INTRODUCTION
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Pain is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey and Bogduk, 1994). It is a multidimensional experience that is essential for the defense mechanism of an individual.

Under normal conditions, primary afferent pain fibers activate particular central pathways that engage protective mechanisms at several functional levels. The nociceptors located at the terminals of the thinly myelinated Aδ-fiber or unmyelinated C-fiber primary afferent neurons transduce noxious chemical, mechanical or thermal stimuli into depolarizing currents that ultimately induce action potentials (Millan, 1999). The action potentials are then conducted to the higher centers in the central nervous system (CNS) through release of neurotransmitters and are accompanied by a variety of responses including withdrawal reflexes, conscious perception of pain and emotional effects such as sufferings. A great paradox of pain is that acute, nociceptive, pain is a necessary defense mechanism that warns against existing or imminent damage to the body, whereas chronic pain can be so deleterious to the individuals. As a defense mechanism, nociceptive pain (sometimes referred to as ‘good’ pain) is essential for survival. By contrast, chronic pain (‘bad’ pain) serves no known defensive, or other helpful, function. Acute pain is produced by the physiological functioning of the normal nervous system whereas chronic pain is a reflection of aberrant functioning of a pathologically altered nervous system (Woolf and Salter, 2000; Renn and Dorsey, 2005).

Pain has been broadly put into three major categories: physiological, inflammatory and neuropathic pain. Physiological pain is generally acute and induced only by noxious stimuli as a natural process that alerts the individuals of imminent tissue damage. Inflammatory pain is the result of tissue damage and is reversible once the underlying cause has been healed. Inflammatory pain may be either acute or chronic depending on severity of damage. On the other hand, neuropathic pain is caused or initiated by a primary lesion or dysfunction in the nervous system and persists even after healing of the initial damage (Dworkin et al., 2003; Kinloch and Cox, 2005). This type of pain is the most debilitating type of chronic pain.

Neuropathic pain can occur in a variety of traumatic diseases including phantom limb, spinal cord injury and nerve compression. Moreover, neuropathic pain
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is a common secondary symptom in diseases like diabetes, cancer and herpes zoster infection as well as due to use of cytotoxic drugs. The presence of peripheral neuropathic pain is often characterized by stimulus-independent persistent pain or abnormal sensory perception of pain such as allodynia (pain perception on exposure to normally non-noxious stimuli) and hyperalgesia (exaggerated pain sensations as a result of exposure to mildly noxious stimuli) (Dworkin et al., 2003). The dominant theme in neuropathic pain research has been investigating alterations in neurons and neuronal functioning in the peripheral and central nervous systems. Indeed, it is widely known that nerve injury produces molecular and cellular changes that result in multiple forms of neuronal plasticity and anatomical reorganization at various levels of the peripheral and central nervous systems (Zimmermann, 2001; Woolf, 2004).

It is well reported that both peripheral and central mechanisms contribute to the pathophysiology of neuropathic pain. The changes in ectopic and spontaneous discharge, alteration in ion channel expression, collateral sprouting of primary afferent terminals, sprouting of sympathetic nerve into the dorsal root ganglion (DRG) and nociceptor sensitization by proinflammatory mediators are the peripheral mechanisms involved following peripheral nerve injury. Central mechanisms include central sensitization, disinhibition of dorsal horn inhibitory neurons, reorganization of neuronal circuits in dorsal horn, changes in descending pain facilitation and pain inhibition (Woolf and Salter, 2000; Taylor, 2001; Salter 2005).

Painful neuropathy is also one of the most common complications arising from diabetes mellitus (Tesfaye and Kempler, 2005). Indeed, spontaneous pain, allodynia and hyperalgesia are frequently encountered in diabetic patients (Benbow et al., 1994), but underlying pathophysiological mechanisms remain unclear. Indeed, the mechanisms underlying diabetic neuropathic pain are complex, and both peripheral and central components of the sensory systems are likely involved. In addition to abnormalities of peripheral afferent nerves, altered sensory processing in the spinal cord may contribute to the development of diabetic neuropathic pain. Further, activation of the polyol pathway, immune mechanisms, peripheral nerve ischemia (Vinik, 1999; Kimura et al., 2005) and/or hyperactivity of dorsal root ganglion neurons (Eaton et al., 2001) have been hypothesized, but better insight into the genesis of neuropathy is needed for improving its treatment.

