

THE ANALGESIC MECHANISMS OF BUPRENORPHINE

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**"Common sense tells us that the things of the earth exist
only a little, and that true reality is only in dreams."**

Charles Baudelaire

(1821-1867)

Les Paradis artificiels, dedication (1860).

To my father and my mother
To Anasthasie and my kids

ABSTRACT

Buprenorphine, a derivative of thebaine, is a semi-synthetic opiate and a partial μ -opioid receptor agonist. Buprenorphine is also a weak κ -opioid receptor antagonist. Buprenorphine is used clinically as an analgesic and for maintenance therapy of opiate-dependent subjects because it produces limited withdrawal symptoms. Recent evidence suggests that the mechanisms of the analgesic effect of buprenorphine are to large extent different from classical μ -receptor agonists. In this thesis, the analgesic mechanisms of buprenorphine are evaluated. Using animal models of neuropathic pain we showed that systemic administration of buprenorphine alleviates mechanical and cold allodynia in rats with injury to the sciatic nerve, infraorbital nerve or the spinal cord. The effect of buprenorphine is similar or superior to morphine in all three models and better than the anticonvulsant gabapentin in infraorbital nerve injured rats. Buprenorphine inhibits spinal cord hyperexcitability induced by repetitive C-fiber stimulation (central sensitization) in decerebrate, spinalized rats at a moderate dose, an effect that is not blocked by naloxone or shared by morphine. In sciatic nerve injured rats, there is a similar tolerance development to the antinociceptive effect of morphine and buprenorphine. In contrast, tolerance developed much slower to the antiallodynic effect of buprenorphine than to morphine and there is no cross-tolerance to buprenorphine in rats made tolerant to morphine. The antinociceptive effect of buprenorphine varies significantly in four inbred strains of mice (DBA2, C3H, C56BL/6 and 129). Significant between-strain differences were also seen for the antinociceptive effect of systemic morphine. The ranking order of potency was largely similar between morphine and buprenorphine in mice. On the other hand, the metabolism and antinociceptive effect of morphine, but not buprenorphine, is different in two sub-strains of Sprague-Dawley rats. The results presented in this thesis show that buprenorphine is effective in rat models of neuropathic pain. The effect of buprenorphine in nerve-injured rats may be mediated by mechanisms different from those of classic μ -opioid receptor agonists and may be related to an inhibition of central sensitization. There are genetic factors in sensitivity to buprenorphine that do not involve differences in metabolism. Buprenorphine may provide a clinical alternative in treating neuropathic pain.

Key words: buprenorphine, morphine, cross-tolerance, μ -opioid receptors, inbred mice.

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LIST OF PAPERS AND PUBLICATIONS

This thesis is based on the following list of papers, which will be referred in the text according to their Roman numerals:

I. Kouya, P.F., Hao, J.-X. and Xu, X.-J. Buprenorphine alleviates neuropathic pain-like behaviors in rats after spinal cord and peripheral nerve injury. Europ. J. Pharmacol. 450 (2002) 49-53

II. Kouya, P.F., Bulka, A., Hao, J.-X., Wiesenfeld-Hallin, Z. and Xu, X.-J. Pharmacological characterization of an orofacial neuropathic pain model after photochemically-induced infraorbital nerve injury: effects of morphine, buprenorphine and gabapentin. Manuscript (Submitted).

III. Kouya, P.F., Hao, J.-X. and Xu, X.-J. Tolerance to the antinociceptive and antiallodynic effect of buprenorphine and morphine in a rat model of mononeuropathy. Manuscript (Submitted).

IV. Kouya, P.F. and Xu, X.-J. Buprenorphine reduces central sensitization after repetitive C-fiber stimulation in rats. Neurosci. Lett. 359 (2004) 127-129

V. Kouya, P.F., Wu, W.-P., Wiesenfeld-Hallin, Z. and Xu, X.-J. Strain differences in the antinociceptive effect of intrathecal R-phenylisopropyl-adenosine (R-PIA) and systemic buprenorphine: comparison with morphine. Manuscript (Submitted).

VI. Bulka, A, Kouya, P.F., Böttiger, Y, Svensson, J.O., Xu, X.-J. and Wiesenfeld-Hallin, Z. Comparison of the antinociceptive effects of morphine, methadone, buprenorphine and codeine in two substrains of Sprague- Dawley rats. Europ. J. Pharmacol. 492 (2004) 27-34

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LIST OF ABBREVIATIONS

ADR	Adverse drug reaction
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
cAMP	Cyclic 3', 5'adenosine monophosphate
CS	Conditioning Stimulation
DF	Descending facilitatory
DRG	Dorsal root ganglia
ECG	Electrocardiogram
ED50	Effective dose 50
EMG	Electromyography
FLSD	Fisher protected least significant different
GABA	Gamma- amino butyric acid
HT	High threshold
IASP	International Association for Study of Pain
IoN	Infraorbital nerve
i.p.	Intra peritoneal
LT	Low threshold
MAD	Median absolute deviation
M3G	Morphine 3 glucoronid
M6G	Morphine 6 glucoronid
NMDA	N-Methyl-D-Aspartat glutamate receptor
NOP	Nociceptin/orphanin FQ peptide
PAG	Periaqueductal gray
PTX	Pertussis toxin
QTL	Quantitative traite loci
R-PIA	R-N- (phenyl isopropyl)-adenosine
s.c.	Subcutaneous
SD-rats	Sprague Dawley rats
S.E.M	Standard error of the mean
SSRI	Serotonin specific reuptake inhibitor
TCAs	Tricyclic antidepressant
WDR	Wild dynamic range

INTRODUCTION

1. Pain: concept and evolution

Pain is defined by the International Association for 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). Today, we appreciate pain as a warning signal to safeguard our body from external harm or disease progress. However, in ancient times, pain was often thought to be related to demons and sins and as punishments for the wicked and purifying trials for the good. Indeed, the word "pain" was derived from the Latin word "poena" meaning "punishment". For the woman in labor, pain has been thought to be the spiritual experience that would transform her into a self-sacrificing mother (<http://www.library.ucla.edu/libraries/biomed/his/painexhibit/panel2.htm>).

Aristotle (c:a 350 B.C.) was probably one of the first great thinkers to relate pain to body function when he suggested that pain is an intense form of touch, one of the five senses of the body. He also proposed that the heart was the center for these senses (Zimmermann, 2005). The importance of the nervous system for experiencing pain was later on proposed by, for example the Greco-Roman physician Galen (120-210 A.D.), reaffirmed and illustrated during the Renaissance by Leonardo Da Vinci (1452-1519) and Andreas Vesalius (1514-1564). From the 17th century to the modern era, a continuous growth in our understanding of the anatomy and physiology of pain transmission has developed through the work of for example Rene Descartes (1596-1650), Thomas Willis (1622-1675), Silas Weir Mitchell (1830-1914), Max von Frey (1852-1932); Henry Head (1861-1940), Charles Sherrington (1857-1952), John Bonica (1917-1994) and Patrick Wall (1925-2001). The modern conceptions of pain are derived in part from the concept of "nociception" as proposed by Sherrington (1906) and from the gate control theory by Melzack and Wall (1965). There is also tremendous progress made in anaesthesia and analgesia with the invention of surgical anesthesia with nitrous oxide or ether, local anesthesia with cocaine and the synthesis of morphine and aspirin (Zimmermann 2005).

2. Neurophysiology of sensory pathways and pain modulation

2.1. Primary afferents, dorsal horn and ascending pathways

Small calibre unmyelinated C primary afferent fibres and medium calibre myelinated A δ afferents are activated by noxious (tissue-damaging) thermal, mechanical or chemical stimuli and convey nociceptive information principally to superficial lamina I and II of the spinal cord dorsal horn (Coggeshall and Willis, 1991). Rapidly conducting large myelinated primary afferent A β fibres, which are preferably activated by innocuous mechanical stimuli, terminate primarily in the deeper lamina III and IV. Primary afferent inputs either synapse onto projecting neurones, which relay information to the brain, or indirectly activate projecting neurones via excitatory interneurons. Dorsal horn interneurons are mainly of three types: high threshold (HT) neurons that respond only to noxious stimuli and are predominantly localised in the superficial lamina I; low threshold (LT) neurons that respond preferably to innocuous stimulation and wide dynamic range (WDR) neurons that encode innocuous and noxious information in a graded fashion (Besson and Chaouch, 1987; Coggeshall and Willis, 1992). The LT and WDR neurons are primarily encountered in deeper laminae. The sensitization of WDR neurons by repetitive noxious stimulation plays a key role in the induction of long-term inflammatory and neuropathic pain states (Willis, 1985; Baramauskas and Nistri, 1998; Doubell *et al.*, 2000).

The projecting neurons in the dorsal horn of the spinal cord send axons transmitting nociceptive information to the structures of the brainstem and diencephalon, including the thalamus, periaqueductal grey (PAG), the parabrachial region, the reticular formation of the medulla and amygdaloid complex (Millan, 1999; Willis, 1991). The thalamus is the main relay structure for sensory information destined to the cortex. The different projections to the thalamic nuclei and from them to the cortex define the functional circuitry of pain processing (Millan, 1999). The lateral nuclear complex of the thalamus consists of VPL (ventroposteriorlateral), VPM (ventroposteriormedial) and VPI (ventroposteriorinferior) nuclei. Neurons of the WDR type predominate in VPL and VPM nuclei (Millan, 1999), responding to thermal and mechanical stimuli. Their projections to the secondary sensory cortex (SII) have been related to the sensory-discriminative and affective-cognitive aspects of pain (Apkarian *et al.*, 1994; Millan, 1999).

2.2. Descending system

In the spinal cord, descending pathways may interact with the terminals of nociceptive primary afferent fibres directly or via inhibitory interneurons. They may also affect the activity of excitatory interneurons in the dorsal horn. There are both facilitatory and inhibitory influences exerted by the brain to the spinal cord and there is no absolute anatomical separation between structures involved in the initiation of mechanisms of descending inhibition or facilitation. The loci for descending control lie in the rostroventral medulla (RVM) and the nucleus tractus solitarius (NTS) (Fields, 1994; Watkins *et al.*, 1998), which are further, controlled and modulated by the hypothalamus and the PAG. The hypothalamus is extensively interlinked with the NTS, PAG and RVM together with the corticolimbic structures implicated in the affective and cognitive dimensions of pain (Millan, 1999). The medial preoptic nucleus of the hypothalamus nucleus projects to the PAG and also to the RVM probably via a glutamatergic pathway (Murphy *et al.*, 1999; Jiang and Behbehani, 2001). Stimulation of these structures suppresses the response of WDR neurones in the dorsal horn to noxious stimulation (Carstens, 1987; Workman and Lumb, 1997) partly via the descending noradrenergic pathway (Aimone *et al.*, 1988; Behbehani *et al.*, 1986; Dafny *et al.*, 1996).

