptosis and significantly decreased the average number of TUNEL-positive cells ( $p < 0.01$ ). This finding is in line with our recent results concerning the lumbar region of the spinal cord (Hassanzadeh et al.). In addition, we found that the group that received morphine and riluzole for 13 days had developed tolerance; they showed an increase in the number of apoptotic cells, as under control conditions. This result indicates that after the completion of tolerance in both the control and the treated groups, apoptosis had already developed. Previous studies have indicated that certain addictive drugs, such as morphine, could induce apoptosis in cultured neuronal cell lines as well as human cells (Singhal et al. 1998; Singhal et al. 1999). More recently, it has been shown that in vivo neuronal apoptosis occurs in the rat's spinal cord dorsal horn after chronic morphine treatment that was associated with the expression of activated caspase- 3 and the involvement of mitogen-

activated protein kinase (MAPK) (Mao et al. 2002), suggesting that chronic morphine may lead to changes within the central nervous system.

Our more recent studies demonstrated that prolonged morphine administration induces up-regulation of proapoptotic elements such as caspase-3 and down regulation of the anti-apoptotic factors Bcl-2 and HSP70 in the rat cerebral cortex and spinal cord (Hassanzadeh et al.; Hassanzadeh et al.; Tikka and Koistinaho 2001; Gabra et al. 2005; Hassanzadeh K et al. 2011). Importantly, up-regulation of caspase-3 and Bax was inhibited when morphine was co-administered with the noncompetitive NMDAR antagonist MK-801, thereby supporting a link between NMDAR activation and intracellular changes in caspase-3 and Bax in response to prolonged morphine administration (Jordan et al. 2007).

Interestingly, our results demonstrated that neuroprotective agents such as serotonin<sub>1A</sub> receptor agonist, minocycline (Habibi-Asl 2009; Habibi-Asl et al. 2009a), selegiline,... could prevent morphine induced tolerance and apoptosis. The stimulation of serotonin<sub>1A</sub> (5HT<sub>1A</sub>) receptors induces a variable level of neuroprotection in different animal models of central nervous system injury such as ischemia, (Prehn et al. 1993; Semkova et al. 1998; Schaper et al. 2000; Kukley et al. 2001; Torup et al. 2000) N-methyl-D-aspartate (NMDA) excitotoxicity, (Oosterink et al. 1998; Oosterink et al. 2003) acute subdural hematoma, (Alessandri et al. 1999) and traumatic brain injury (Kline et al. 2001). Furthermore, in vitro evidence indicates that 5HT<sub>1A</sub> agonists are able to protect neurons from apoptosis induced by staurosporine (Suchanek et al. 1998), glutamate (Semkova et al. 1998), or serum deprivation (Ahlemeyer and Kriegstein 1997; Ahlemeyer et al. 1999). There are different hypotheses on the mechanisms involved in 5HT<sub>1A</sub>-mediated neuroprotection, including neuronal membrane hyperpolarization that reduces excitability, (Ahlemeyer and Kriegstein 1997; Krüger et al. 1999), reduced glutamate release, (Mauler et al. 2001) and blockade of voltage-sensitive Na channels (Melena et al. 2000).

Other neuroprotective mechanisms have also been proposed for 5HT<sub>1A</sub> agonists such as stimulation of the anti-apoptotic proto-oncogene B-cell lymphoma protein 2 (BCL-2) expression through the mitogen-activated protein kinase (MAPK/ERK) signaling pathway (Kukley et al. 2001) and suppression of the proapoptotic protein caspase-3 in a MAPK- and protein kinase C alpha-dependent manner (Adayev et al. 2003).

More recently we examined the effect of 8-OH-DPAT, a specific 5-HT<sub>1A</sub> receptor agonist, on morphine induced tolerance to an analgesic effect in rat. We found that Intra-dorsal raphe nucleus (DRN) administration of the 5-HT<sub>1A</sub> receptor agonist, 8-OH-DPAT, prevented morphine-induced apoptosis after tolerance to the analgesic effect. On the other hand, the total analgesic effect of morphine significantly increased in animals treated with morphine and 8-OH-DPAT in comparison with the control group. In addition, the results indicated that administration of both 5HT<sub>1</sub> agonist (8-OH-DPAT) and antagonist (NAN-190) together with morphine prevent the antiapoptotic activity of the 5HT<sub>1A</sub> agonist. This means that after antagonizing the 5HT<sub>1A</sub> receptor, the apoptosis process has already developed. Another mechanism contributes to the morphine tolerance is microglial activation. Studies showed that NMDA-induced neuronal death involved proliferation and activation of microglial cells and that neuroprotective agents such as minocycline completely prevented NMDA toxicity and the preceding activation and proliferation of microglial cells. These results support the notion that microglial activation contributes to excitotoxic neuronal death, which can be inhibited by anti-inflammatory compounds, such as minocycline (Tikka and Koistinaho 2001). The mechanism underlying the role of glial cells in the effects of morphine on naive mice is unclear. It is possible that morphine acts directly on microglia,

triggering alterations in their morphology, metabolism, and function (Watkins et al. 2005). Mika et al. concluded that the effect of minocycline on morphine tolerance is related to microglia. Their results provide evidence that systemic administration of minocycline in mice influences morphine's effectiveness and delays the development of morphine tolerance by attenuating microglial activation and its markers (Mika et al. 2009).

In summary, we believe that adding the neuroprotective agents to analgesic drugs specially opioids, increase the analgesic effect and prevents the hyperalgesia and tolerance to their analgesic effects.

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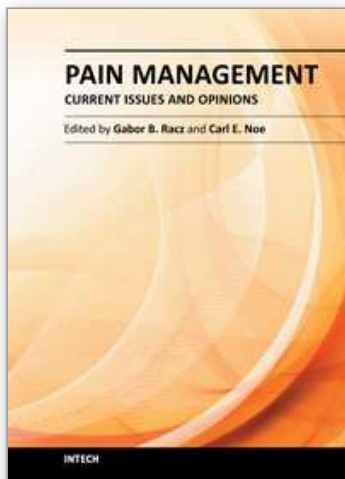
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