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The Analgesic Profiles of Opioids in Human Experimental Pain Models

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The present thesis is partly based on four studies, which are referred to in the text by Roman numerals. The studies have been carried out in the period from 2005-2009 at Mech-Sense, Department of Gastroenterology, Aalborg Hospital in collaboration with Centre for Sensory-Motor Interactions (SMI), Aalborg University.

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Aalborg, Denmark, 8th of July, 2009

Anne Estrup Olesen

Abbreviations

AMPA: α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate

AUC: area under the curve

CGRP: calcitonin gene-related peptide

CNS: central nervous system

EEG: electroencephalograph

GABA: gamma-aminobutyric acid

M3G: morphine-3-glucuronide

M6G: morphine-6-glucuronide

NK1: neurokinin 1

NMDA: N-methyl-D-aspartic acid

PAG: periaqueductal grey

PB: parabrachial area

PDT: pain detection threshold

PK-PD: pharmacokinetic-pharmacodynamic

PTT: pain tolerance threshold

RVM: rostral ventromedial medulla

SP: substance P

TRPV1: transient receptor potential vanilloid type 1

VAS: visual analogue scale

VLM: ventrolateral medulla

WDR: wide dynamic range

1. Introduction

Chronic pain of moderate to severe intensity occurs in 19% of adult Europeans, seriously affecting the quality of their social and working lives. Very few are managed by pain specialists and nearly half receives inadequate pain management (Breivik et al. 2006). Therefore, further research in and focus on pain physiology and treatment are warranted.

1.1 Clinical versus experimental pain

Studies of clinical pain are limited by bias due to e.g. cognitive, emotional and social aspects of the disease as illustrated in Figure 1. Therefore, pain is a multi-dimensional, highly individual perception, difficult to quantify and validate in clinical settings. The great individual differences and concomitant symptoms that especially follow visceral pain make it difficult to study basic pain manifestations or the effect of pharmacological treatment. Thus, the effect on e.g., nausea and anxiety may be difficult to distinguish from pain relief in the clinical settings. When applying experimental pain in healthy volunteers some of these bias are overcome and the quality and intensity of painful stimuli can be reported reproducibly (Stahl et al. 2006b). Therefore, experimental human pain models appear to be better suited to test analgesic compounds, but also to study pain mechanisms.

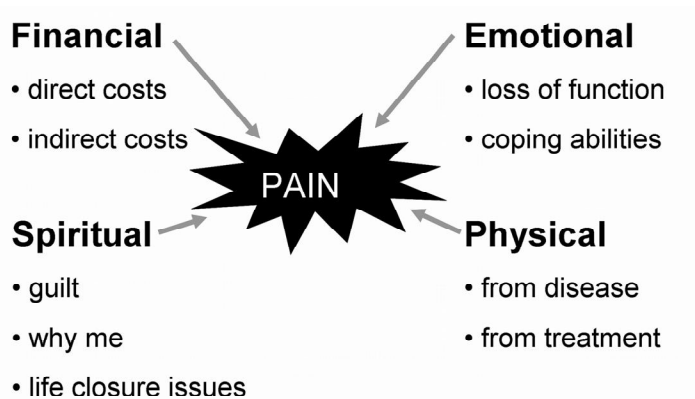


Figure 1. A schematic overview of factors that can bias the outcome in clinical pain studies. Pain is a multi-dimensional experience affected not only by physical but also financial, spiritual and emotional factors.

In standardized experimental pain studies it is possible to control precisely the localisation, intensity, duration and modality of the stimulus (Figure 2) (Drewes, Gregersen, & Arendt-Nielsen 2003). The evoked sensations can be assessed with subjective methods quantitatively (e.g. by using

a visual analogue scale) and qualitatively (e.g. by using the McGill Pain Questionnaire), and stimulus-response relationships can be investigated. Objective, physiological responses for the pain can be recorded with e.g., the nociceptive reflex and cerebral evoked potentials.

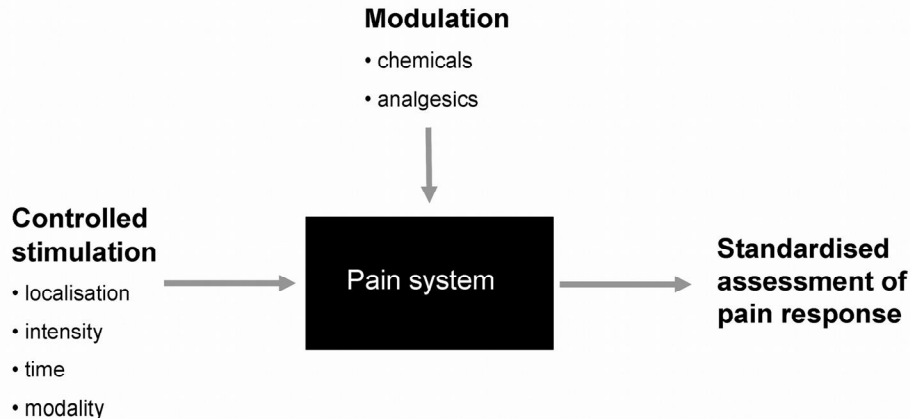


Figure 2. A schematic overview of an experimental pain model. Illustration of the context in experimental pain. The pain system can be considered a black box between the stimulation (inputs) and the pain response (outputs). In standardized experimental pain studies it is possible to control precisely the localisation, intensity, duration and modality of the stimulus. It is possible to modulate the pain system by e.g. chemicals or analgesics and most important it offers the possibility to assess a standardized pain response.

The disadvantages of experimental models are the short lasting acute stimuli and hence the limited psychological involvement. The experimental stimuli may therefore not mimic clinical pain sufficiently as pain experienced and reported by healthy volunteers is different from clinical pain (Arendt-Nielsen, Curatolo, & Drewes 2007). Especially, single-modality experimental pain stimuli may be inadequate in pharmacological studies. The most reliable experimental test situations are probably established by multi-modal testing using different experimental pain stimuli in the same setup. For visceral stimulations the different stimuli activate superficial and deeper layers of the gut as illustrated in Figure 3. Hereby, the complex sensory experiences present in painful visceral diseases can be mimicked. Moreover, sensitization, a hallmark of clinical pain, can also be evoked experimentally in healthy volunteers. It can be applied to different tissues such as skin where capsaicin has been used for inducing sensitization (Dirks et al. 2002; Poyhia & Vainio 2006) and viscera where both capsaicin (Hammer & Vogelsang 2007), and acid or the combination has been used (II) (Brock et al. 2009; Drewes et al. 2003a; Willert et al. 2007). Peripheral inflammatory conditions can be induced experimentally with sensitization patterns correlating to clinical inflammatory pain (Schmelz 2009). Hereby, it is possible to mimic pathological conditions such as allodynia (pain produced by normally innocuous stimuli) and hyperalgesia (increased response to

normally painful stimuli). Experimental pain models of hyperalgesia are more difficult to control than simple and short lasting pain stimuli, but may initiate some of the inflammatory processes and changes in the pain system that are seen in the clinic (Frokjaer et al. 2005;Sami et al. 2006). For example sensitization of the oesophagus can evoke generalized hyperalgesia in remote organs and in the skin (Brock et al. 2009;Frokjaer et al. 2005;Sarkar et al. 2006;Willert et al. 2007).

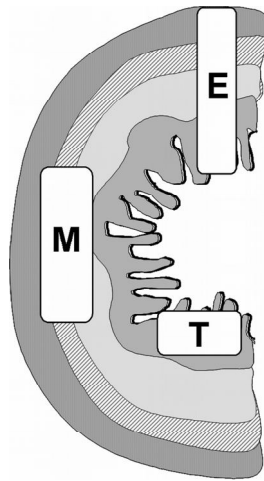


Figure 3. Schematic illustration of the proposed gut layers, which are preferentially affected with: (T) Thermal stimuli (mucosa and submucosa, dark grey); (M) Mechanical stimuli (circular and longitudinal muscle – light and hatched grey); (E) Electrical stimuli (all layers depending on stimulus intensity).

1.2 Opioids

Opioids have been used for thousands of years for the treatment of pain. Opioid is the term used broadly to describe all compounds that work at the opioid receptors. Most of the most common opioids are agonists, and create their effect by stimulating the opioid receptors. Differences in activity and efficacy appear to be related to the relative stimulation of the various opioid receptors (μ , κ , δ etc.) as well as genetic differences in opioid receptor sensitivity (Trescot et al. 2008). The majorities of the clinically relevant opioids have their primary activity at the initial “morphine receptor” or “ μ -receptors” and are therefore considered “ μ -agonists” (Trescot et al. 2008). Opioid analgesics are used in moderate to severe pain of both benign and malign origin. Opioids are strong analgesics and very effective in many different types of pain. The degree of analgesia is limited by development of tolerance or side effects as these factors might complicate the clinical use of opioids. Opioid treatment causes a number of side effects as constipation, sedation and respiratory depression that may also indirectly interfere with their effects in the clinic (Mather & Smith 1999).