2.3. Central sensitization

Increased excitability of central nervous system in the form of, for example, windup and central sensitization has been recognized as an important mechanism in pain modulation. Windup was first described by Mendell and Wall (1965; 1966) as a gradual increase in neuronal discharges after repetitive stimulation of primary afferent fibers. Thus, when a peripheral nerve was stimulated at sufficient intensity to activate C-fibers, repetition of the stimulus at high frequencies resulted in a progressive build-up in the amplitude of the response in some dorsal horn neurons. Central sensitization refers to the activity-dependent increase in spinal cord excitability after intense activation of the C-fibers as first described by Wall and Woolf (1984). They showed that a conditioning stimulation (CS) of, and only of, C-fibers in nerves innervating skin or muscle produced a facilitation of the spinal nociceptive flexor reflex lasting from several minutes up to hours. Since then, central sensitization has been observed in many studies of spinal nociceptive systems under normal conditions, after inflammation and nerve injury. This manifests at the single cell level as a change in receptive field properties with a reduction of threshold, an increase in responsiveness and spatial extent and recruitment of novel inputs (Cook *et al.*, 1987). Behaviourally, it is manifested as

abnormal or heightened sensitivity with a spread of hypersensitivity to uninjured sites (secondary hyperalgesia) and the generation of pain by low threshold A β mechanoreceptors i.e. allodynia (Torebjörk *et al.*, 1992). The occurrence of wind-up and central sensitization is related to the ability of C-fibers to generate slow after-depolarization in dorsal horn neurons (Woolf and Thompson, 1992) and the release of substance P and glutamate from primary afferent fibers (Dickenson *et al.*, 1987; Urch *et al.*, 2001).

2.4. The flexor reflex

Withdrawal responses are usually the result of the activation of reflex circuits organized within the spinal cord or trigeminal nucleus and they can be modified by control emanating from higher centres in the brain. Limb withdrawal is the most obvious form of response to a noxious stimulus and the hind limb withdrawal flexor reflex has particular significance in pain research as it is often used as an index of nociceptive responsiveness in animals (Le Bars *et al.*, 2002; Schomburg 2000; Schouenborg 1992; 2002). Classical work by, for example Sherrington (1906), has shown that the flexor reflex involves contractions of one group of muscles, the flexors and concomitant relaxation in a separate set of muscles, the extensors. It has been well established in humans that the magnitude of the flexor reflex correlates well with pain sensation (Willer 1977) and the flexor reflex is also widely used to study central sensitization (Wall and Woolf, 1984).

3. Neuropathic pain and animal models

Neuropathic pain is defined as pain resulting from injury or dysfunction of the nervous system (Braune and Schady 1993, Bennett, 1994). Neuropathic pain can occur after peripheral nerve trauma (deafferentation, entrapment, and amputation), infection (post-herpetic neuralgia, human immunodeficiency syndrome-associated neuralgia), pressure due to tumor growth (neoplasia) and metabolic disturbance (diabetic neuralgia). Neuropathic pain may occur long after the initial injury has healed and is often chronic which invades the patient's life, affecting their behaviour, work, daily task, emotional state and social interactions (Braune and Schady 1993) and neuropathic pain remains difficult to treat.

Clinical research on neuropathic pain is difficult as these are not common disorders and valid patient samples homogenous for important variables are difficult to obtain (Bennett 1994). Moreover, in order to produce animals models of neuropathic pain in an experimental setting, identical injuries need to be introduced to the nervous tissues, which does not occur in

humans. The genetic variability present in human patients, who may underlie the variability in the occurrence of neuropathic pain, is eliminated by using inbred animal strains. This makes possible studies of the role of genetic factors in pain. Furthermore, invasive methods are usually used to address the underlying mechanisms and novel unproven treatments are tested, which is unethical in humans. All these speak for the need to produce analogues of human neuropathic pain conditions in laboratory animals. One of the earliest models is ligation and transection of the hind limb nerves in rodents as described by Wall and Devor (Wall *et al.*, 1979). This lesion produces immediate and irreversible interruption of nerve conduction followed by degeneration of the axons distal to the lesion and sprouting of the proximal axonal stumps in an attempt to regenerate. Behaviourally, rodents exhibited self-mutilation of the denervated area of the limb, a behavior known as autotomy (Wall *et al.*, 1979). Despite controversy (Rodin and Kruger 1984), autotomy is now generally regarded as a model of neuropathic pain, such as phantom limb pain or anaesthesia dolorosa (Coderre *et al.*, 1986; Kauppila, 1998).

The clinical situation of complete section of a major peripheral nerve is uncommon and the majority of neuropathic pain cases involve partial or minor nerve injury (Bennett, 1994). Furthermore, one of the features of neuropathic pain is allodynia and hyperalgesia, which cannot be studied with the axotomy model since the peripheral area affected by the nerve injury is totally deafferented (Bennett, 1994). That is why models of partial nerve injury have also been developed. These may involve mechanical (Bennett and Xie 1988; Seltzer *et al.*, 1990; Kim and Chung, 1992), ischemic (Gazelius *et al.*, 1996, Kupers *et al.*, 1998), inflammatory (Sorkin *et al.*, 1997) or cytotoxic (Aley *et al.*, 1997; Cliffer *et al.*, 1998) lesions. Despite such variable causes of injury, these models share a great deal of similarity as they all produce partial degeneration and demyelination of the nerve along with a period (weeks to months) of behavioral abnormalities characterized by mechanical allodynia and in some cases, cold allodynia and heat hyperalgesia. In addition to somatic hind- and forelimb nerves, there are also models of neuropathic pain affecting other areas of nervous system such as models of central pain following spinal cord injury (Xu *et al.*, 1992) and orofacial neuropathic pain (Vos *et al.*, 1998).

4. Opioids

Opioids are a group of naturally occurring or synthetic chemicals resembling the active component of the opium poppy. Opioids exert their biological effect through endogenous

opioid receptors, which belong to the superfamily of G-protein coupled receptors with a seven transmembrane domains. Opioid receptor activation leads primarily to neuronal inhibition through multiple effectors including the inhibition of adenylyl cyclase (AC) and voltage-gated calcium channels, as well as stimulation of inwardly rectifying potassium channels. The coupling of opioid receptors to these effectors is achieved by activation of Gi/G_o pertussis toxin (PTX)-sensitive G proteins (Wheeler–Aceto and Cowan, 1991). There are four subtypes of opioid receptors cloned so far, identified as μ , δ , κ and nociceptin/orphanin FQ peptide (NOP) (<http://www.iuphar-db.org/iuphar-rd/list/ListO.htm>). It is generally believed that μ -receptor selective agonists display the strongest antinociceptive activity, but also the highest abuse liability. The δ -receptor agonists have less addictive potential, but are also less effective in producing antinociception. The use of κ -agonists for pain treatment is mostly limited to the peripheral action because of its strong dysphoric properties. Agonists of NOP receptor have not been tested yet for their clinical analgesic effects.

4.1. Morphine and its metabolites

Morphine is the most widely used opioid analgesic originally isolated as an alkaloid base from opium by Sertürner in 1805 (Fig. 1). The analgesic effect of morphine is mediated by activation of the μ -receptor. Morphine has at least eight metabolites (Boerner and Roe, 1975; Hanks et al., 1987) and of these, most interest has been focused on morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). These metabolites represent 15% and 50% respectively of total metabolites and can significantly accumulate in long-term morphine-treated patients with renal impairment (Hanna *et al.*, 1993). It has been assumed that M6G also binds to the μ -receptor, but recent evidence suggests that there may be a separate receptor for M6G (Brown *et al.*, 1997). M6G has an antinociceptive effect in both animals and humans (Shimomura *et al.*, 1971; Osborne 1988: Osborne *et al.*, 1992) and it is about twice as potent as morphine in animal models (Pasternak *et al.*, 1987). Furthermore, M6G appears to cause less respiratory depression and constipation than the parent drug (Schmidt *et al.*, 1994; Thompson *et al.*, 1992). M3G has only low affinity to the opioid receptors and no analgesic effect (Christrup, 1997). It produces hyperalgesia and allodynia in animals (Smith *et al.*, 1990) and may function as a physiological antagonist of morphine (Ekblom *et al.*, 1993).

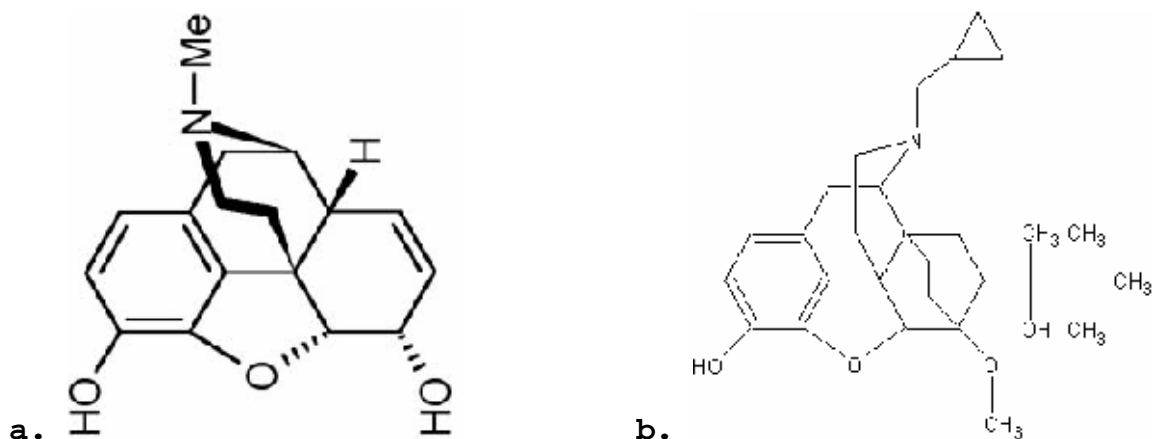


Fig.1. Chemical structure of morphine (a) and buprenorphine (b)

4.2. Buprenorphine

Buprenorphine is a derivative of the morphine alkaloid thebaine and is a member of the 6,14-endo-ethanotetrahydrooripavine class of compounds that includes other potent analgesics such as diprenorphine and etorphine (Bentley *et al.*, 1967; Cone *et al.*, 1984) (Fig. 1). In vivo and in vitro studies have shown that buprenorphine is a partial agonist of the μ -receptor and an antagonist of the κ -receptor (Martin *et al.*, 1976). It is believed that buprenorphine produces analgesia, respiratory depression and miosis through activation of the μ -receptor (but see below). As a partial agonist, buprenorphine may have a wider safety profile compared to full μ -agonists, especially with regard to respiratory depression. Buprenorphine is converted in human liver to norbuprenorphine by the hepatic microsomal cytochrome P4503A4. The oral bioavailability of buprenorphine is low because of extensive first-pass hepatic metabolism (Jasinski 1982; Bullingham *et al.*, 1984). The administration of buprenorphine by the sublingual or transdermal route thus allows for the bypassing of hepatic metabolism. Buprenorphine is approved for use as an analgesic for several of types of pain. It is also been used as maintenance therapy of opioid dependent subjects.