The orofacial region is one of the most densely innervated (by the trigeminal nerve) areas of the body, which focuses some of the most common acute pains, i.e.
those accompanying the pathological states of the teeth and the related structures. However, the mechanisms underlying these pains are still poorly understood, partly due to the relative scarcity of investigations devoted to the face and the mouth, compared to the rest of the body (Sessle, 2005). In particular, there are relatively few behavioral models in laboratory animals dedicated to the study of nociception in the trigeminal region. Some years ago, a new formalin model in the rat has been developed to assess nociceptive processes in the orofacial region (Dallel et al., 1989) that allows to study biochemical, molecular, pharmacological, anatomical approaches to study pathophysiological mechanisms involved in orofacial pain.

Chronic pain conditions such as fibromyalgia are characterized by widespread muscle pain and joint tenderness (Bennett, 2005). A recent report of enhanced temporal summation (wind up) in fibromyalgia patients, consistent with central sensitization (Staud, 2002), suggests that the etiological development of chronic musculoskeletal pain in humans may share a number of common underlying mechanisms with other chronic pain conditions including those of neuropathic origin (Zimmermann, 2001). It is generally accepted that chronic fatigue syndrome is also associated with musculoskeletal pain. However, the clear understanding on the mechanisms involved in this pain remains obscure. Adding to this, there is a general lack of animal models to study nociceptive mechanisms involved in this pain. Recently, Sluka et al. (2001) and Radhakrishnan et al. (2003) have developed a model of acid or carrageenan-induced pain in rodents, respectively, which has been suggested to have greater face validity to pain of musculoskeletal origin in humans and allow to study various possible mechanisms involved in the disease pathogenesis.

It has been well reported that prolonged tissue damage or nerve injury often leads to chronic pain characterized by the genesis of hyperalgesia and/or allodynia, which persists after healing. Many animal models have been developed in an attempt to understand the etiologies of various pain syndromes following an inflammatory insult. These models include application of various chemical irritants to induce peripheral inflammation (complete Freund’s adjuvant, carrageenan, mustard oil and formalin), all of which elicit spontaneous pain and/or thermal hyperalgesia and tactile allodynia (Le Bars et al., 2001). The models described above have broadened our knowledge of the mechanisms underlying pain following the induction of peripheral inflammation, but few studies have examined pain mechanisms following the induction of central inflammation. It is now well established that peripheral
administration of lipopolysaccharide (LPS; also known as endotoxin) interacts with various cell types to release cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α in the periphery and central nervous system, which can in turn precipitate hyperalgesia. Central administration of endotoxin can also initiate the production of cytokines known to modulate nociception (Watkins et al., 1994, 1995; Reeves et al., 2000). Painful neuropathy turns out to be a therapeutic challenge, as it often remains refractory to classical analgesics. Currently, the most commonly used medications include tricyclic antidepressants (Coluzzi and Mattia, 2005) and anticonvulsants (Wiffen et al., 2005), alone or in combination. Alternative therapies are therefore needed. This led notably to using paroxetine, a serotonin reuptake blocker with clearcut inhibitory activity at norepinephrine reuptake (Owens et al., 2000), which displays moderate but significant efficacy to reduce pain associated with diabetic neuropathy, without causing the autonomic side effects generally met with tricyclic antidepressants (Sindrup and Jensen, 1999). Nevertheless, these drugs have a rather limited efficacy and often produce undesirable side effects. Similar to neuropathic pain in humans, chronic musculoskeletal pain conditions remain somewhat refractory to treatment with currently available analgesics, reinforcing the need to understand exact pathophysiological mechanisms. Further, the exact pathophysiological mechanisms involved in the development of mechanisms of orofacial pain and LPS-induced hypersensitivity are not clear.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed and widely used drugs in the management of pain, especially pain associated with inflammatory conditions. They act by inhibiting cyclooxygenase (COX), an enzyme that catalyzes the conversion of arachidonic acid (AA) into prostaglandins (PGs) and show analgesic effect, anti-inflammatory and antipyretic properties. Three isoforms of COX have been identified and the physiological function of COX-1 and -2 are clearly known (Simmons et al., 2004). Recently, a splice variant of COX-1, COX-1b (also known as COX-3) has been identified, however, its function is not known as yet (Chandrasekharan et al., 2002; Snipes et al., 2005). Classically, in most peripheral tissues, COX-1 is constitutively expressed and is predominantly responsible for house keeping functions and for a basic level of PGs. In contrast, COX-2 is inducibly expressed after tissue injury, mitogens, and endotoxins (Svensson and Yaksh, 2002). It has been demonstrated that unlike many
peripheral tissues, the majority of neurons and radial glia in the rat spinal cord constitutively express high levels COX-2 protein (Ghilardi et al., 2004). Many different types of spinal neurons express COX-2, such as the majority of lamina I neurokinin (NK)-1 expressing neurons (Todd et al., 2002), which correspond to spinothalamic and spinoparabrachial neurons and which are involved in the ascending conduction of pain. In contrast, most glial cells such as oligodendrocytes and microglia do not express detectable levels of COX-2. However, astrocytes located in the white matter express a very high level of COX-2 suggesting a specifically regulated expression of constitutive COX-2 by cells in the spinal cord (Okuno et al., 2004).