The main therapeutic effects of commonly used opioids are mediated by opioid receptors, which are located predominantly in central nervous system (CNS) (Trescot et al. 2008). However, peripheral opioid receptors have also been demonstrated (Labuz et al. 2007;Stein 1993;Trescot et al. 2008). Several clinical investigations now focus on the development of new peripheral opioid agonists, unable to penetrate the blood brain barrier, in order to induce effective peripheral analgesia with reduced central side effects typically associated with opioids (I) (Janson & Stein 2003).

1.3 Hyperalgesia

Chronic pain is often associated with inflammation and hyperalgesia which can lead to changes in the pain systems (Anand et al. 2007;Curatolo, Arendt-Nielsen, & Petersen-Felix 2006). Two types of hyperalgesia exist: 1) primary hyperalgesia which is mediated by peripheral mechanisms and 2) secondary hyperalgesia which is related to central sensitization (Coderre et al. 1993). Referred pain and hyperalgesia are often reported in the muscle and skin within the same spinal cord dermatome as injured organs. However, evidence that referred pain is also in part dependent on CNS changes is provided by findings that referred pain and hyperalgesia spread to areas which do not share the same dermatome (Coderre et al. 1993).

During inflammatory processes, opioid receptors are transported from dorsal root ganglia towards the peripheral sensory nerve endings. At the same time, immune cells containing endogenous opioid peptides accumulate within the inflamed tissue (Janson & Stein 2003). Altered expression of specific opioid receptors have been reported in inflammatory states (Pol, Alameda, & Puig 2001;Pol, Palacio, & Puig 2003;Sengupta et al. 1999;Stein 1993). Therefore, opioid induced peripheral effects are particularly prominent under painful inflammatory conditions, both in animals and in humans. Animal studies have shown that opioid-binding to κ -receptor sites are increased in inflammation compared to binding to the μ -receptor which could be caused by a more pronounced up-regulation of κ -receptors (Iadarola et al. 1988;Sengupta et al. 1999). Hyperalgesia, often do not respond adequately to treatment with classical μ -opioid agonists (De Schepper et al. 2004;Stein, Schafer, & Machelska 2003). Therefore, opioids with different pharmacological profiles such as those working on the κ -receptor may be advantageous in the treatment of pain conditions with hyperalgesia (III) (De Schepper et al. 2004;Gallantine & Meert 2008;Stein, Schafer, & Machelska 2003).

1.4 Hypothesis

Peripheral κ -opioid receptors in the gut have been suggested as an important feature of the visceral pain system (De Schepper et al. 2004) and a possible target for attenuating peripheral nociception (Burton & Gebhart 1998; Riviere 2004). In addition, because of the absence of respiratory depression, constipation, and abuse liability, peripherally selective κ -opioid agonists should be safer and better tolerated than classical μ -opioid agonists (Riviere 2004; Stein 1993). It has been shown that κ -agonists may be effective in the treatment of visceral pain (Eisenach, Carpenter, & Curry 2003). CR665, is a tetrapeptide agonist at the κ -opioid receptor, substantially excluded by the blood-brain barrier, with essentially no activity at other opioid receptor subtypes (Binder et al. 2001; Vanderah et al. 2008). Therefore, it was *hypothesized* that CR665 could attenuate visceral pain in humans.

Opioids may have different pharmacological profiles in the treatment of visceral pain. For example, oxycodone and morphine have different pharmacological profiles (Riley et al. 2008). Morphine binds to the μ -opioid receptor with stronger affinity than oxycodone and rodent experiments have indicated that oxycodone is a partial κ -agonist (Holtman & Wala 2006; Nielsen et al. 2007; Nozaki et al. 2005; Nozaki, Saitoh, & Kamei 2006; Ross & Smith 1997) and therefore, could be more effective in treatment of visceral pain. Analgesic effects of morphine and oxycodone were previously investigated in experimental pain in healthy volunteers, where they were equipotent in modulation of skin and muscle pain, but oxycodone showed better effect on visceral pain (Staahl et al. 2006a). This demonstrated a distinct pharmacological profile of the two opioids and a better analgesic profile of oxycodone in visceral pain. This emphasized that important differences exist between the pain physiology in acute pain from different tissues and great care should be taken when using data from pain stimulation in the skin to predict drug effects on pain in deeper tissues. This led to the *hypothesis* that the effect of a peripheral restricted κ -agonist on visceral pain could be demonstrated in a multi-modal and multi-tissue experimental pain model.

Moreover, inflammation could affect the opioid system and hereby the pharmacological profiles of different opioids. Chronic pancreatitis is characterized by long lasting inflammation, hyperalgesia and pain (Drewes et al. 2008) and subsequently an experimental pain study was performed in patient with chronic pancreatitis to investigate differentiated effects of morphine and oxycodone. Oxycodone was more effective than morphine attenuating experimental skin, muscle and visceral pain in patients (Staahl et al. 2007), indicating a more pronounced differential pharmacological profile when inflammation was present. This led to the *hypothesis* that a

translational experimental pain model including hyperalgesia could bridge findings from studies in healthy volunteers to patients.

As changes in the pain system and the opioid system occurs after sensitization it is interesting to investigate the effect of opioids in a controlled experimental pain study of hyperalgesia. Analgesic testing in experimental pain models has so far been done almost exclusively in acute pain, thus more research on anti-hyperalgesic effects of opioids are warranted. It is crucial to apply several pain modalities to ensure that many pain mechanisms are activated to further mimic of the clinical pain situation. It was *hypothesized* that perfusion of the human oesophagus with acid and capsaicin could induce generalized sensitization and hyperalgesia and that differentiated effects of morphine and oxycodone could be demonstrated in this model.

Furthermore, modelling pharmacokinetic-pharmacodynamic (PK-PD) can reveal further questions on the analgesic effect. It was *hypothesized* that modelling PK-PD of oxycodone could reveal the contribution of peripheral analgesia in hyperalgesia. Therefore, the experimental pain studies should be followed by PK-PD analysis.

1.5 Aims

The overall objectives of this project were to test the effect a peripheral restricted κ -agonist and compare it to the effect of oxycodone and placebo in a multi-modal and multi-tissue experimental pain model (study I), apply an experimental pain model of visceral hyperalgesia (study II) to test the effect of the two opioids morphine and oxycodone (study III) and further to evaluate the PK-PD relationship for oxycodone in this experimental pain model of visceral hyperalgesia (study IV).

The aims were:

1. To assess the effects of a peripherally-selective tetrapeptide κ -opioid receptor agonist, CR665, on experimental pain from multi-modal stimulation of skin, muscle, and viscera, and to compare these effects with those of oxycodone (mainly a centrally-acting opioid).
2. To develop and explore an experimental pain model of visceral and generalized hyperalgesia
3. To investigate whether morphine and oxycodone – compared to placebo – had a differentiated effect after experimentally induced hyperalgesia
4. To evaluate the PK-PD relationship for oxycodone in the model of experimental hyperalgesia

2. The pain system

The International Association for the study of Pain has defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Pain involves a psychological component which can alter its perception and therefore, undergo extensive processing through the nervous system, and particularly the brain. Therefore, pain is considered a perception and not a sensation.

2.1 The primary afferent

Nociception begins in the periphery, where the peripheral terminals of sensory fibres (primary afferents) respond to a myriad of stimuli and translate this information into the dorsal horn of the spinal cord, where the central ends of these fibres terminate (D'Mello & Dickenson 2008). There are three main types of sensory fibres in the peripheral nervous system: 1) A β -fibres, 2) A δ -fibres and 3) C-fibres. A β -fibres are large in diameter, highly myelinated, allowing them to quickly conduct action potentials from their peripheral to central terminals. These fibres include touch receptors that are activated by low pressure giving innocuous signals whereas A δ - and C-fibres are nociceptors or “pain-fibres” that respond to noxious stimuli which may be mechanical, thermal or chemical (Julius & Basbaum 2001). A δ -fibres are small in diameter and thinly myelinated fibres and transmit a stimulus slower than A β -fibres but much faster than the large non-myelinated C-fibres. Activation of nociceptors results in a graded receptor potential, giving noxious signals that reflect the stimulus intensity. There are sensory fibres in viscera, skin and joints that do not respond to high intensity stimulation under normal circumstances. However, the sensory fibres become spontaneously active and often also respond to innocuous mechanical stimuli after the tissues are irritated or inflamed by exogenously applied irritants or inflammatory substances (Sengupta & Gebhart 1994). These fibres have been termed “silent nociceptors”.