In recent years evidence has emerged to suggest that the antinociceptive effect of buprenorphine may be mediated by additional mechanisms different from activation of the μ -receptors. Thus, the effect of buprenorphine is not sensitive to PTX pre-treatment, which uncouples G-proteins from the μ -receptors (Wheeler–Aceto and Cowan, 1991). In contrast,

buprenorphine is particularly sensitive to changes in an ATP-sensitive K⁺ channel (Ocana *et al.*, 1995). Moreover, receptor binding of buprenorphine is not typical for an opioid (Rothman *et al.*, 1995) and in several assays buprenorphine exhibited unexpectedly strong efficacy, such as in the formalin test in neonatal rats (McLaughlin and Dewey, 1994), the cold tail flick test (Wang *et al.*, 1995) and inhibition of diffuse noxious inhibitory control (Guirimand *et al.*, 1995).

4.3. Other opioids

Because of their therapeutic potential in the treatment of pain, opioids have spurred many pharmacological studies and the development of a great number of novel synthetic derivatives. Most effort has been aimed to increase the selectivity of opioid receptors agonists and antagonists to opioid receptor subtypes with the hope that one could discriminate the effects mediated by these receptors. Currently, a wide range of μ -opioid receptor agonists is available clinically to treat acute and chronic pain, including hydromorphone, oxycodone hydrocodone, dihydrocodeine and methadone (Gourlay, 1999). Some randomised controlled trials seem to indicate that some of these agents might have a more favourable side-effect profile than traditional opioids such as morphine.

Methadone is a synthetic opioid drug that in many ways is different from morphine. Methadone, while being a potent μ -receptor agonist, also has reasonable affinity to the δ -opioid receptor (see Gourlay 1999 for review). Unlike morphine, methadone has no active metabolites and the clearance of methadone from the body is considerably slower than morphine, resulting in a long half-life, up to 12- 14 h (Fainsinger *et al.*, 1993; Mercadante, 1997; Gourlay, 1999). Clinical evidence suggests that methadone is atypical as an opioid since it often produces analgesic effect in patients refractory to other μ -opioid agonists (Crews *et al.*, 1993; Leng and Finnegan, 1994; see Morley and Makin 1998 for review). Methadone also possesses N-methyl-D aspartate (NMDA) receptor antagonist activity (Ebert *et al.*, 1995). Another opioid that has been studied in this thesis is codeine, which is a weak opioid. Codeine is used clinically as an analgesic and a centrally acting antitussive. Codeine exerts its effect by being metabolised to morphine (He *et al.*, 1998).

4.4. Opioid tolerance

Repeated administration of opioids leads to the development of tolerance to some of their pharmacological effects, including antinociception. Tolerance is defined as a loss of effect so that a higher dose is required for obtaining equivalent analgesia (McQuay 1999). This is one of the limiting factors in the clinical application of opioids (Jasinski *et al.*, 1978; Nestler, 1996). The mechanisms of tolerance may be due to changes in opioid receptors, as well as in anti-opioid mechanisms. At the single cell level, chronic exposure to opioid agonists results in several adaptive changes such as the down-regulation of opioid receptors, receptor internalization and uncoupling from inhibitory G-proteins (Mao *et al.*, 2002). These processes may lead to receptor desensitization. Another adaptive response to sustained opioid exposure is an up-regulation or super-sensitization of the cAMP signal transduction system (Mao *et al.*, 2002). Long-term opioid exposure also results in the phosphorylation of opioid receptor proteins, their G proteins and several related effector proteins (Mao *et al.*, 1995). At the systems level, opioid tolerance is known to be associated with increased activity of endogenous anti-opioid mechanisms, including the neuropeptide cholecystikinin (CCK) (Welin *et al.* 1994; Cesselin 1995; Wiesenfeld-Hallin and Xu, 1996), NMDA receptors (Mao *et al.*, 1995) and glial activation (Watkin *et al.*, 2005). Opioid tolerance may also be associated with changes in activity of endogenous opioid peptides (Persson *et al.*, 1989).

Tolerance development is influenced by many factors, including intrinsic efficacy of the agonists, manner of drug administration, testing procedures and other environmental factors (Siegel, 1976; Sherman *et al.*, 1982; Advokat and Isaac, 1983; Dafters and Odber, 1989; Sosnowski and Yaksh 1990). It is generally accepted that opiate drugs that are selective to one type of opiate receptor will show cross-tolerance (Carter and Tiffany, 1996; Wagner *et al.*, 1997; Walker *et al.*, 1997). On the other hand, cross-tolerance between agonists of different opiate receptors is minimal (Young and Khazan, 1984).

5. Pharmacology of neuropathic pain

As mentioned above, neuropathic pain is difficult to treat. Drugs that are widely used to treat other types of pain, including opioids and NSAIDs have no or only limited effect in neuropathic pain. In particular, opioid sensitivity in neuropathic pain remains unclear. Although it has been suggested that opioids are ineffective in treating neuropathic pain (Arner and Meyerson, 1988), recent experimental and clinical studies seem to indicate that opioids,

including morphine, are effective in treating neuropathic pain, but their potency may be reduced in comparison to nociceptive pain (Rowbotham *et al.*, 1999; Ossipov *et al.* 1999).

Opioid sensitivity in neuropathic pain is determined by several factors. First, nerve injury induces down-regulation of pre- and postsynaptic μ -receptors in DRG and the spinal cord (Zhang *et al.*, 1998). Second, increased activation of NMDA receptors after nerve injury may reduce opioids-mediated antinociception (Mao *et al.*, 1995). Third, changes in peptide expressions may also play a role. Thus, expression of peptides such as CCK and dynorphin, which block the effects of μ -opioids, is upregulated in the spinal cord after nerve injury. Finally, neuropathic pain is often manifested as mechanical allodynia mediated by large diameter A β -afferents which may not be sensitive to μ -opioids since μ -receptors are located selectively on the terminals of small diameter afferents (Jones *et al.*, 2003; Aicher *et al.*, 2000).

Others classes of drugs that are commonly used to treat neuropathic pain include tricyclic antidepressants (TCAs), anticonvulsants and systemically administered local anesthetics. TCAs have been successfully used for the treatment of neuropathic pain for a long time. They act through inhibition of the reuptake of noradrenalin and serotonin in the nervous system and modulate sodium channels. The TCAs may however be associated with a number of several side effects. The specific serotonin reuptake inhibitors (SSRI) have not shown any efficacy in treating neuropathic pain compared to TCAs (Mackin, 1997; Beydoun and Kutluay, 2002).

Anticonvulsants modify voltage dependent sodium and calcium channels, thereby stabilising neuronal membranes. Adverse effects such dizziness, sedation fatigue; ataxia and confusion have been reported in medication with anticonvulsants (Campbell *et al.*, 1966). Gabapentin is perhaps the best studied anticonvulsant used for treating neuropathic pain. It was originally developed as a structural GABA analog, but it has no GABA-like action (Backonja *et al.*, 2000). Gabapentin and its recently developed analogue pregabalin act through binding to the δ -subunit of voltage-dependent calcium channels (Triggle, 2003).

6. Genetic differences in pain and analgesia

Pain perception in humans and animals display considerable interindividual variability. Recent clinical and experimental studies suggest that such variability is to a large extent determined by genetic variance (Mogil *et al.*, 1996a). Similarly, sensitivity to analgesics is

also affected by genetic factors. This has been studied in the case of morphine. Thus, response to the analgesic effect of opioids has been found to vary in humans and among inbred strains of rodents (Mogil 1999, Mogil *et al.*, 1996a), indicating significant inheritance. Many factors may be involved in the genetic differences in response to opioids and one example is the inherited pharmacokinetic variance produced by the polymorphism in enzyme activity of cytochrome P4502D6 leading to variability in the response to codeine (Desmeules *et al.*, 1991) Genetic differences in response to opioids may also be due to variability in the level of opioid receptors in the nervous system. Indeed, using quantitative trait locus (QTL) mapping, the antinociceptive effect of morphine on the hot plate test has been linked to 4 QTLs (Bergeson *et al.*, 2001 ; Uhl *et al.*, 1999) and the largest of these QTLs is attributed to the gene *Oprm* encoding the μ -opioid receptor (Bergeson *et al.*, 2001).

Studies of genetic differences in analgesic responses to drugs have important theoretical and practical implications. For example, the occurrence of adverse drug reactions (ADR) is associated with mortality and substantial cost of medical care. Potential risk factors for ADRs including age, gender, and organ function, especially liver and kidneys as well as life style (smoking and alcohol intake). Genetic variables however can also modify pharmacokinetics, pharmacodynamics and responsiveness to drugs and may thus also contribute to ADRs. Moreover, better understanding of genetic variation in drug responses may lead to improved treatment regime based on individual pharmacogenomics.

AIMS OF THE STUDY

The general aim of this study is to examine the mechanisms of the analgesic effect of buprenorphine with emphasis on neuropathic pain and genetic differences. The specific aims are:

1. To establish antinociceptive effect of buprenorphine in models of neuropathic pain after injury to the sciatic nerve, infraorbital nerve and spinal cord and to compare the effect of buprenorphine with that of morphine and the anticonvulsant gabapentin.
2. To study tolerance and cross-tolerance between buprenorphine and morphine in rats with peripheral nerve injury.
3. To explore the mechanisms of buprenorphine's antinociceptive action by evaluating its effect on central sensitization
4. To study the role of genetic factors in buprenorphine-mediated antinociception

MATERIALS AND METHODS

1. Animals

All experiments have been approved by the local animal research ethics committee and were performed according to the guidelines of IASP. Outbred Sprague Dawley rats (SD) from either Möllergård (Möllergård, Denmark, SD-Mö) or B&K, Universal (Sweden, SD-BK) were used in studies I-IV and VI. Four inbred mouse strains, DBA/2Ntac (DBA2), 129S6SvEvTac (129), C3H/HeNTac (C3H) and C57BL/6Ntac (C57BL/6), from Taconic (Denmark) were used in study V. All animals were housed in standard laboratory conditions, with constant room temperature and 12 h light/dark cycle. Food and water was accessible *ad libitum*.