Prostaglandins are potent sensitizing agents, which are able to modulate multiple sites in the nociceptive pathway enhancing transduction (peripheral sensitizing effect) and transmission (central sensitizing effect) of nociceptive information (Uda et al., 1990; Willingale et al., 1997). Prostaglandin (PG) E\textsubscript{2} is thought to be a principle mediator of the hypersensitivity (Turnbach et al., 2002) but several prostanoids show similar effects (Ferreira and Lorenzetti, 1981; Uda et al., 1990). The central hyperalgesic properties of prostanoids have been established mainly in experimental studies with intrathecal administration of PGE\textsubscript{i}, PGE\textsubscript{2}, PGF\textsubscript{2a}, PGI\textsubscript{2} and thromboxane (TX) B\textsubscript{2} which evoke thermal and mechanical hyperalgesia (Uda et al., 1990; Minami et al., 1992, 1994; Turnbach et al., 2002) and mimic the central sensitization associated with peripheral inflammation (Vanegas and Schaible, 2001). These effects appear to be mediated by several subtypes of prostanoid receptors (Tilley et al., 2001) including EP\textsubscript{1}, EP\textsubscript{3} (Minami et al., 2001) and IP receptors (Murata et al., 1997). The observation that IP, EP\textsubscript{1} and EP\textsubscript{3} receptors are co-expressed in peripheral and central structures involved in the nociceptive pathway is consistent with this hypothesis (Bos et al., 2004).

It has been reported that up-regulation of spinal COX-2 takes place during peripheral inflammation. In experimental animals, the induction of neuronal and non-neuronal COX-2 in ipsi- and contralateral portions of the cervical and lumbar cord has been observed after hindpaw injection of several inflammmogens, such as carrageenan (Hay and de Belleroche, 1997; Ibuki et al., 2003), zymosan (Tegeder et al., 2001a) and complete Freund’s adjuvant (CFA) (Hay and de Belleroche, 1998). However, this does not exclude COX-1 in the spinal cord also being a source of spinal PGs in
peripheral inflammation and, indeed, this has been demonstrated in COX-2-deficient knockout mice (Ballou et al., 2000). Recent findings have also shown that there is an upregulation of COX-1 immunoreactivity that is co-expressed along with glial cells in gracilus nucleus of the spinal cord and indicates an important role of COX-1 in spinal nociceptive processing and sensitization after surgery (Zhu et al., 2003). Accumulating data indicates the possible involvement of peripheral and/or central inflammatory components. Thus, there may be possibility of involvement of COX isoforms in generating PGs, the potent pro-inflammatory mediators and subsequent induction of exaggerated pain states.