2.1.1 Cutaneous nociceptors

Several types of nociceptors exist in the human skin. A δ -fibres are responsible for conducting the “first pain”, which is the characteristic sharp pain felt immediately after noxious stimuli. In the skin A δ -fibres are mainly specialized for detection of dangerous mechanical and thermal stresses and for triggering a rapid nociceptive response and protective reflexes (Byers & Bonica 2001). C-fibres are responsible for conducting the “second pain” which is the dull, burning sensation felt after the “first

pain”. C-fibres are mainly polymodal but some C-fibres are specialized for detecting a single stimulus as heat or pinch (Craig 2003). C-fibres detect strong mechanical, thermal and chemical stimulation.

2.1.2 Muscular nociceptors

In muscle innervations thick myelinated afferents terminate as organized endings (muscle spindles, tendon organs); whereas A δ and C fibres terminate as free nerve endings. Most of these endings are located in the wall of arterioles in the muscle belly and in the surrounding tissues (Schaible 2006). The peripheral apparatus of muscle pain consists of nociceptors that can be excited by endogenous substances and mechanical stimuli. The nociceptors are free nerve endings supplied by thin myelinated and nonmyelinated afferents with conduction velocities less than 30 m/s. At the molecular level, nociceptors have receptors for algescic substances, such as bradykinin, serotonin, and prostaglandin E₂ (Graven-Nielsen & Mense 2001). Numerous sensory A δ - and C-fibres in muscle are only activated by noxious mechanical or chemical stimuli. Approximately 80% of the C-fibres are polymodal and possess a higher threshold to mechanical stimuli than A-fibres. The C-fibres adapt slowly to stimuli and only a few responds to stretch (Byers & Bonica 2001). Muscle pain is often aching and cramping and difficult to localize, and it is often referred to other deep tissue such as other muscles, tendon, fascia, joint and ligaments. In contrast to cutaneous pain, muscle pain typically elicits a drop in blood pressure as well as sweating and nausea, suggesting differences in the processing of nociceptive inputs from skin and deep tissue (Schaible 2006).

2.1.3 Visceral nociceptors

All of the abdominal and thoracic viscera have afferent innervation associated with parasympathetic of sympathetic nerves, participating in reflex control of cardiopulmonary, gastrointestinal and genitourinary functions, by conveying information from the viscera to the central nervous system. Most of such information is not perceived and does not reach consciousness (e.g., responses to intraluminal nutrients, baroreceptor input, lung inflation, normal gastrointestinal motility, and so forth) (Gebhart 2000a). The terminals (receptors) of primary visceral afferent neurons are located in mucosa, muscle, and serosa (mesentery) of hollow organs. Accordingly, visceral afferent neuron terminals are placed to respond to luminal and local chemical stimuli and to mechanical (usually distending) stimuli (Gebhart 2000a). The visceral afferent innervation is sparse relative to somatic innervations. In contrast with afferent fibres arising from somatic structures, the number of spinal

visceral afferent fibres is estimated to be less than 10% of the total spinal afferent input from all sources.

Sensations from the gastrointestinal tract are mediated through free nerve endings of small myelinated A δ and unmyelinated C fibres (Gebhart 2000b). The proportion of unmyelinated fibres increases from the oral to anal end of the gastrointestinal tract (Sengupta & Gebhart 1994). Visceral pain is unique in that there are no first (fast) and second (slow) components of pain; instead, pain is often poorly localized, deep and dull (Julius & Basbaum 2001). Sensory innervations of the gastrointestinal tract involves all layers of a viscous (mucosa, muscle, and serosa) and visceral nociceptors are generally polymodal and exhibit chemosensitivity, thermosensitivity, and mechanosensitivity (Sengupta & Gebhart 1994). Electrophysiological studies of visceral afferents have shown that most of the fibres encode stimuli in both the noxious and innocuous range. About 25-37% of mechanoreceptors can be classed as high-threshold mechanoreceptors, which only encode stimuli in the noxious range (Gebhart 2000a; Sengupta & Gebhart 1994). Different receptor classes have also been demonstrated in humans (Drewes et al. 2005a). Both A δ - and C-fibres are susceptible to sensitization, but in contrast to somatic tissue both low – and high threshold receptors sensitize (Craig 2003),

2.2 The spinal neuron

The spinal cord is the first relay in the pain pathways from the periphery to the brain. The dorsal horn of the spinal cord is the major receiving zone for primary afferent axons that transmit information from sensory receptors in the skin, viscera, joints and muscle of the trunk and limbs to the nervous system. Primary afferents release a variety of chemical mediators, but all appear to use glutamate as their principal neurotransmitter, and on entering the dorsal horn they form excitatory synapses with the secondary neurons (Todd & Koerber 2006). Nociceptive afferents mainly terminate in laminae I and II of the dorsal horn; whereas, A β -fibres predominantly innervate the deeper laminae III-VI (D'Mello & Dickenson 2008). The termination of visceral afferents on neurons in laminae I, II, V and X is spread over several segments and can include the contralateral spinal cord (Gebhart 2000b).

When proposing the “Gate control theory” Melzack and Wall (1965) suggested that inhibitory interneurons located in the superficial part of the dorsal horn played a crucial role in controlling incoming sensory information before it was transmitted to the brain through ascending pathways (Melzack & Wall 1965). This theory thus explains how stimulus that activates only nonnociceptive

nerves can inhibit pain. The pain seems to be lessened when the injured area is rubbed because activation of nonnociceptive fibres inhibits the firing of nociceptive fibres in the laminae. In transcutaneous electrical stimulation, this mechanism can be used where nonnociceptive fibers are selectively stimulated with electrodes and thereby decrease pain. Later, Wall demonstrated that structures in the brain stem tonically inhibit nociresponsive neurons in the spinal cord (Wall 1967). Moreover, stimulation produced analgesia has demonstrated that the descending systems can selectively modulate pain at spinal level (Mayer & Price 1976).

The spinal cord contains various neuronal cell types. Some cells are termed nociceptive-specific (NS) cells and are mostly found superficially and synapse with A δ and C-fibres only. These cells fire action potentials when a painful stimulus is detected at the periphery. Cells which receive input only from A β -fibres are proprioceptive and only respond to touch (D'Mello & Dickenson 2008). Other neurones in the spinal cord are termed wide dynamic range (WDR) and receive input from all three types of sensory fibre, and therefore respond to the full range of stimulation. WDRs fire action potentials in a graded fashion depending on stimulus intensity and also exhibit wind-up, a short lasting form of synaptic plasticity. Repetitive, strong or long-lasting painful stimulation produce wind-up of WDR neurones, whereby increase of their evoked response and post-discharge is induced (D'Mello & Dickenson 2008). Excitatory aminoacids like glutamate and neuropeptides like substance P (SP) and calcitonin gene-related peptide (CGRP), all mediate nociceptive signalling in the spinal cord. They are released from primary afferents and activate various receptors. The most important receptors are α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors, but amongst others also the kinate receptors have a function when nociceptive signals are conveyed (Dickenson 1995). A simplified overview of the pharmacological mechanisms is given in Figure 4.

Interneurons within the spinal cord can be divided in two classes. Inhibitory interneurons, which use gamma-aminobutyric acid (GABA) and/or glycine as a transmitter and excitatory interneurons which are glutamateric (D'Mello & Dickenson 2008). These interneurons can decrease or increase the response of nociceptive specific cells and WDRs, thus influencing the output of the dorsal horn. A simplified overview of this dual modulation of nociceptive processing in the dorsal horn is illustrated in Figure 5. Serotonergic input to the dorsal horn as illustrated in Figure 5 is one example of control of the activity of descending pathways. Noradrenergic and dopaminergic mechanisms will influence other descending pathways by dual modulation as well leading to both inhibition and facilitation (Millan 2002). Glutamate exerts an excitatory effect on a number of receptors found on

post-synaptic spinal neurones, leading to membrane depolarization via three distinct receptor subclasses: AMPA, NMDA and the G-protein coupled metabotropic family of receptors (D'Mello & Dickenson 2008).