2. Photochemically-induced ischemic injury

2.1. Sciatic nerve

Male rats weighing 200-250 g were anaesthetized with chloral hydrate (Sigma, 300mg/kg, i.p). The common sciatic nerve was dissected free from the surrounding tissue over a distance of about 1 cm at mid-thigh level. The photosensitizing dye, Erythrosin B (Aldrich, 32.5 mg/kg) was injected intravenously and the exposed nerve was irradiated with the knife-edge beam of an argon laser with an average power of 170 mW at the wavelength of 514 nm (Innova model 70, USA). Aluminium foil was placed under the nerve to isolate the surrounding tissue and to reflect light. Paraffin oil was applied on the nerve to prevent it from drying out during irradiation. As a result of the interaction between the dye and the laser beam, vascular occlusion and localized focal ischemia was produced, causing nerve injury (Cameron *et al.*, 1990; Gazelius *et al.* 1996; Kupers *et al.*, 1998).

2.2. Infraorbital nerve (IoN)

The procedure is similar to sciatic nerve irradiation and male rats were used. Under chloral hydrate anesthesia, the left IoN was exposed by a 1 cm long skin incision in the *regio masseterica* and carefully separated from the surrounding vasculature with a glass hook. The exposed nerve was irradiated with the laser for 6 min after injection of erythrosin B. The injection was repeated after 5 min. After the irradiation the incision was closed in layers and the rats were returned to their cages for recovery.

2.3. Spinal cord injury

Spinal cord injury was produced in female SD rats weighing 200 g. The rats were anesthetized with chloral hydrate and a midline incision was made on the skin overlying vertebral segments T12-L1. The animals were positioned beneath an argon laser beam and irradiated for 10 min with the beam directed towards vertebral segment T12 or T13 (spinal segments L3-5). Immediately prior to and 5 min after the start of the irradiation, erythrosin B was injected intravenously through the tail vein at the same dose as for the sciatic and IoN irradiation. The laser beam, same intensity as for the sciatic and IoN experiments, covered the entire width of the vertebrae and the length of the irradiation beam was approximately 1-2 mm. After irradiation, the wound was closed in layers and the rats were allowed to recover. The bladder was emptied manually for 1 week.

3. Behaviour studies

3.1 Normal animals

Hot plate test

In studies I and VI the antinociceptive effect of buprenorphine and other opioids was tested in normal rats with the hot plate test. Prior to drug administration the rats were trained on the hot plate for 5 days in order to obtain stable baseline response latency, usually between 3-5 s. A hot plate (IITC; Woodland Hills, CA, USA) maintained at $54\pm 0.1^\circ\text{C}$ was used and the latency of licking one of the hind paws was measured with an accuracy of 0.1 s. The cut-off time was 30 seconds, after which the animals were removed to avoid tissue damage.

Tail flick test

In studies III and V, the antinociceptive effect of buprenorphine and morphine was studied in rats or mice with the tail flick test. The animals were gently held and a radiant heat source (Ugo Basile, Italy) was focused 1-2 cm from the tip. The tail flick latency was recorded automatically and the cut-off time was 10 s.

3.2. Sciatic nerve injured rats

Mechanical stimulation

Rats were placed in a plastic cage with a metal mesh floor and calibrated nylon monofilaments (von Frey hairs, Stoelting, USA) with different bending forces were applied to the plantar surface of the hind paw of each animal. The withdrawal threshold was taken as the force at which the animal withdrew the paw at least 3 out of 5 stimuli.

Cold stimulation

The animals were placed on a metal mesh floor. The response to cold was tested with ethyl chloride, which was briefly sprayed on the plantar surface of the hind paw. The responses were scored as following: 0= no response, 1= startle-like response, no hind paw withdrawal, 2= briefly withdrawal of the stimulated hind paw, 3= sustained or repeated withdrawal of the stimulated paw, briefly licking or shaking and 4= prolonged withdrawal, shaking and licking of the hind paw, vocalization and generalized aversive reaction.

3.3. IoN injured rats

Mechanical sensitivity of the face was tested with a graded series of von Frey filaments. During testing the rat was gently held by the experimenter and the von Frey filaments were applied in ascending order within the IoN territory on the hairy skin of the vibrissae pad. The skin on innervation territory of the IoN was stimulated with each filament four times at 1/s. The response threshold was taken as the force at which the rat presented any of the following aversive responses: withdrawal, struggle/escape or attack (Vos *et al.*, 1998). The cut-off value was set at 40.14 g.

3.4 Spinal cord injured rats

In spinally injured rats, the mechanical allodynia was assessed by examining the vocalization thresholds to von Frey hair stimulation. During testing, the rats were gently restrained in a standing position and the von Frey hairs were pushed onto the skin until the filament became bent. The frequency of the stimulation was about 1/s and at each intensity 5-10 stimuli were applied. The intensity of stimulation which induced consistent vocalization (>70 % response rate) was considered as a pain threshold. Responses to cold were tested with ethyl chloride spray applied to the shaved skin area. The response was graded with a score of 0 = no observable response; 1 = localized response (skin twitch and contraction), no vocalization; 2 = transient vocalization, moderate struggle and 3 = sustained vocalization and aggression.

4. Induction of tolerance to morphine and buprenorphine

In study III, the development of tolerance was examined in two groups of rats ($n=10$ in each group) starting 7 d after sciatic nerve injury. Morphine (10 mg/kg) or buprenorphine (0.3 mg/kg) was injected twice daily between at 09:00 and 17:00 for 17 days. The withdrawal threshold of the ipsilateral paw to mechanical stimulation and tail flick latency were tested

every two days before and 30 min after drug administration. The development of cross-tolerance was assessed by testing the effect of morphine in rats treated chronically with buprenorphine and vice versa.

5. Electrophysiological study of the flexor reflex

Both male and female rats were used. The rats were briefly anaesthetised with chloral hydrate (300 mg/kg). A tracheal cannula was inserted and the rats were artificially ventilated. Rectal temperature and electrocardiogram (ECG) were monitored and core temperature was maintained at 36-37°C with a heating pad. The rats were mounted on a stereotaxic spinal unit and the forebrain and the midbrain were removed by aspiration. The spinal cord was exposed by a laminectomy at mid-thoracic level and transected at Th8-9. The posterior biceps femoris/ semitendinosus muscle was exposed at one side and two stainless needle electrodes were inserted for recording of the electromyogram (EMG). The hamstring flexor reflex was elicited by subcutaneous electrical stimulation of the tarsal surface innervated by the sural nerve through a pair of needle electrodes using rectangular pulses of 1 ms of 5 mA, sufficient to activate C-fibers. The frequency of stimulation during recording of the baseline reflex was 1/min and the elicited EMG activity was integrated over 2 seconds. To study central sensitization, a conditioning stimulation (CS, 1 Hz for 20 seconds) was administered at the same intensity as the test stimuli after establishing a stable baseline reflex and the single test stimulation was then given 30 s and 1 min after the CS to determine the extent of spinal cord sensitization.

6. Implanting chronic i.t. catheters in mice

Chronic i.t. catheters were prepared and implanted in mice according to methods described previously (Wu *et al.*, 2004). The proper location of the catheter was verified by observing bilateral paralysis following i.t. administration of lidocaine (Xylocain 20 mg/ml, AstraZeneca, Sweden) and the motor function of the mice were examined 1 day after implantation by observing if there was any motor dysfunction, weakened extension withdrawal reflex of the hind limb and reduced toe spread. Animals with motor dysfunction were sacrificed.

7. Measurements of the metabolism of morphine and buprenorphine

The animals were decapitated 15 or 60 min after administration of 0.3-mg/kg buprenorphine and 30 or 120 min after 10-mg/kg morphine. The samples ($n=6$ in each group) were

centrifuged and stored at -20°C until analysis. Plasma concentrations of morphine and its metabolites M3G and M6G and buprenorphine and its metabolite norbuprenorphine were measured by liquid chromatography with mass spectrometry detection.

8. Drugs

Buprenorphine (Temgesic, Schering-Plough, Sweden), morphine hydrochloride (Apoteksbolaget, Sweden), methadone hydrochloride (Apoteksbolaget, Sweden), codeine phosphate (Apoteksbolaget, Sweden), gabapentin (Sigma, USA), naloxone (Sigma, USA) and R-PIA (Sigma, USA) were dissolved or diluted in physiological saline. All drugs, except R-PIA, were injected systemically (s.c. or i.p.). R-PIA which was injected i.t...

9. Statistics

All data were analyzed with the statistic program StatView on PC or Macintosh computers. The data from the von Frey and cold tests are expressed as median \pm median absolute deviation (M.A.D) and analysed with Friedman ANOVA followed by Wilcoxon signed-ranks test, Dunnet's test and Mann Whitney U-test. The data for the hot plate and tail flick tests was calculated and expressed as mean \pm S.E.M and analyzed with ANOVA followed by Fisher's Protected Least Significant Difference (FLSD) test. Paired and unpaired t-tests were also used. In some studies, % maximal possible effect was used that was calculated based on the formula

$$\% \text{ MPE} = \frac{\text{Latency after drug} - \text{baseline}}{\text{Cut-off} - \text{baseline}} \times 100$$

RESULTS

1. The antinociceptive effect of buprenorphine in normal SD-Mö rats (paper I)

Buprenorphine at doses of 0.03, 0.1 and 0.3mg/kg produced significant dose-response increase in the hot plate latency in normal SD-Mö rats (Fig.1). The effect of buprenorphine at the two higher doses was more potent and prolonged. The peak effect of 0.3 mg/kg buprenorphine was stronger than 0.1 mg/kg, but of shorter duration. No signs of side effects such as sedation or motor impairment were noted following buprenorphine at any doses.

2. The anti-allodynic effect of buprenorphine in SD-Mö rats with sciatic nerve injury (paper I)

Paw withdrawal threshold was significantly decreased after sciatic nerve ischemic injury starting 1 day after injury and was maintained at day 3 and 7 (Fig. 2A). The response to cold was increased with a time course similar to mechanical hypersensitivity. Cumulative doses of buprenorphine (0.03 mg/kg + 0.07 mg/kg + 0.2 mg/kg) administered at 30 min intervals reduced the mechanical and cold hypersensitivity after sciatic nerve injury (Fig. 2B, C). The effect on mechanical hypersensitivity was already significant at the dose of 0.03 mg/kg and at the doses of 0.1 or 0.3 mg/kg, the hypersensitivity was totally reversed. The effect after 0.3 mg/kg cumulative buprenorphine lasted 90-120 min. Saline produced no effect.