The study of the mechanisms of neuropathic pain is largely based on animal models. The rat models of traumatic nerve injury are common in experimental pharmacology for studying mechanisms involved and effects of various agents in neuropathic pain. It is well known that PGs involve in central nociceptive processing. Intriguingly, spinal administration of PGs produces hyperalgesia and allodynia that resembles neuropathic symptoms (Uda et al., 1990; Minami et al., 1992, 1994, 2001). Indeed, central sensitization mechanisms are predominantly involved in the development and maintenance of hypersensitivity (Taylor, 2001; Zimmerman, 2001). Growing body of evidence indicates that subcutaneous or spinal administration of COX inhibitors reversed the established neuropathic pain in rats with chronic nerve constriction suggests the possible involvement of central PGs in neuropathic pain (Parris et al., 1996; Syriatowicz et al., 1999). Consistent with these reports, EP1 receptor antagonists also reduced hypersensitivity in mononeuropathic rats (Kawahara et al., 2001; Syriatowicz et al., 1999), further demonstrating the involvement of PGs. On the contrary, it has also been reported that intrathecal and systemic administration of COX inhibitors failed to reverse hypersensitivity in rats with spinal nerve ligation (Lashbrook et al., 1999; Hafferan et al., 2003b). Recent studies have shown that PGE2 EP receptors and COX isoforms are differentially up-regulated in injured nerves following nerve injury (Ma and Eisenach, 2002, 2003, 2004; Zhu and Eisenach, 2003). Despite enormous data on understanding of the role of PGs in mononeuropathic pain, little is known about the relative role of COX isoforms and the effects of COX inhibitors in the development and maintenance of neuropathic pain following peripheral nerve injury. Further, there are various etiological differences in the pathogenesis of traumatic, metabolic, cancer, and chemotherapy-induced neuropathic pain. Basic research in different animal models of neuropathic pain

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indicates that multiple pathophysiological mechanisms, i.e., neurophysiological, morphological and biochemical, may be at play in neuropathic pain conditions. Etiologically different clinical conditions may harbor similar pathophysiological mechanisms. Similarly, etiologically similar clinical conditions may harbor different pathophysiological mechanisms. Thus, further studies are needed to understand the exact role of COX isoforms in neuropathic pain of different etiologies.

There are various painful conditions associated with or without inflammation, such as musculoskeletal pain, muscle pain, orofacial pain, post-operative pain. In fact, there are relatively few behavioral models in animals developed and validated to the study of these painful conditions. Recently animal models have been developed for various nociceptive conditions such as the orofacial formalin test for studying orofacial pain of trigeminal origin (Clavelou et al., 1995), incision-induced pain for studying post-operative pain (Brennan et al., 1996), intramuscular acidic saline or carrageenan for studying muscular pain (Sluka et al., 2001; Radhakrishnan et al., 2003). All these models are well characterized and validated to study disease pathogenesis and to investigate effects of various mediators involved. Growing body evidence suggests that PGE$_2$ synthesized in the brain may also be involved in the modulation of trigeminal nociception. Stimulation of the intracranial duramater with PGE$_2$ produces sensitization of brain stem trigeminal neurons (Bolton et al., 2001; Burstein et al., 1998). It has been reported that brain-derived PGE$_2$ induces mechanical hyperalgesia and hypoalgesia of brain stem trigeminal neurons through the E-prostanoid receptors, EP$_3$ and EP$_1$ respectively (Oka et al., 1997). Thus, it is possible that the AA-COX-PGs pathway is critically involved in orofacial pain. However, there are no studies reported the relative role of COX isoforms and effects of COX inhibitors in these models so far.