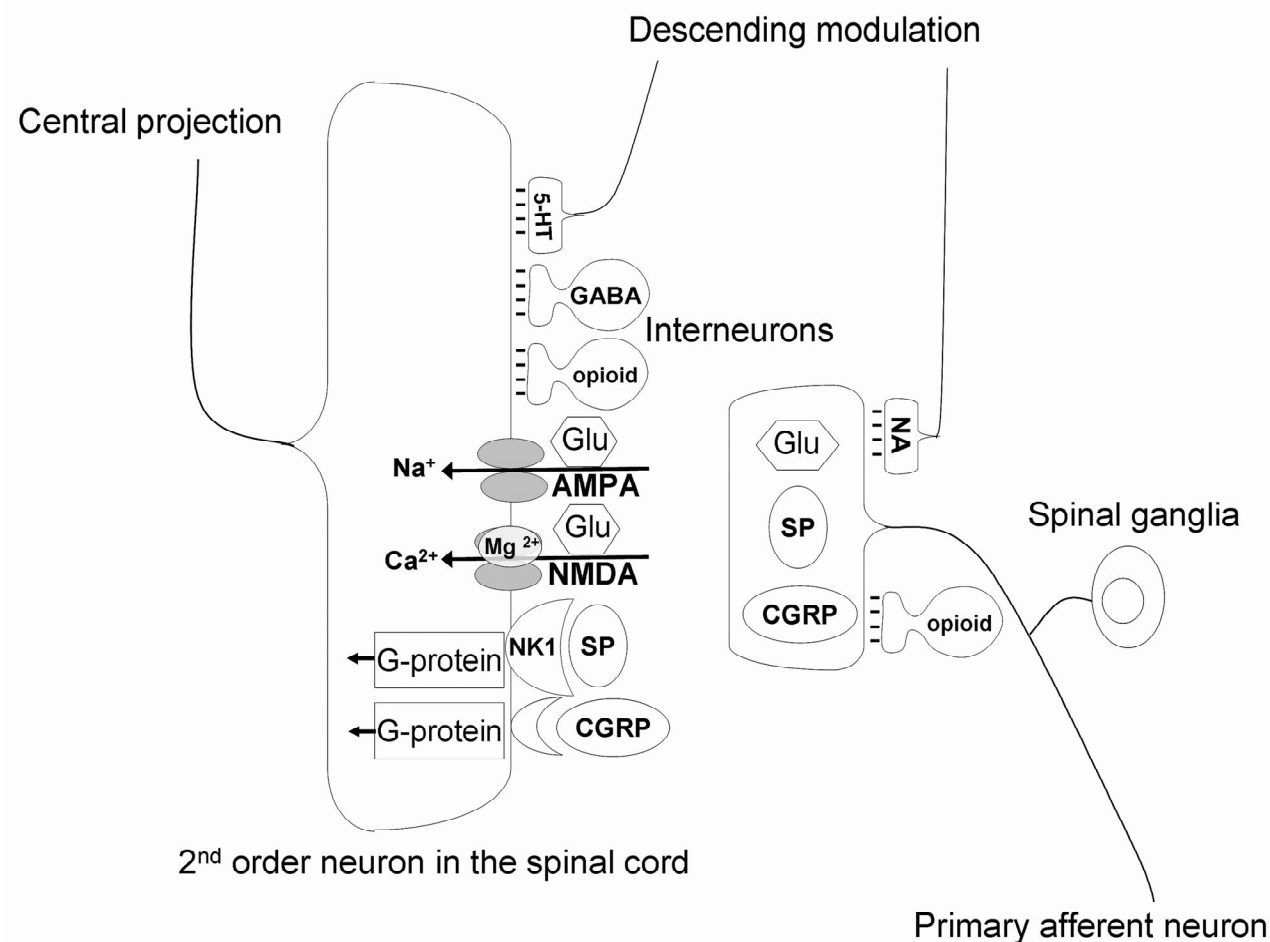


Figure 4. Illustration of the most important mediators in the spinal dorsal horn synapse. Left side of the figure shows the post-synaptic terminal, right side is the presynaptic terminal. The presynaptic terminal release the excitatory amino acid glutamate (Glu) and neuropeptides as substance P (SP) and calcitonin gene related peptide (CGRP). These substances work on receptors in the postsynaptic terminal causing ion influx and activation of various cascades. SP activates the neurokinin-1 (NK1) receptor. Glutamate is released from sensory afferents in response to acute and more persistent noxious stimuli and it is fast α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor activation that is responsible for setting the initial baseline response of spinal dorsal horn neurones to both noxious and tactile stimuli. However, if a repetitive and high-frequency stimulation of C-fibres occurs, there is an amplification and prolongation of the response of spinal dorsal horn neurones to subsequent inputs, wind-up. This enhanced activity result from activation of the N-methyl-D-aspartic acid (NMDA) receptor. In acute or low-frequency inputs to the spinal cord, activation of the NMDA receptor is not possible since under normal conditions the ion channel of this receptor is blocked by the normal level of magnesium ions found in nervous tissues. This magnesium plug of the channel requires a sustained depolarization of the membrane in order to be removed and allow the NMDA receptor channel to be activated and opened. Here it is likely that the co-release of neuropeptides as substance P (SP) and CGRP is responsible for a prolonged slow depolarization on the neurone and subsequent removal of the NMDA block. These events all mediate nociceptive signalling to supraspinal sites. Descending modulation function via the noradrenergic (NA) and serotonergic (5-HT) pathways, pre- and postsynaptically. The descending modulation by NA and 5-HT is mainly inhibitory. However, it can be facilitatory as well depending on the receptor subunit classes. Interneurons secreting gamma-aminobutyric acid (GABA) and endogenous opioid peptides modulate nociceptive transmission in the dorsal horn.

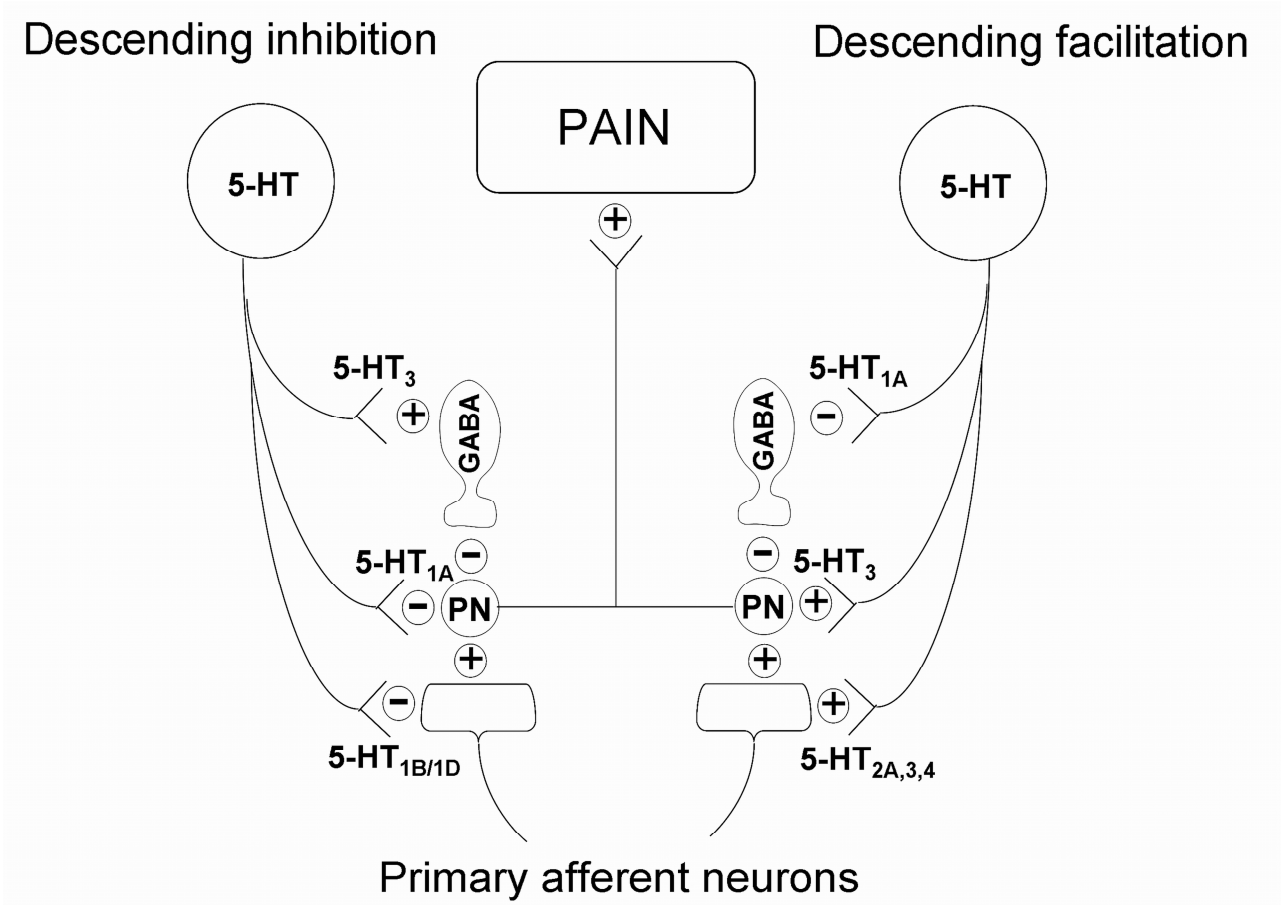


Figure 5. Significance of different classes of serotonin (5-HT) receptors and of their divergent neuronal localisation to the modulation of nociceptive processing in the dorsal horn. On the left side, mechanisms mediating descending inhibition are indicated and on the right side, those expressing descending facilitation are shown. Actions are exerted at terminals of primary afferent neurons, projection neurons (PN) and inhibitory interneurons with gamma-aminobutyric acid (GABA) as transmitter. Note that 5-HT_{1A} and 5-HT_{1B/1D} receptors suppress neuronal activity, whereas cellular excitability is enhanced by 5-HT₂, 5-HT₃ and 5-HT₄ receptors.