3. The anti-allodynic effect of buprenorphine in SD-Mö rats with spinal cord injury (paper I)

After spinal cord injury, rats developed chronic pain-like behaviour manifested as increased response to mechanical or cold stimulation. As in rats with sciatic nerve injury, cumulative doses of buprenorphine alleviated the mechanical and cold allodynia-like behavior in spinally injured rats (Fig. 3). The mechanical and cold allodynia was totally alleviated by 0.1 and 0.3-mg/kg buprenorphine respectively. Saline produced no effect.

4. The anti-allodynic effect of buprenorphine in SD-Mö rats with IoN injury in comparison to morphine and gabapentin (paper II)

After IoN injury, rats developed mechanical hypersensitivity in the innervation region of the IoN, starting within 3 days and peaking on day 7. Response threshold decreased from about 40 g to below 10 g. The mechanical hypersensitivity was stable thereafter for about 1 month.

Cumulative administration of morphine or buprenorphine significantly alleviated mechanical hypersensitivity and at 5mg/kg morphine or 0.1mg/kg buprenorphine, the mechanical hypersensitivity was totally abolished (Fig. 3).

The cumulative effect of morphine on IoN injury-induced hypersensitivity was also seen with single dose administration at 2 or 5 mg/kg (Fig. 1A). In contrast, gabapentin alleviated mechanical hypersensitivity only at a dose of 100 mg/kg, but not at 30 mg/kg, significantly, starting 2 h after injection and lasting at least 6 hours (Fig. 1B). However, this dose of gabapentin produced motor deficits, expressed as impaired coordination and righting reflex. Furthermore, even repeated administration of gabapentin at 30 mg/kg once daily starting 1 week after IoN injury also did not alleviate mechanical hypersensitivity (Fig. 2).

5. Development of tolerance to morphine and buprenorphine in rats with sciatic nerve injury (paper III)

In sciatic nerve injured rats, 10mg/kg morphine, 0.3 or 1 mg/kg buprenorphine produced marked antinociception in the tail flick test (Fig.1A) and reduced mechanical allodynia in the paw on the nerve injured side (Fig. 1B). Following chronic morphine or buprenorphine administration, tolerance to the antinociceptive effect of both drugs developed similarly as tested on the tail flick (Fig. 2). In contrast, the development of tolerance to mechanical allodynia was different for the two drugs. Tolerance developed to the anti-allodynic effect of morphine by day 7 whereas the effect of buprenorphine was maintained up to day 15 following repeated administration (Fig. 3B).

6. Cross-tolerance to morphine or buprenorphine in rats with sciatic nerve injury (paper III)

In rats treated with chronic morphine or buprenorphine for 17 days, cross tolerance was assessed on day 18. In the tail flick test, there was complete cross-tolerance to the antinociceptive effect of morphine and buprenorphine (Fig. 4A). In contrast, in rats chronically treated with morphine, buprenorphine completely reversed mechanical hypersensitivity (Fig. 4B). Morphine was also significantly, albeit weakly, increased paw withdrawal threshold to mechanical stimulation in nerve injured rats chronically treated with buprenorphine (Fig. 4B).

7. Effect of morphine and buprenorphine on the flexor reflex and central sensitization (paper IV)

Subcutaneous administration of buprenorphine or morphine at 0.03 or 1 mg/kg respectively produced no or little depression of the flexor reflex whereas at 0.1 or 2 mg/kg buprenorphine and morphine produced a moderate (about 30%) depression of the flexor reflex (Fig. 1). C-fiber CS of the sural nerve produced a significant increase in reflex magnitude with peak facilitation observed 30 s or 1 min after the CS. The increase in reflex magnitude lasted 2-5 min. When delivered 20-30 min prior to CS, buprenorphine at 0.1 mg/kg, but not at 0.03 mg/kg, significantly reduced reflex facilitation-induced by C-fiber CS whereas morphine at 1 or 2 mg/kg produced no effect (Paper IV, Fig. 2). The effect of buprenorphine was not prevented by 0.5mg/kg naloxone.

8. Strain differences in the antinociceptive effect of buprenorphine in mice: comparison with morphine and the adenosine A-1 agonist R-PIA (paper V)

Four inbred strains of mice (129, DBA/2, C57BL/6 and C3H) were tested with the tail flick test. There were significant differences in baseline tail flick latency with the 129 and DBA/2 mice having longer latency to respond than the C57BL/6 and C3H mice (Paper V, Fig.1). I.p. morphine, buprenorphine or i.t. R-PIA produced dose-dependent antinociception in all strains and there were significant strain differences for all three drugs tested (Figs 2-4). However, the rank order of potency was quite similar for drugs, with DBA/2 strain being least sensitive for the antinociceptive effect of all three drugs (Table 1).

Table 1 ED50 for the antinociceptive effect of morphine (MO), buprenorphine (BU and R-PIA in the four inbred mice strains

Strain	MO (mg/kg)	BU (mg/kg)	R-PIA (nmol)
C3H	0.4	0.01	0.9
C57BL/6	0.5	0.02	2.9
129	0.7	0.01	1.0
DBA/2	1.0	0.08	4.0

9. Differential opioids antinociception in two substrains of SD rats (paper VI)

Morphine, methadone, buprenorphine and codeine were injected i.p. in SD rats delivered by two vendors (SD-BK and SD-Mö) and the antinociceptive effect was tested with the hot plate test. The effect of morphine and methadone was significantly stronger in SD-BK than in SD-Mö rats (Figs 1-2, 5). The effect of buprenorphine and codeine was similar in the two substrains (Figs. 3-5).

10. Metabolism of morphine and buprenorphine in two substrains of SD rats (paper VI)

The metabolism of morphine into M3G was significantly higher in SD-Mö than in SD-BK 30 min and 120 min after administration (Fig. 6). The plasma concentration of the active metabolite M6G was very low in both strains, although the level was significantly higher in the SD-BK than SD-Mö rats (Paper VI, Fig. 6). Mean plasma levels of buprenorphine and norbuprenorphine did not differ between the two substrains (Fig. 7).

DISCUSSION

1. Buprenorphine is effective in rat models of neuropathic pain

In work presented in this thesis, we showed that systemic administration of buprenorphine effectively alleviated neuropathic pain-like behaviours (antiallodynia) in three different rat models of neuropathic pain: after injury to the sciatic nerve, infraorbital nerve and spinal cord. The antiallodynic effect of buprenorphine is comparable to its antinociceptive effect, is dose-dependent, of long duration and devoid of side effects. Buprenorphine is at least as efficacious as morphine in the models of peripheral nerve injury (papers I, II, Kauppila *et al.*, 1998) and is superior to morphine in the model of spinal cord injury pain (paper I, Yu *et al.*, 1997). Buprenorphine is also superior to the effect of gabapentin in all three models tested (Paper II, see also Hao *et al.*, 2001). Thus, it is suggested that buprenorphine may be effective in treating clinical neuropathic pain. An anti-allodynic effect of buprenorphine in rodent models of neuropathic pain has also been reported recently (Christoph *et al.*, 2005), confirming our results. There is also clinical evidence that buprenorphine may be effective in neuropathic pain when other opioids failed (Benedetti *et al.*, 1998; Likar and Sittl, 2005).

2. The mechanisms of action for buprenorphine in neuropathic pain

As stated in the Introduction, some of the analgesic mechanisms of buprenorphine may be different from those of classical μ -opioid agonists (McCormack, 1998). The results presented in this thesis support this hypothesis. Furthermore, we suggest that this is particularly true after nerve injury when buprenorphine is used to treat neuropathic pain-like behaviours. Thus, tolerance developed similarly to the antinociceptive effect of morphine and buprenorphine and there was complete cross-tolerance. In contrast, tolerance developed much slower to the antiallodynic effect of buprenorphine than morphine and there was no or reduced cross-tolerance. It is likely that the antinociceptive effect of buprenorphine is mediated by its partial agonism of the μ -opioid receptor. This is also supported by the similar pattern of antinociception between morphine and buprenorphine in four inbred strains of mice (paper V). On the other hand, the mechanisms of antiallodynic effect of buprenorphine are different from that of morphine and other classical μ -agonists.

As also discussed in the Introduction, central sensitization is one of the potential mechanisms underlying neuropathic pain. Thus, alleviation of this pain condition may be related to a

reduction in central sensitization. Previous studies concerning the effect of morphine on central sensitization have been conflicting and it is difficult to draw conclusions from some of these studies when morphine produced marked depression of the baseline response (Carpenter et al. 2000). That is why we have assessed the effect of moderate doses of morphine and buprenorphine and showed that at doses producing comparable depression of baseline reflex magnitude, buprenorphine, but not morphine, reduced reflex facilitation induced by repetitive C-fiber stimulation. This indicates that the ability to reduce central sensitization may be one of the underlying mechanisms for the effect of buprenorphine on neuropathic pain. Interestingly, a recent human study has shown that buprenorphine-induced analgesia and anti-hyperalgesia have different profiles, with the anti-hyperalgesic effect outlasting the analgesic effect, a phenomenon that is opposite to classical μ -agonists (Koppert *et al.*, 2005). These results suggest that buprenorphine may be more effective than pure μ -agonists in reducing central sensitization, which agrees with the results presented in paper IV.