Over the past decades, and in the current Decade of Pain Control and Research (2001–2010), pain research has undergone major changes, from a system level to cellular, subcellular and molecular levels. A growing literature supports the idea that some forms of exaggerated pain may involve glial activation. It is well known that sensitization of spinal cord dorsal horn neurons by neuroactive substances such as substance P (SP), glutamate, and nitric oxide (NO) leads to hypersensitivity (Haley and Wilcox, 1992). Although classic views of pain facilitation have only focused on neurons, these neuroactive substances also activate non-neuronal cells, also known as glia (Kreutzberg, 1996; McMahon et al., 2005). It has been demonstrated that glial
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cells such as microglia and astrocytes release a wide variety of neuroactive substances, including several known to activate spinal cord pain transmission neurons (Hanani et al., 2005; Salter, 2005). Recent developments in understanding that spinal microglia participate in the pathogenesis of exaggerated pain provides a framework for addressing the major questions for the future therapeutic targets. Further, the signal(s) for activating microglia and astroglia in spinal cord is unknown but recent evidence raises the possibility that neuron–microglia signaling in the brain could be a candidate. Thus further studies are needed to address the role of different glial cells in exaggerated pain states.

The development of new pain strategies involves combining widely used analgesic drugs that target both central and peripheral pain pathways in order to enhance or preserve antinociception and decrease side effects at reduced and tolerable doses of individual drugs (Mehlisch, 2002; Desmeules et al., 2003). Further, when two or more drugs are combined, they can show an independent action, and the effects will be the sum of those of each drug individually (no-interaction or additive effects). However, the drugs may interact, and the observed effects of the combination may be more or less than expected, in which case an interaction is usually present synergy or antagonism, respectively.

Tramadol is an orally active, clinically effective, centrally acting analgesic. The analgesic efficacy and potency of acutely administered tramadol is comparable to that of codeine, pentazocine, or dextropropoxyphene (Yalcin and Aksu, 2005), while its antinociceptive potency is only 5 to 10 fold lower than that of morphine (Lehmann et al., 1990). Unlike typical opioid analgesics, tramadol has not been associated with significant opioid-like side effects, such as respiratory depression, constipation, or sedation. Tramadol, as opposed to morphine, is not likely to induce tolerance and physical dependence (Raffa et al., 1992; Dayer et al., 1994).

Gabapentin, 1-(aminomethyl) cyclohexane acetic acid (Neurontin®), is widely used against certain forms of epilepsy (Strolin Benedettí et al., 2005) and neuropathic pain (Blackburn-Munro and Erichsen, 2005). Although several clinical trials have meanwhile confirmed its clinical effectiveness in a variety of pain syndromes, the mechanisms in particular of its analgesic action are still unknown. The only high affinity binding site of gabapentin is the accessory α2δ subunit of high voltage-gated Ca2+ channels (Luo et al., 2001; 2002). Despite the lack of a clear mode of action, it is
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widely accepted that gabapentin exerts its analgesic effect through an interaction with nociceptive processing in the spinal cord (Coderre et al., 2005). Therefore, studying combination of NSAID and tramadol or gabapentin in widely accepted animal models of nociception would give more insight into possible beneficial interactions.

Thus, the present work reported in this thesis was undertaken to investigate the effects of COX inhibitors to address the role of PGs and COX isozymes in the pathophysiology of neuropathic pain of different etiologies and various inflammatory nociceptive conditions, particularly orofacial pain and musculoskeletal disorders. Attempts were also made to address the role of non-neuronal cells in acute pain and chronic inflammatory and neuropathic pain. The studies were also directed to investigate the possible therapeutically beneficial antinociceptive interactions between COX inhibitors and tramadol or gabapentin, widely used analgesics in the management of pain. All the observations are presented under different chapters.