2.3 The supraspinal level

The output from the dorsal horn to higher centres in the brain is carried by spinal projection neurones along ascending pathways. For example, neurokinin 1 (NK1) neurons in laminae I respond to noxious stimulation and have been shown to project to areas in the brain such as thalamus, the periaqueductal grey (PAG) and in particular the parabrachial area (PB). These neurons also project into brainstem areas such as the rostral ventromedial medulla (RVM) (D'Mello & Dickenson 2008). A large number of projection neurons are also found in lamina III-VI and these projects predominantly to the thalamus. As illustrated in Figure 6 several structures in the brain

make up the “pain matrix”. The pain-modulating circuit can facilitate as well as inhibit nociceptive transmission (Fields 2004). This dual control results from the activity of more neuronal subpopulations. Two functional important classes of neurones are present in PAG, RVM and ventrolateral medulla (VLM) (Heinricher et al. 2009). One class is OFF-cells that show a pause in firing after pain stimulation causing decreased pain inhibition. The other class: ON-cells, shows a burst of activity after pain stimulation causing increased pain activation (Fields 2004). Consistent with their role in pain modulation RVM on- and off-cell axons project directly and selectively to dorsal horn laminae that relay nociceptive signals.

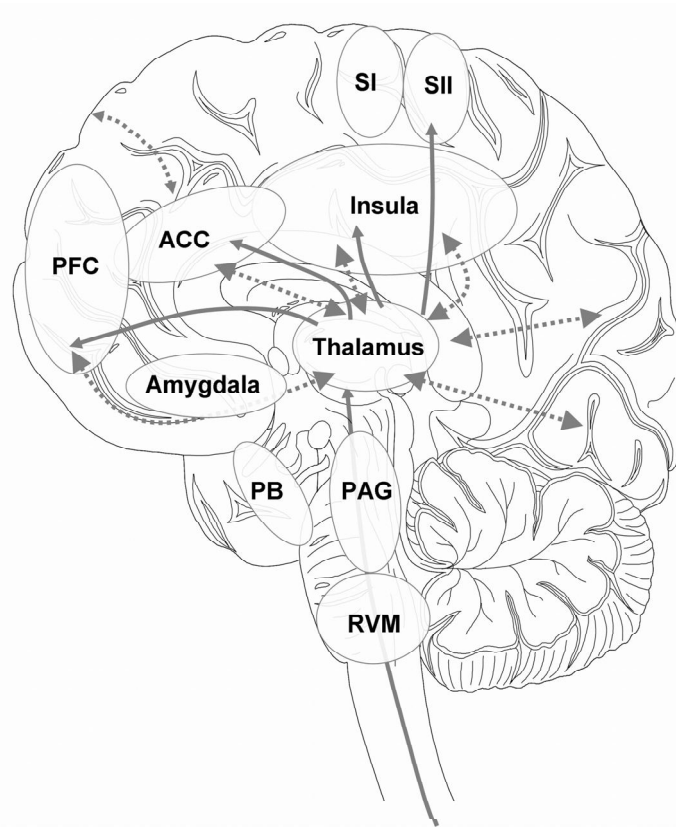


Figure 6. Cortical and sub-cortical regions involved in pain perception, their interconnectivity and ascending pathways. Not a single but various regions in the brain make the “pain matrix”; primary and secondary somatosensory cortices (SI and SII), prefrontal cortex (PFC), anterior cingulate cortex (ACC), Insula, thalamus, amygdala, parabrachial area (PB), the periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM).

2.4 Sensitization

Sensitization of the pain system can be presented as allodynia or hyperalgesia. Allodynia is a painful response to a usually non-painful stimulus whereas hyperalgesia is an extreme and more painful reaction to a stimulus which is normally painful. Sensitization can result from either lowering of nociceptor activation thresholds (peripheral sensitization) or increased responsiveness of spinal cord pain transmission neurons (central sensitization).

2.4.1 Peripheral sensitization

Peripheral sensitization is produced following mild injury, and this is a factor in producing primary hyperalgesia (Byers & Bonica 2001). Injury results in local release of numerous chemicals as for example histamine, prostaglandins, serotonin, protons and substance P, which mediate or facilitate the inflammatory process. Some chemicals can directly activate nociceptors, while others act indirectly via inflammatory cells. These inflammatory mediators reduce the transduction threshold of primary afferents and recruit previously silent nociceptors. Some mediators lead to a sensitization of the nociceptor response to natural stimuli and play a role in primary hyperalgesia (Meyer et al. 2005). Peripheral injury can produce central changes which are maintained even after the inputs from the injury are removed (Coderre et al. 1993). Therefore, once hyperalgesia is established, it does not need to be maintained by inputs from the injured peripheral tissue.

2.4.2 Central sensitization

Clinically, central sensitization has been demonstrated to contribute to pain hypersensitivity in the skin, muscle, joints and viscera (Anand et al. 2007). Deep pain in particular visceral pain is more potent than cutaneous pain inducing central sensitization.

With central sensitization, pain can be produced by activity in non-nociceptive primary sensory fibres. (Julius & Basbaum 2001). The role of brainstem structures, like RVM, in sensitization is illustrated in a study by Fields et al. Here prolonged nociceptor inputs including thermal and chemical stimulation, inflammation or nerve injury produced a state of generalized hyperalgesia that was reversed by lesions or reversible inactivation of the RVM (Fields 2004). Thus, the activation of RVM neurons can generate either facilitation or inhibition of pain transmission under different conditions. ON-cells in the RVM can be activated indirectly by noxious stimulation via glutamateric terminals leading to activation of ON-cells. The selective activations of ON-cells enhance responses to noxious stimulations leading to facilitation of pain transmission in the pain system (Fields 2004).

Repetitive and high-frequency stimulation of C-fibres produces amplification and prolongation of the response of spinal dorsal horn neurones to subsequent inputs, so-called wind-up. Enhanced activity of spinal dorsal horn neurones results e.g., from the activation of the NMDA receptor (Figure 4). The role of the NMDA receptor is well established in hyperalgesia and enhancement of pain signalling seen in more persistent pain states including inflammation and neuropathic conditions (D'Mello & Dickenson 2008). Central facilitation manifests within seconds of an appropriate nociceptive conditioning stimulus and can outlast the stimulus for several hours (Anand et al. 2007). Wind-up of dorsal horn neuron activity can be mimicked by the application of L-glutamate or NMDA to the dorsal horn (Coderre et al. 1993).

Hyperalgesia in chronic pain is often, at least partly, seen as a state of hypersensitivity of CNS that amplifies nociceptive input arising from damaged tissues (Curatolo, Arendt-Nielsen, & Petersen-Felix 2006). Changes in the CNS after peripheral tissue injury have been shown in animals (Roza, Laird, & Cervero 1998) and in human studies (Azpiroz et al. 2007; Chang et al. 2000; Drewes et al. 2006; Verne, Robinson, & Price 2001; Wilder-Smith & Robert-Yap 2007). In healthy volunteers experimentally induced peripheral inflammation in muscle and viscera have also evoked both peripheral and central sensitization as a result of increased excitability of the CNS (Arendt-Nielsen & Svensson 2001; Brock et al. 2009; Drewes et al. 2003a; Sami et al. 2006; Sarkar et al. 2006). Chemical perfusion of the oesophagus can for example induce central and generalized sensitization. However, human visceral pain models with chemical stimulation of the gastrointestinal tract are not investigated in details and offer several methodology problems. Therefore, study II introduced a model to evoke experimental hyperalgesia of the oesophagus with a combination of acid and capsaicin. The model was able to evoke consistent local hyperalgesia in the oesophagus as well as generalized hyperalgesia spreading to somatic tissue (III) and later it was demonstrated by Brock et al. that this model induced central sensitization (Brock et al. 2009).

Referred pain: Due to the extensive convergence of visceral and somatic input on the same neurons at various levels of the CNS, the changes in neuronal excitability triggered by the afferent visceral barrage can change the central processing of normal afferent inputs from the referred somatic pain area. This can explain the observed alterations in the size, localization and stimulus response of the referred pain area seen in animal studies in chronic pain states and in human studies of visceral pain (Giamberardino 1999). Therefore, in experimental pain studies referred pain areas can act as proxies for central changes. Referred pain and hyperalgesia are often reported in the muscle and skin within the same spinal cord dermatome as injured organs. However, evidence that

referred pain partly depend on changes in CNS, during chronic pain, is provided by findings that referred pain and hyperalgesia spread to areas which do not share the same dermatome (Coderre et al. 1993). Referred pain and hyperalgesia are believed to result from either: 1) reduction in threshold of nociceptors or 2) increase in the excitability of CNS neurons involved in pain transmission. The fact that pain and hyperalgesia can spread to areas far removed from the injured region implies that central changes, as opposed to convergence, are involved in the spread of hyperalgesia (Coderre et al. 1993).