The effect of morphine and buprenorphine on the baseline reflex reflects their opioid action whereas the effect of buprenorphine on the facilitation of the flexor reflex seems to be non-opioid in nature since it was not reversed by naloxone. Central sensitization, including facilitation of the flexor reflex induced by C-fiber CS, is mediated by activation of the NMDA receptors (Eide *et al.*, 2000) and some opioids are known to have NMDA receptor blocking effect (Ebert *et al.*, 1995). It is possible that buprenorphine shares such property. This may also reflect the effect of buprenorphine on neuropathic pain and its application in treating drug dependence and withdrawal since in both conditions NMDA receptor antagonists are useful (Inturrisi, 1997)

3. Genetic factors in antinociception mediated by morphine, buprenorphine and R-PIA

The baseline tailflick latency and the antinociceptive effect of systemic morphine differ significantly among four inbred strains of mice, which agree with previous studies (Mogil *et al.*; 1996a; Mogil, 1999). Some of our observation, such as the relative sensitivity to morphine in DBA/2 mice compared to C57BL/6 mice, differs from previous reports (Mogil *et al.*, 1996b; Wilson *et al.*, 2003). This may be due to the fact that the mice were from different vendors or other environmental factors, such as experimental conditions which are known to have significant impact (Mogil *et al.*, 1996b; Wilson *et al.*, 2003). In this study all mice were supplied from the same vendor at the same time and the experiments were carried out in the way that environmental variables were minimized. It is thus most likely that the results reflect

inherited differences in nociception and analgesic responses. We also found strain differences in response to i.p. buprenorphine or i.t. R-PIA and the pattern of these differences was to a large extent similar to systemic morphine, with the only exception that the C57BL/6 mice appear to be relatively resistant to buprenorphine and R-PIA. This suggests that the genetic factors mediating morphine, buprenorphine and R-PIA-induced antinociception may be similar despite the largely independent mechanisms of action of these compounds, particularly between morphine and R-PIA (μ -opioid vs. adenosine A₁R receptor activation). Interestingly, it has been established previously that the antinociceptive effect of i.t. morphine may be mediated in part by a spinal release of adenosine (Sawynok and Liu, 2003). The similar pattern of genetic differences in antinociception between morphine and buprenorphine support the notion that it is mediated by similar mechanisms (see above).

No correlation was seen between baseline tail flick latency and responses to either morphine, buprenorphine or R-PIA. This may imply that the μ -opioid receptor or A₁R are not physiologically important in determining tail flick latency. However, the number of strains studied is low. It is also unclear whether a genetic correlation can be established between the responses to these compounds with the development of pain after nerve injury. This question is interesting since it may shed light on the mechanisms of neuropathic pain.

4. Opioid antinociception in two substrains of SD rats

Sensitivity to the antinociceptive effect of morphine and methadone in the hot plate test was significantly reduced in SD-Mö rats, compared to SD-BK rats whereas no such difference was seen for buprenorphine and codeine. This could be due to either genetic and environmental factors and/or interactions between them. Results presented in Paper VI indicate that such differences may also have pharmacodynamic backgrounds. Thus, the observed differences in these sub-strains in plasma levels of morphine and its metabolites correspond to the analgesic effects in that SD-Mö rats had significantly lower mean plasma level of morphine and significantly higher ratio of M3G/morphine at 30 min. post dosing, with tendency to higher ratio at 120 min, compared to SD-BKs. The morphine metabolite M3G has an excitatory effect which reduces analgesia (Osborne *et al.*, 1992) and the higher M3G/morphine ratio in Mö rats can thus underlie their weaker response to morphine compared to SD-BKs. Levels of the potent analgesic active metabolite M6G were very low in both sub-strains and would not have contributed to the analgesic effect (Gong *et al.*, 1992). Thus, SD-Mö rats metabolize morphine to M3G to a greater extent compared with the SD-BKs, and this difference in the

pharmacokinetic profiles between the two sub-strains could in part explain the difference in the analgesic effects to morphine.

Methadone has no active metabolites (Boulton *et al.*, 2001). Thus, difference in pharmacokinetics cannot explain the reduced effect of methadone in SD-Mö compared to SD-BK rats. Previous work in this laboratory has shown that some of differences of the effect of opioids in these two sub-strains of SD rats may also be due to different level of activation of the NMDA receptors (Bulka *et al.*, 2002). The lack of difference for the effect of buprenorphine thus may be due to a lack of difference in metabolism as shown in Paper VI, but also to the different analgesic mechanisms under some conditions between morphine and buprenorphine as discussed above.

CONCLUSIONS

Buprenorphine produces antinociceptive effect in normal rodents and an anti-allodynic effect after nerve injury. The antiallodynic effect of buprenorphine is observed across several models of neuropathic pain, is devoid of major side effects and showed minimal tolerance. The effect of buprenorphine is equal or superior to morphine and gabapentin. Thus, buprenorphine may be effective clinically as a treatment for neuropathic pain.

The antinociceptive effect of buprenorphine under normal conditions is probably mediated by mechanisms similar to classical μ -opioid agonists. However, buprenorphine may exert effects through mechanisms independent of activation of the μ -receptors, possibly related to interaction with NMDA or other anti-opioid systems. Such effect of buprenorphine may explain its action on central sensitization, neuropathic pain and in certain substrains of rats where the effects of other opioids are reduced.

The antinociceptive effect of buprenorphine is affected by both genetic and environmental factors, which may be different or similar to morphine, depending on the situation.

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REFERENCES

- Advokat, C., Isaac, L. (1983). Nociceptive assessment modifies behavioural tolerance without altering brain morphine concentration. *Eur. J. Pharmacol.* 92:135-138
- Aicher, S.A., Punnoose, A., Goldberg, A. (2000). μ -opioid receptors often colocalize with the substance P receptor (NK1) in trigeminal dorsal horn. *J. Neurosci.* 20:4345-4354.
- Aimone, L.D., Bauer, C.A., Gebhart, G.F. (1988). Brain-stem relays mediating stimulation-produced antinociception from the lateral hypothalamus in the rat. *J. Neurosci.* 8:2652-2663.
- Aley, K.O., Reichling, D.B. Levine, J.D. (1997). Vincristine-hyperalgesia in the rat: a model of painful peripheral neuropathy in humans. *Neuroscience* 73: 259-265.
- Apkarian, A.V., Stea, R.A. and Bolanowski, S.J. (1994). Heat-induced pain diminishes vibrotactile perception: a touch gate. *Somatosensory Motor Res.* 11:259-267.
- Arner, S., Meyerson, B.A. (1988). Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. *Pain.* 33:11-23.
- Backonja, M.M. (2000). Anticonvulsants (antineuropathics) for neuropathic pain syndromes. *Clin. J. Pain.* 16:S67-72.
- Baranauskas, G., Nistri, A. (1998). Sensitization of pain pathways in the spinal cord: cellular mechanisms. *Progress Neurobiol.* 54: 349-365.
- Behbehani, M.M., Zemlan, F.P. (1986). Response of nucleus raphe magnus neurons to electrical stimulation of nucleus cuneiformis: role of acetylcholine. *Brain Res.* 369: 110-118.
- Benedetti, F., Vighetti, S., Amanzio, M., Casadio, C., Oliaro, A., Bergamasco, B., Maggi, G. (1998). Dose-response relationship of opioids in nociceptive and neuropathic postoperative pain. *Pain.* 74:205-211.
- Bennett, G.J., Xie, Y.K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain.* 33: 87-107.
- Bennett, G.J. (1994). Neuropathic pain. In: Wall, P.D., Melzack, R. (Eds). *Textbook of Pain.* Edinburgh, Churchill Livingstone, pp. 201-224.
- Bentley, K.W., Hardy, D.G., Meek, B. (1967). Novel analgesics and molecular rearrangements in the morphine-thebaine group. II. Alcohols derived from 6,14-endo-etheno- and 6,14-endo-ethanotetrahydrothebaine. *J. Am. Chem. Soc.* 89: 3273-3280.
- Bergeson, S.E., Helms, M.L., O'Toole, L.A., Jarvis, M.W., Hain, H.S., Mogil, J.S., Belknap, J.K. (2001). Quantitative trait loci influencing morphine antinociception in four mapping populations. *Mamm Genome.* 12: 546-553.
- Besson, J.M., Chaouch, A. (1987). Peripheral and spinal mechanisms of nociception. *Physiol. Rev.* 67: 67-186.
- Beydoun, A., Kutluay, E. (2002). Oxcarbazepine. *Expert Opin. Pharmacother.* 3: 59-71.
- Boerner, U., Roe, R.L. (1975). Letter: The formation of norcodeine from morphine in man. *J. Pharmacy Pharmacol.* 27: 215-216.
- Boulton, D.W., Arnaud, P., DeVane, C.L. (2001). Pharmacokinetics and pharmacodynamics of methadone enantiomers after a single oral dose of racemate. *Clin. Pharmacol. Ther.* 70:48-57.
- Braune, S., Schady, W. (1993). Changes in sensation after nerve injury or amputation: the role of central factors. *J. Neurol. Neurosurg. Psychia.* 56: 393-399.

- Brown, G.P., Yang, K., Ouerfelli, O., Standifer, K.M., Byrd, D., Pasternak, G.W. (1997). 3H-morphine-6beta-glucuronide binding in brain membranes and an MOR-1-transfected cell line. *J. Pharmacol. Exp. Ther.* 282: 1291-1297.
- Bulka, A., Plesan, A., Xu, X-J., Wiesenfeld-Hallin, Z. (2002). Reduced tolerance to the anti-hyperalgesic effect of methadone in comparison to morphine in a rat model of mononeuropathy. *Pain.* 95:103-109.
- Bullingham, R.E., McQuay, H.J., Moore, R.A. (1984). Studies on the peripheral action of opioids in postoperative pain in man. *Acta Anaesthesiol. Belgica.* 35: 285-290.
- Cameron, T., Prado, R., Watson, B.D., Gonzalez-Carvajal, M., Holets, V.R. (1990). Photochemically induced cystic lesion in the rat spinal cord. I. Behavioral and morphological analysis. *Exp Neurol.* 109: 214-223.
- Campbell, F.G., Graham, J.G., Zilka, K.J. (1966). Clinical trial of carbamazepine (tegretol) in trigeminal neuralgia. *J. Neurol. Neurosurg Psychiatr.* 29:265-267.
- Carpenter, K.J., Chapman, V., Dickenson, A.H. (2000). Neuronal inhibitory effects of methadone are predominantly opioid receptor mediated in the rat spinal cord in vivo. *Eur. J. Pain.* 4:19-26.
- Carstens, E., Gilly, H., Schreiber, H., Zimmermann, M. (1987). Effects of midbrain stimulation and iontophoretic application of serotonin, noradrenaline, morphine and GABA on electrical thresholds of afferent C- and A-fibre terminals in cat spinal cord. *Neuroscience* 21: 395-406.
- Carter, B.L., Tiffany, S.T. (1996). Cross-tolerance of associative and nonassociative morphine tolerance in the rat with mu and kappa-specific opioids. *Psychopharmacology* 123: 289-296
- Cesselin, F. (1995). Opioid and anti-opioid peptides. *Fund. Clin. Pharmacol.* 9:409-433.
- Christoph, T., Kogel, B., Schiene, K., Meen, M., DeVry, J., Friderichs, E. (2005). Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain. *Eur. J. Pharmacol.* 507: 87-98.
- Christrup, L.L. (1997). Morphine metabolites. *Acta Anaesthesiol. Scand.* 41: 116-122.
- Cliffer, K.D., Suiuciak, J.A., Carson, S.R. (1998). Physiological characterization of Taxol-induced large-fiber sensory neuropathy in the rat. *Ann Neurol.* 43:46-55.
- Coderre, T.J., Grimes, R.W., Melzack, R. (1986). Deafferentation and chronic pain in animals: an evaluation of evidence suggesting autotomy is related to pain. *Pain* 26: 61-84.
- Coggeshall, R.E., Willis, W.D. (1991). Sensory Mechanisms of the Spinal Cord. *Plenum Press, New York*
- Coggeshall, R.E., Willis, W.D., Cameron, A.A. (1992). Evidence that fine primary afferent axons innervate a wider territory in the superficial dorsal horn following peripheral axotomy. *Brain Res.* 575:151-154.
- Cone, E.J., Gorodetzky, C.W., Yousefnejad, D., Darwin, W.D. (1984). The metabolism and excretion of buprenorphine in humans. *Drug Metabol. Disposition* 12: 577-581.
- Cook, A.J., Woolf, C.J., Wall, P.D., McMahon, S.B. (1987). Dynamic receptive field plasticity in rat spinal cord dorsal horn following C-primary afferent input. *Nature* 325: 151-153.
- Crews, J.C., Sweeney, N.J., Denson, D.D. (1993). Clinical efficacy of methadone in patients refractory to other mu-opioid receptor agonist analgesics for management of terminal cancer pain. *Cancer.* 72: 2266-2272
- Dafny, N., Dong, W.Q., Prieto-Gomez, C., Reyes-Vazquez, C., Stanford, J., Qiao, J.T. (1996). Lateral hypothalamus: site involved in pain modulation. *Neuroscience* 70: 449-460.