3. Opioids

WHO (World Health Organization) has developed a three-step "ladder" to illustrate standard guidelines for pain control drug therapy (Figure 7). If pain occurs, there should be prompt oral administration of drugs in the following order: nonopioids (aspirin and paracetamol); then, as necessary, mild opioids (codeine); then strong opioids such as morphine, until the patient is free of pain. To calm fears and anxiety, or to treat special pain conditions as for example neuropathic pain, additional drugs – “adjuvants” – should be used. This thesis will focus only on opioids for treatment of moderate to severe pain as for example morphine and oxycodone.

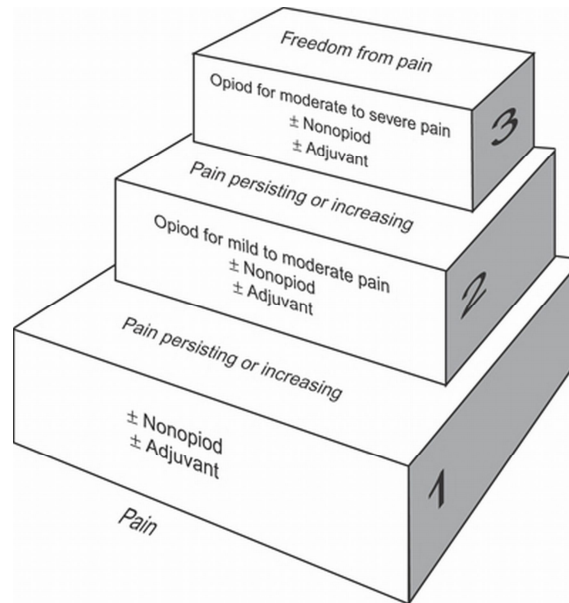


Figure 7. WHO's pain ladder. If pain occurs, there should be prompt oral administration of drugs in the following order: nonopioids (aspirin and paracetamol); then, as necessary, mild opioids (codeine); then strong opioids such as morphine, until the patient is free of pain. To calm fears and anxiety, additional drugs – “adjuvants” – should be used. To maintain freedom from pain, drugs should be given “by the clock”, that is every 3-6 hours, rather than “on demand” This three-step approach of administering the right drug in the right dose at the right time is inexpensive and 80-90% effective.

3.1 Opioid pharmacology

Opium refers to a mixture of alkaloids from the opium poppy. Opiates are naturally occurring alkaloids such as morphine. Opiates, the prototype of which is morphine, are potent analgesic and addictive drugs that act through opioid receptors. The opioid system plays a major role in pain-modulating systems.

3.1.1 Opioid receptors

Opioids mainly exert their analgesic effect in the CNS, but it is now well known that opioid receptors are synthesized in the dorsal root ganglia and transported towards both central and peripheral nerve terminals. Several subtypes of opioid receptors exist. 1) μ -receptors, 2) δ -receptors, 3) κ -receptors and 4) the opioid receptor like-1 (ORL-1) a receptor with 65% structure homology to the other members of the opioid family (Fioravanti & Vanderah 2008). The majority of the clinical relevant opioids have their primary activity at the μ -receptor. Each receptor consists of an extracellular N-terminus, 7 transmembrane helical twists, 3 extracellular and intracellular loops, and an intracellular C-terminus and is G-protein coupled receptors. Activating opioid receptors located on the presynaptic terminals of the nociceptive C-fibres and A δ fibres will release a part of the G-protein, which diffuses within the intracellular space until it reaches its target (either an enzyme or an ion channel). This results in indirect inhibition of voltage-dependent calcium channels, decreasing cAMP (Adenosine monophosphate) levels and blocking the release of pain neurotransmitters such as glutamate, SP, and CGRP from the nociceptive fibres, resulting in analgesia (Figure 8) (Trescot et al. 2008). At post-synaptic sites the opioids block the voltage dependent potassium channels and hence dampen the excitability of the neuron.

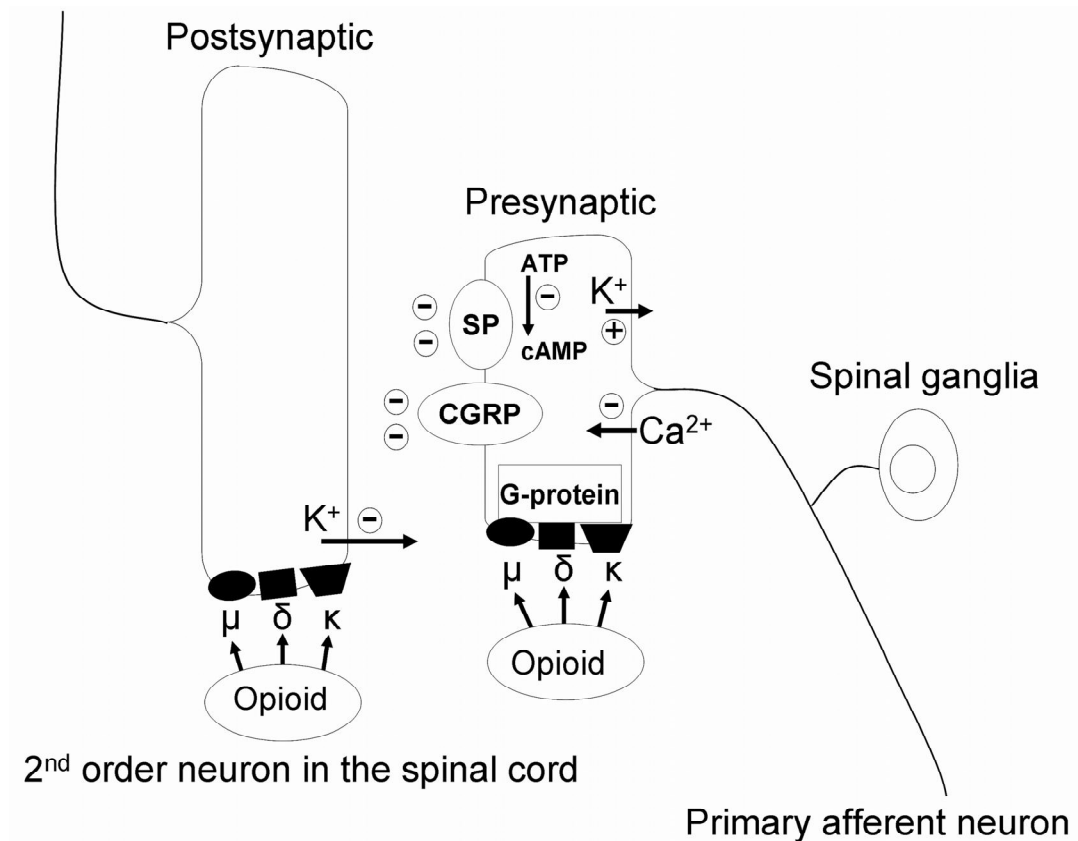


Figure 8. Molecular mechanisms for the opioid action in the spinal cord. The presynaptic action involves inhibition of calcium influx, by enhancing outward movement of potassium or by inhibiting adenylate cyclase the enzyme which converts ATP to cAMP. Hereby, the release of neurotransmitters as substance P (SP) and calcitonin gene-related peptide (CGRP) is inhibited. The majority of opioid receptors are located presynaptic (70%). Postsynaptic action involves inhibition of potassium ion efflux, which decreases the neuron excitability.

The peripheral level: Opioid receptors are present on peripheral sensory nerve fibres and their terminals (Stein 1995). Opioid receptors are synthesized in the dorsal root ganglion and axonal transport is responsible for delivering macromolecules from the cell body to the nerve terminals. Opioid peptides activate opioid receptors on peripheral terminals, where they decrease the excitability of the neuron (Stein 1995). Ligands with preference for μ -receptors are generally the most potent inducers of peripheral analgesia but δ - and κ -ligands are active as well (Stein 1995). Both animal and human studies have confirmed that μ -, δ - and κ -receptors are present on small diameter primary afferents (Stein, Schafer, & Machelska 2003). The κ -opioid receptor is also found in peripheral tissues including afferent fibres and several studies have suggested that κ -agonists can attenuate visceral nociception via a peripheral action (Field et al. 1999; Sengupta, Su, & Gebhart

1996;Su, Sengupta, & Gebhart 1997). Furthermore, previous studies have demonstrated that κ -agonists possess anti-inflammatory activity (Wilson, Nayanar, & Walker 1996). Animal experiments have shown that κ -receptors is a key feature of the visceral afferents (Burton & Gebhart 1998;Sengupta et al. 1999). Contrary to the situation in somatic tissue, visceral κ -receptors seem be of some importance to analgesia in non-inflammatory pain. The findings have been supported by human experiments in healthy volunteers where oxycodone, an opioid with partial effect at the κ -receptors showed improved analgesia in visceral pain compared to morphine (μ -agonist) (Stahl et al. 2006a). Therefore, study I explored the effect of a peripheral restricted κ -opioid agonist (CR665) in a human experimental multi-modal, multi-tissue pain model. As hypothesized, CR665 had a selective effect on visceral pain.