- Dafters, R., Odber, J. (1989). Effects of dose, interdose interval, and drug-signal parameters on morphine analgesic tolerance for current theories of tolerance. *Behav. Neurosci.* 103:1082-1090
- Desmeules, M.P., Gascon, P., Dayer, M. Magistris. (1991). Impact of enviromental and genetic factors on codeine analgesia. *Eur. J. Clin. Pharmacol.* 41:23-26.
- Dickenson, A.H., Sullivan, A.F. (1987). Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 26: 1235-1238.
- Doubell, T.P., Baron, J., Skaliora, I., King, A.J. (2000). Topographical projection from the superior colliculus to the nucleus of the brachium of the inferior colliculus in the ferret: convergence of visual and auditory information. *Eur. J. Neurosci.* 12: 4290-4308.
- Ebert, B., Andersen, S., Krogsgaard-Larsen, P. (1995). Ketobemidone, methadone and pethidine are non-competitive N-methyl-D-aspartate (NMDA) antagonists in the rat cortex and spinal cord. *Neurosci. Lett.* 187:165-168.
- Eide, P.K. (2000). Wind-up and the NMDA receptor complex from a clinical perspective. *Eur. J. Pain.* 4:5-15.
- Ekblom, M., Gardmark, M. Hammarlund-Udenaes, M. (1993). Pharmacokinetics and pharmacodynamics of morphine-3-glucuronide in rats and its influence on the antinociceptive effect of morphine. *Biopharmaceu. Drug Disp.* 14: 1-11.
- Fainsinger, R., Schoeller, T., Bruera, E. (1993). Methadone in the management of cancer pain: a review. *Pain.* 52:137-147.
- Fields, H. (1994). Pain modulation and the action of analgesic medications. *Ann. Neurol.* 35: 42-45
- Gazelius, B., Cui, J.G., Svensson, M., Meyersson, B., Linderoth, B.(1996). Photochemically induced ischaemic lesion of the rat sciatic nerve. A novel method providing high incidence of mononeuropathy. *Neuroreport.*7:2619-2623.
- Gong, L., Middleton, R.K. (1992). Sublingua ladministration of opioids. *Ann. Pharmacother.* 26:1525-1527.
- Gourlay, GK. (1999). Different opioids-same action?. *In: Opioid Sensitivity of Chronic Noncancer Pain.* Kalso, E., McQuay., Wiesenfeld-Hallin. Z. (Eds), IASP Press, pp97-115
- Guirimand, F., Chauvin, M., Willer, J.C., LeBars, D. (1995). Buprenorphine blocks diffuse noxious inhibitory controls in the rat. *Eur. J. Pharmacol.* 294: 651-659.
- Hanks, G.W., Hoskin, P.J., Aherne, G.W., Turner, P., Poulain, P. (1987). Explanation for potency of repeated oral doses of morphine? *Lancet.* 2: 723-725.
- Hanna, MH., D'Costa, F., Peat, S.J., Fung, C., Venkat, N., Zilkha, T.R., Davies, S. (1993). Morphine-6-glucuronide disposition in renal impairment. *Br. J. Anaesth* 70: 511-514.
- Hao, J-X., Xu, X-J., Urban, L., Wiesenfeld-Hallin, Z. (2001). Repeated administration of gabapentin alleviates allodynia-like behaviors in spinally injured rats. *Neurosci. Lett.* 280: 211-214.
- He, H., Shay, S.D., Caraco, Y., Wood, A.J. (1998). Simultaneous determination of codeine and its seven metabolites in plasma and urine by high-performance liquid chromatography with ultraviolet and electrochemical detection. *J. Chromatogr. B. Biomed. Sci. Appl.*708:185-193
- Inturrisi, C.E., McLemore, G.L., Kest, B. (1997). The effects of LY293558, an AMPA receptor antagonist, on acute and chronic morphine dependence. *Brain Res.*778: 120-126.

- Jasinski, D.R., (1982). Progress report of the NIDA Addiction Research Center. *NIDA Res. Monograph. 41: 45-52.*
- Jasinski, D.R., Pevnick, J.S., Griffith, D. (1978). Human pharmacology and abuse potential of the analgesic buprenorphine: a potential agent for treating narcotic addiction. *Arch. Gen. Psychiat. 35: 501-516.*
- Jiang, M., Behbehani, M.M. (2001). Physiological characteristics of the projection pathway from the medial preoptic to the nucleus raphe magnus of the rat and its modulation by the periaqueductal gray. *Pain. 94:139-147.*
- Jones, T.L., Sweitzer, S.M., Wilson, S.P., Yeomans, D.C. (2003). Afferent fiber-selective shift in opiate potency following targeted opioid receptor knockdown. *Pain 106:365-371*
- Kauppila, T. (1998) Correlation between autotomy behavior and current theories of neuropathic pain. *Neurosci. Behav. Rev. 23: 111-129.*
- Kauppila, T., Xu, X-J., Yu, W., Wiesenfeld-Hallin, Z. (1998). Dextromethorphan potentiates the effect of morphine in rats with peripheral neuropathy. *NeuroReport 9: 1071-1074.*
- Kim, S.H., Chung, J.M. (1992). An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain 50: 355-363.*
- Koppert, W., Ihmsen, H., Korber, N., Wehrfritz, A., Sittl, R., Schmelz, M., Schuttler J. (2005). Different profiles of buprenorphine-induced analgesia and anti hyperalgesia in a human pain model. *Pain 118:15-22.*
- Kupers, R., Yu, W., Persson, J.K., Xu, X.J., Wiesenfeld-Hallin, Z.(1998). Photochemically-induced ischemia of the rat sciatic nerve produces a dose-dependent and highly reproducible mechanical, heat and cold allodynia, and signs of spontaneous pain. *Pain 76:45-59.*
- Le Bars, D. (2002) The whole body receptive field of dorsal horn multireceptive neurones. *Brain Res. Rev. 40: 29-44.*
- Leng, G., Finnegan, M.J. (1994). Successful use of methadone in nociceptive cancer pain unresponsive to morphine. *Palliat Med. 8:153-135*
- Likar, R., Sittl, R. (2005). Transdermal buprenorphine for treating nociceptive and neuropathic pain: four case studies. *Anesth Analg. 100:781-785*
- Mackin, G.A. (1997). Medical and pharmacologic management of upper extremity neuropathic pain syndromes. *J. Hand Ther. 10: 96-109.*
- Mao, J., Price, D.D., Mayer, D.J. (1995). Experimental mononeuropathy reduces the antinociceptive effects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain. *Pain 61: 353-364.*
- Mao, J., Sung, B., Ji, R.R., Lim, G. (2002). Chronic morphine induces downregulation of spinal glutamate transporters: implications in morphine tolerance and abnormal pain sensitivity. *J. Neurosci. 22: 8312-8323.*
- Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., Gilbert, P.E. (1976). The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther. 197: 517-532.*
- McCormack, K., Prather, P., Chapleo, C. (1998). Some new insights into the effects of opioids in phasic and tonic nociceptive tests. *Pain 78: 79-98.*
- McLaughlin, C.R., Dewey, W.L. (1994). A comparison of the antinociceptive effects of opioid agonists in neonatal and adult rats in phasic and tonic nociceptive tests. *Pharmacol. Biochem. Behav. 49: 1017-1023.*
- McQuay, H. (1999). Opioids in pain management. *Lancet. 353:2229-2232.*