The spinal level: The dorsal horn of the spinal cord is a major area in which opioids exert their analgesic action. The net effect of opioids in the spinal cord is to decrease the ascending nociceptive traffic. Animal studies have shown that the main receptor types are present in the dorsal horn; μ - and κ -receptors are mainly located around C- and A δ -fibre terminals in laminae I and II (Cesselin et al. 1999;Minami & Satoh 1995). The absolute densities of the μ , δ and κ binding sites in laminae I and II vary from one study to another, but, at least in rodents the μ receptor is found to be the most abundant (Cesselin et al. 1999). It has been suggested that the κ -opioid receptor as well as μ -opioid receptor, might play important roles in the modulation of nociceptive information at the postsynaptic sites of primary afferents (Minami & Satoh 1995). The δ - receptors are located in the upper laminae as well as the deeper laminae of the dorsal horn, where motor neurones terminate (Minami & Satoh 1995). This receptor type is furthermore present in the ventral horn.

The supraspinal level: Opioid receptors are distributed throughout the central nervous system. The wide distribution of opioid receptors in the brain accounts for the multiplicity of pharmacologic responses elicited by e.g. morphine administration. For example, μ -receptors are responsible for supraspinal analgesia, respiratory depression, euphoria, sedation, opioid induced bowel dysfunction, and physical dependence. A limited number of brain sites supports opioid analgesia, the most important and best studied being PAG and RVM (Heinricher & Morgan 1999). Figure 9 gives an overview of the supraspinal effects of opioids. μ -receptors are found in both RVM and PAG. In RVM μ -receptor mediated effects are paramount in opioid analgesia (Heinricher & Morgan 1999). κ -receptors are found within the PAG, but the antinociceptive effect of opioids acting within the PAG appear to be mediated primarily by μ -receptors (Heinricher & Morgan 1999). However, κ -receptors are responsible for spinal analgesia, sedation, dyspnoea, dependence, dysphoria,

respiratory depression and physiological effects from aquaretic activity. δ -receptors are widespread located in the brain and their effects are not well understood. They may be responsible for psychomimetic and dysphoric effects (Trescot et al. 2008). The PAG itself does not project to the dorsal horn but sends a large projection to the RVM. In addition to the PAG and RVM, a number of other supraspinal sites support opioid analgesia, but the physiology of opioid-sensitive neurons and whether opioids produce their effects in these regions via activation of the PAG-RVM axis or through independent pathways have not yet been fully investigated. The knowledge about the deeper brain structures is mainly based on findings in rodents.

In the RVM opioids works by inhibition of stimulatory centres and stimulation of inhibitory centres. For example, when μ -opioid receptor agonists are administered systemically, either into the PAG or locally in RVM, on-cells become silent and off-cell firing accelerates and becomes continuous by removing GABA inhibition (illustrated in Figure 8). Selective blockade of off-cell activation prevents morphine's anti-nociceptive effect. Therefore, off-cell activation is necessary for the pain inhibitory effects of μ -opioid receptor ligands given systemically or supraspinally (Fields 2004). This dual action is thought to increase the descending inhibitory control arising from RVM, which has a net effect of inhibition of nociceptive processing in the spinal cord (Figure 9).

Brain areas constituting the brain network for acute pain are: primary and secondary somatosensory, insular, anterior cingulate, prefrontal cortices and thalamus. Moreover, these areas are rich in opioid receptors (Apkarian et al. 2005). Activation in anterior cingulate may indicate an active pain modulating role of this structure. Furthermore covariation of activity between the anterior cingulate and the PAG during pain and opioid analgesia, but not during pain alone has been shown (Sprenger et al. 2005).

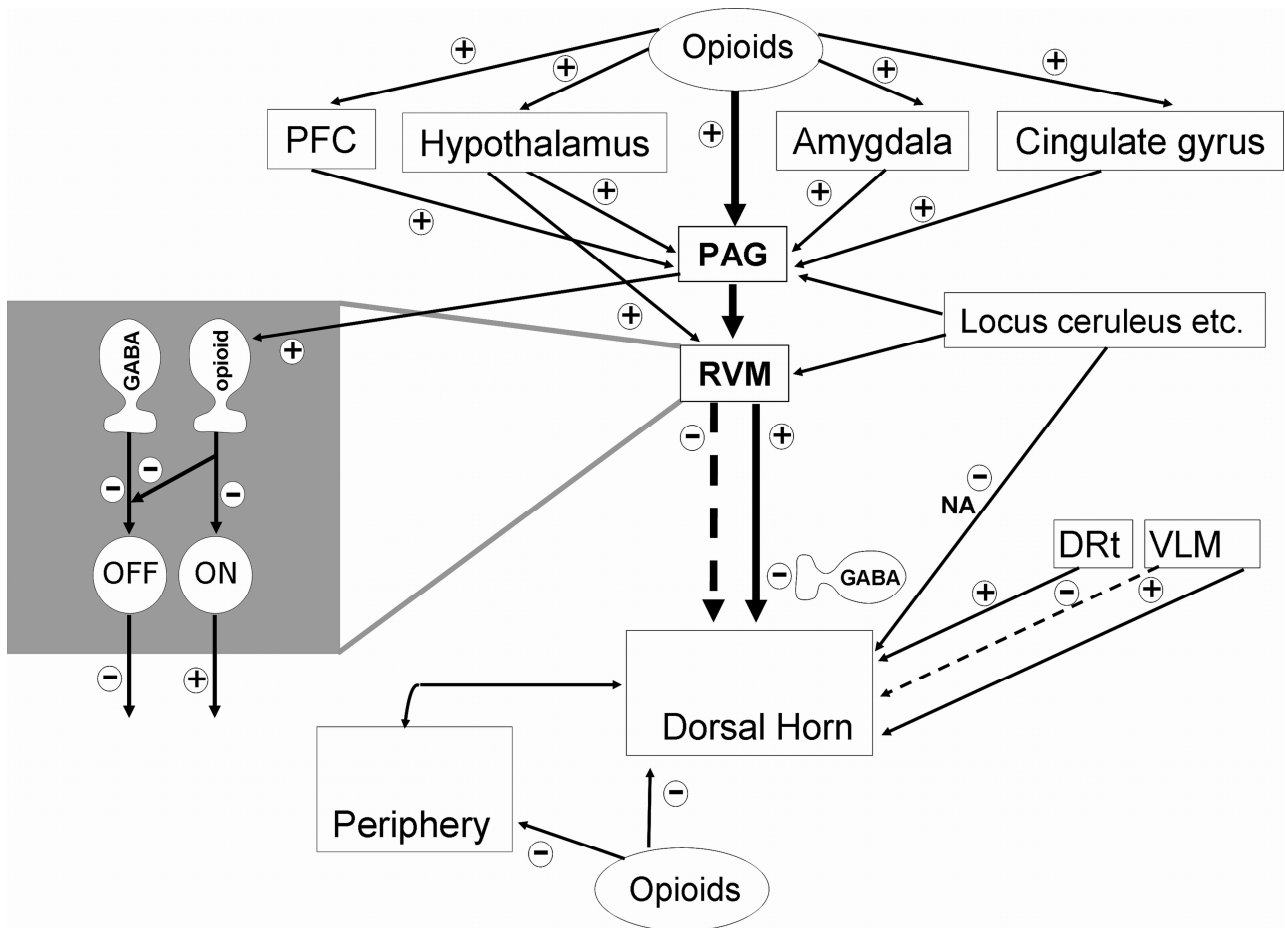


Figure 9. The descending control system, showing the main site of action of opioids on pain transmission. The pathways shown in this diagram represent a considerable oversimplification, but depict the general organisation of the supraspinal control mechanisms. Opioids excite neurons in brain areas as prefrontal cortex (PFC), hypothalamus, amygdala and cingulate gyrus and hereby indirectly excite neurons in PAG. Opioids also directly excite neurons in PAG, which project to the RVM. From the RVM neurons run to the substantia gelatinosa of the dorsal horn, and exert an inhibitory or excitatory influence on transmission. In addition to the PAG-RVM system, two areas of the caudal medulla, the dorsal reticular nucleus (DRt) and caudal lateral ventrolateral medulla (VLM) have also been implicated in descending control of dorsal horn nociceptive processing and opioids also excite neurons in these areas. DRt is thought to be facilitating, and VLM primarily inhibitory, although it may, like RVM, have both an inhibitory and facilitatory influence probably through ON- and OFF-cells. Opioids also act directly on the dorsal horn, as well as on peripheral terminals of nociceptive afferent neurons. As gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter and will inhibit the facilitatory pathways, GABAergic neurons play a role in descending control to spinal cord level. Other brain areas as the parabrachial area, nucleus of the solitary tract and locus ceruleus also control dorsal horn nociceptive processing either directly or indirectly via PAG and RVM. Noradrenaline (NA) act directly on the dorsal horn mainly as an inhibitory neurotransmitter. The left side of the diagram illustrates how opioids work on ON and OFF cells in RVM as an example, where endogenous opioids inhibit opioid receptor-bearing ON-cells and GABAergic inputs to OFF-cells. The inhibition of the GABAergic inputs disinhibits the OFF-cell, which when activated inhibits nociceptive transmission at the level of the dorsal horn.