- Melzack, R., Wall, P.D. (1965) Pain mechanisms: a new theory. *Science* 150: 917-919.
- Mendell, L.M. (1966). Physiological properties of unmyelinated fiber projection to the spinal cord. *Exp. Neurol.* 16: 316-332.
- Mendell, L.M., Wall, P.D. (1965). Responses of single dorsal cord cells to peripheral cutaneous unmyelinated fibres. *Nature.* 206: 97-99.
- Mercadante, S. (1997). Methadone in cancer pain. *Eur. J. Pain.* 1:77-83
- Merskey, H., Bogduk, N. (Eds.) (1994). Classification of Chronic Pain: Description of Chronic Pain Syndromes and Definition of Pain Terms. *Seattle, IASP Press*
- Millan, M.J. (1999). The induction of pain: an integrative review. *Prog. Neurobiol.* 57: 1-164.
- Mogil, J.S., Sternberg, W.F., Marek, P., Sadowski, B., Belknap, J.K., Liebeskind, J.C. (1996a). The genetics of pain and pain inhibition. *Proc. Natl. Acad. Sci. USA.* 93:3048-3055.
- Mogil, J.S., Kest, B., Sadowski, B., Belknap, J.K. (1996b). Differential genetic mediation of sensitivity to morphine in genetic models of opiate antinociception: influence of nociceptive assay. *J. Pharmacol. Exp. Ther.* 276:532-544.
- Mogil, J.S. (1999). The genetic mediation of individual differences insensitivity to pain and its inhibition. *Proc. Natl. Acad. Sci. USA.* 96: 7744-7751.
- Morley, J., Makin, M. (1998). The use of methadone in cancer pain poorly responsive to other opioids. *Pain Rev.* 5: 51-58
- Murphy, N.P., Lee, Y., Maidment, N.T. (1999). Orphanin FQ/nociceptin blocks acquisition of morphine place preference. *Brain Res.* 832: 168-170.
- Nestler, E.J. (1996). Under Siege: The brain on opiates. *Neuron.* 16: 897-900
- Ocana, M., Del Pozo, E., Barrios, M., Baeyens, J.M. (1995). Subgroups among mu-opioid receptor agonists distinguished by ATP-sensitive K⁺ channel-acting drugs. *Br. J. Pharmacol.* 114:1296-1302.
- Osborne, R., Thompson, P., Joel, S., Trew, D., Patel, N., Slevin, M. (1992). The analgesic activity of morphine-6-glucuronide. *Br. J. Clin. Pharmacol.* 34: 130-138.
- Osborne, R. (1988). Analgesic activity of morphine-6-glucuronide. *Lancet.* 1: 828.
- Ossipov, M.H., Lai, J., Malan Jr. P., Forreca, F. (1999). Opioid analgesic activity in neuropathic pain states. In: Opioid Sensitivity of Chronic Noncancer Pain. Kalso, E., McQuay., Wiesenfeld-Hallin. Z. (Eds), *IASP Press*, pp. 163-182
- Pasternak, G.W., Bodnar, R.J., Clark, J.A., Inturrisi, C.E. (1987). Morphine-6-glucuronide, a potent mu agonist. *Life Sci.* 41: 2845-2849.
- Persson, S., Post, C., Alari, L., Nyberg, F. (1989) Increased neuropeptide-converting enzyme activities in cerebrospinal fluid of opiate-tolerant rats.] *Neurosci. Lett.* 107: 318-322.
- Rowbotham, D.J., (1999). The debate over opioids and neuropathic pain. In: Opioid Sensitivity of Chronic Noncancer Pain. Kalso, E., McQuay., Wiesenfeld-Hallin. Z. (Eds), *IASP Press*, pp. 307-318.
- Rodin, B.E., Kruger, L. (1984). Deafferentation in animals as a model for the study of pain: an alternative hypothesis. *Brain Res.* 319: 213-228.
- Rothman, R.B., Xu, H., Wang, J.B. (1995). Ligand selectivity of cloned human and rat opioid mu receptors. *Synapse.* 21: 60-64
- Sawynok, J., Liu, X-J. (2003). Adenosine in the spinal cord and periphery: release and regulation of pain. *Prog. Neurobiol.* 69: 313-340.
- Schmidt, N., Brune, K., Geisslinger, G. (1994). Opioid receptor agonist potencies of morphine and morphine-6-glucuronide in the guinea-pig ileum. *Eur. J. Pharmacol.* 255: 245-247.

- Schomburg, E.D., Jankowska, E., Wiklund-Fernstrom, K. (2000). Nociceptive input to spinal interneurons in reflex pathways from group II muscle afferents in cats. *Neurosci. Res.* 38: 447-450.
- Schouenborg, J., Holmberg, H., Weng, H.R. (1992). Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. *Exp. Brain Res.* 90: 469-478.
- Schouenborg, J. (2002). Modular organisation and spinal somatosensory imprinting. *Brain Res. Rev.* 40: 80-91.
- Seltzer, Z., Dubner, R., Shir, Y. (1990). A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain.* 43: 205-518.
- Sherman, J.E., Proctor, C., Strub, H. (1982). Prior to hot plate exposure enhances morphine analgesia in tolerant and drug-naïve rats. *Pharmacol. Biochem. Behav.* 17:229-232
- Sherrington, C.S. (1906). The integrative Action of the Nervous System. *New York, Charles Scribner's Sons.*
- Shimomura, K., Kamata, O., Ueki, S., Ida, S., Oguri, K. (1971). Analgesic effect of morphine glucuronides. *Tohoku J. Exp. Med.* 105: 45-52.
- Siegel, S. (1976). Morphine analgesic tolerance: its situation specificity supports a Pavlovian conditioning model. *Science.* 193: 323-325
- Smith, M.T., Watt, J.A., Cramond, T. (1990). Morphine-3-glucuronide--a potent antagonist of morphine analgesia. *Life Sci.* 47: 579-585
- Sorkin, L.S., Xiao, W.H., Wagner, R., Myers, R.R. (1997). Tumour necrosis factor-alpha induces ectopic activity in nociceptive primary afferent fibres. *Neuroscience* 81:255-262.
- Sosnowski, M., Yaksh, T.L (1990). Differential cross-tolerance between intrathecal morphine and sufentanil in the rats. *Anesthesiology*73: 1141-1149
- Thompson, P.I., Bingham, S., Andrews, P.L., Patel, N., Joel, S.P., Slevin, M.L. (1992). Morphine 6-glucuronide: a metabolite of morphine with greater emetic potency than morphine in the ferret. *Br. J. Pharmacol.* 106: 3-8.
- Torebjörk, H.E., Lundberg, L.E., LaMotte, R.H. (1992). Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. *J. Physiol.* 448: 765-780.
- Triggle, D.J. (2003). Drug targets in the voltage-gated calcium channel family: Why some are and some are not? *Assay Drug Dev. Technol.* 1: 719-733.
- Uhl, G.R., Sora, I., Wang, Z. (1999). The μ opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses, *Proc. Natl. Acad. Sci. USA.* 96: 7752-7755
- Urch, C.E., Rahman, W., Dickenson, A.H. (2001). Electrophysiological studies on the role of the NMDA receptor in nociception in the developing rat spinal cord. *Dev. Brain Res.* 126: 81-89.
- Vos, B.P., Hans, G., Adriaensen, H. (1998). Behavioral assessment of facial pain in rats: face grooming patterns after painful and non-painful sensory disturbances in the territory of the rat's infraorbital nerve. *Pain* 76: 173-178.
- Wagner, E.J., Zhang, G., Lagrange, A.H., Ronnekleiv, O.K. (1997). Tolerance to mu-opioid receptor agonists but not cross-tolerance to gamma-aminobutyric acid(B) receptor agonists in arcuate A12 dopamine neurons with chronic morphine treatment. *J. Pharmacol Exp. Ther.* 280:1057-1064.
- Walker, E.A., Richardson, T.M., Young, A.M. (1997). Tolerance and cross-tolerance to morphine-like stimulus effects of mu opioids in rats. *Psychopharmacology* 133: 17-28

- Wall, P.D., Devor, M., Inbal, R., Scadding, J.W., Schonfeld, D., Seltzer, Z., Tomkiewicz, M. (1979). Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. *Pain*. 7: 103-113
- Wall, P.D., Woolf, C.J. (1984). Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. *J. Physiol.* 356: 443-458.
- Wang, Q., Tseng, L.F., Colling, K.A. (1995). Differential ontogenesis of thermal and mechanical antinociception induced by morphine and beta-endorphin. *Eur. J. Pharmacol.* 277: 71-76.
- Watkins, L.R., Hutchinson, M.R., Johnston, N.I., Maier, S.F. (2005). Glia: Counter-regulators of opioid analgesia. *Trends. Neurosci. In press*
- Watkins, L.R., Wiertelak, E.P., McGorry, M., Martinez, J., Schwartz, B., Sisk, D., Maier, S.F. (1998). Neurocircuitry of conditioned inhibition of analgesia: effects of amygdala, dorsal raphe, ventral medullary, and spinal cord lesions on antianalgesia in the rat. *Behav. Neurosci.* 112: 360-378.
- Welin, M., Harro, J., Yukhananov, R., Nyberg, F., Orelund, L. (1994) Cholecystokinin receptor binding in morphine analgesia: tolerance, withdrawal and abstinence. *Neuropeptides.* 26:379-83.
- Wheeler-Aceto, H., Cowan, A. (1991). Buprenorphine and morphine cause antinociception by different transduction mechanisms. *Eur. J. Pharmacol.* 195: 411-413.
- Wiesenfeld-Hallin, Z., Xu, X.-J. (1996). The role of cholecystokinin in nociception, neuropathic pain and opiate tolerance. *Regul. Rept.* 65: 23-28
- Willer, J.C. (1977). Comparative study of perceived pain and nociceptive flexor reflex in man. *Pain.* 3: 69-80
- Willis, W. D. (1985). Nociceptive pathways: anatomy and physiology of nociceptive ascending pathways. *Phi. Trans. Royal Soc. London - B: Biol. Sci.* 308: 253-270.
- Willis, W. D. Jr. (1991). Role of the forebrain in nociception. *Prog Brain Res.* 87:1-12.
- Wilson, S.G., Smith, S.B., Chesler, E.J., Melton, K.A., Haas, J.J., Mitton, B., Strasburg, K., Hubert, L., Rodriguez-Zas, S.L., Mogil, J.S. (2003). The heritability of antinociception: common pharmacogenetic mediation of five neurochemically distinct analgesics. *J. Pharmacol Exp. Ther.* 304: 547-559.
- Woolf, C.J., Thompson, S.W. (1992). The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain.* 44: 293-299.
- Workman, B.J., Lumb, B.M. (1997). Inhibitory effects evoked from the anterior hypothalamus are selective for the nociceptive responses of dorsal horn neurons with high- and low-threshold inputs. *J. Neurophysiol.* 77: 2831-2835.
- Wu, W-P., Xu, X-J., Hao, J-X. (2004). Chronic lumbar catheterization of the spinal subarachnoid space in mice. *J. Neurosci. Meth.* 133:65-69
- Xu, X-J., Hao, J-X., Aldskogius, H., Seiger, A., Wiesenfeld-Hallin, Z. (1992). Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury. *Pain* 48: 279-290.
- Young, G.A., Khazan, N. (1984). Differential tolerance and cross-tolerance to repeated daily injections of mu and kappa opioid agonists in rat. *Neuropharmacology* 23: 505-509
- Yu, W., Hao, J-X., Xu, X-J., Wiesenfeld-Hallin, Z. (1997). Comparison of antiallodynic and antinociceptive effects of systemic, intrathecal and intracerebroventricular morphine in a rat model of central neuropathic pain. *Eur. J. Pain* 1: 17-29

- Zhang, X., Bao, L., Shi, T.J., Ju, G., Elde, R., Hökfelt, T. (1998). Dow-regulation of mu-opioid receptors in rat and monkey dorsal root ganglion neurons and spinal cord after peripheral axotomy. *Neuroscience* 82: 223-240.
- Zimmermann, M. (2005). The history of pain concepts and treatment before IASP. In: Merskey, H., Loeser, J.D., Dubner R. (eds). *The Paths of Pain*. IASP Press., Seattle, 2005, pp. 1-22.