3.2 Endogenous opioids

The location of endogenous opioids or endorphins in the CNS was discovered in 1973, and the first endogenous opioid (enkephalin) was discovered in 1975. Their location in the CNS allows them to function as neurotransmitters, and they may play a role in hormone secretion, thermoregulation, and cardiovascular control (Trescot et al. 2008). Enkephalins are relatively selective δ ligands although these peptides also bind to the μ -opioid receptor, but with less affinity. Endorphins bind to the μ and δ receptor. Dynorphins are highly selective at the κ -receptor. An overview of the binding of endogenous opioids to the different opioid receptors is given in Table 1.

Table 1. Endogenous opioids and effect at opioid receptors.

	Mu (μ)	Delta (δ)	Kappa (κ)
Endogenous Opioids			
Enkephalins	Agonist	Agonist	
β -Endorphin	Agonist	Agonist	
Dynorphin A	Agonist		Agonist

3.3 Exogenous opioids

Many of the side effects of exogenous opioids, as well as their effects, may be related to the opioid metabolites. It is generally assumed that most of the metabolism occurs in the liver. The basal rate of metabolism is determined by genetic makeup, gender, age, as well as environment including diet, disease state, and concurrent use of medications. There is no clear evidence of renal metabolism, though the kidney is an important site of excretion. Most opioids are metabolized by glucuronidation by the cytochrome P450 system (Trescot et al. 2008). In the next section, the opioids used in the current studies are described in more details.

3.3.1 Morphine

After oral administration, only approximately 40 to 50 percent of the administered dose reaches the central nervous system, within 30 minutes for the immediate release morphine. The reason for this poor penetration is poor lipid solubility, plasma protein binding, rapid conjugation with glucuronic acid, and ionization of the drug at a physiologic pH (Trescot et al. 2008). The elimination half-life of morphine is approximately 2 hours.

Morphine is metabolized by demethylation and glucuronidation which is the predominant mode of metabolism, producing morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G) in a ratio of 6:1 (Lotsch 2005; Trescot et al. 2008). Five percent of morphine is demethylated into normorphine. M3G in high enough concentrations can potentially lead to hyperalgesia whereas M6G is believed to be responsible for some additional analgesic effect of morphine (Lotsch 2005) (Figure 10). Glucuronidation occurs almost immediately after morphine enters the serum in both hepatic and extra hepatic sites, with evidence that a limited amount of intrahepatic recycling occurs (Trescot et al. 2008).

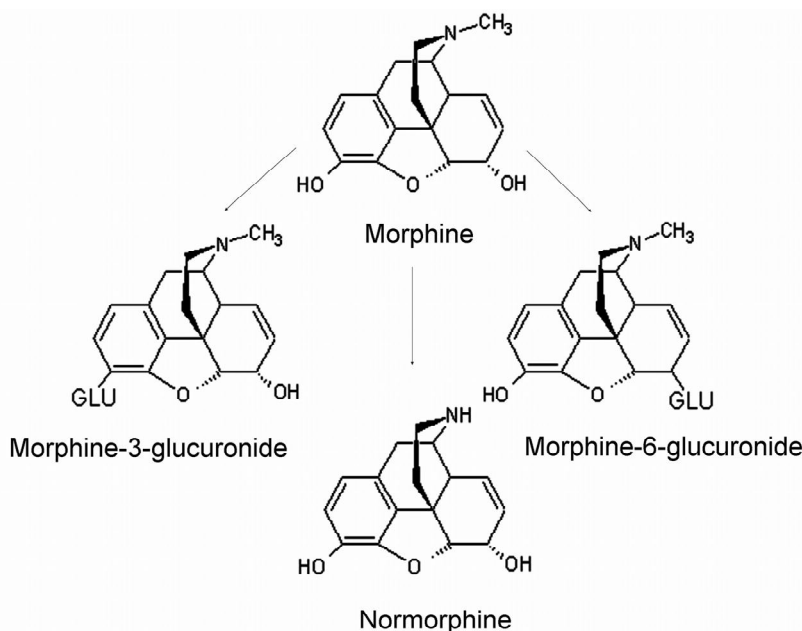


Figure 10. The predominant morphine metabolism involving glucuronidation of morphine into morphine-3-glucuronide, morphine-6-glucuronide and normorphine.

3.3.2 Oxycodone

Oxycodone has activity at multiple opiate receptors. Bioavailability of oxycodone is high in oral dosage, with a half-life of 2.5 to 3 hours. It undergoes extensive hepatic conjugation and oxidative degradation to a variety of metabolites excreted mainly in urine. Animal studies have indicated that oxycodone has more pronounced effect at the κ -receptor compared to morphine (Ross & Smith 1997).

Oxycodone undergoes extensive hepatic conjugation and oxidative degradation to several metabolites excreted mainly in urine. Oxycodone is metabolized by glucuronidation to

noroxycodone with less than 1% of the analgesic potency of oxycodone, and by CYP2D6 to oxymorphone which may have some analgesic effect. Oxymorphone has high affinity for the μ -receptor with negligible interaction with κ - and δ -receptors. Oxymorphone is about 10 times more potent than morphine, and is not affected by CYP2D6 or CYP3A4. Oxymorphone can further undergo glucuronidation to noroxymorphone, which is considered to be the most likely active metabolite as μ -opioid receptor binding affinity is comparable to morphine. However, there are no indication that noroxymorphone exerts central analgesic effects as it does not penetrate the blood-brain barrier (Lalovic et al. 2006) (Figure 11). Therefore, the major contribution to analgesia comes from the parent compound. Because oxycodone is dependent on the CYP2D6 pathway for clearance, it is possible that drug–drug interactions can occur with CYP2D6 inhibitors. Study IV was done to further elucidate the theory about peripheral effect of oxycodone by exploring the PK-PD relationship of oxycodone in hyperalgesia. No effect delay from plasma oxycodone concentrations and dynamic effect was found, indicating an initial peripheral effect of oxycodone. This initial peripheral effect is believed to be followed and supplied by a central effect when oxycodone enters the brain.

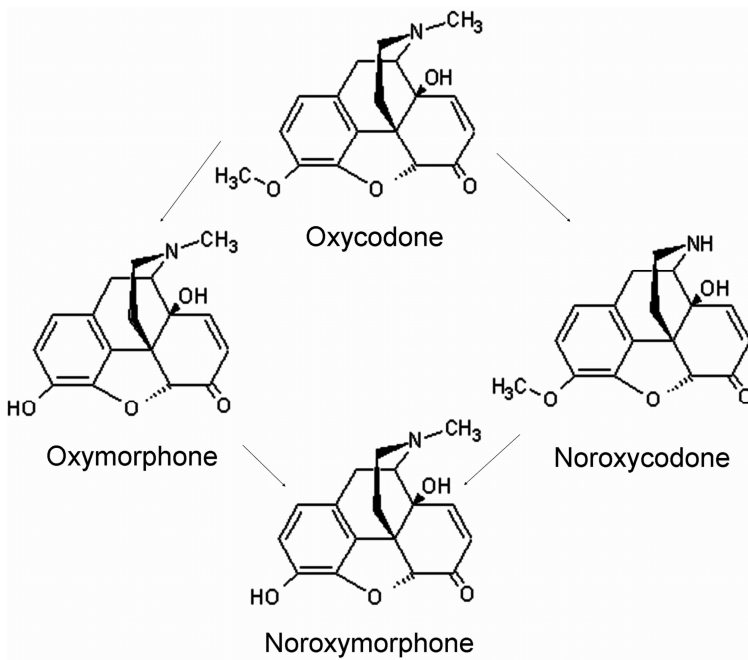


Figure 11. The predominant metabolism of oxycodone to oxymorphone, noroxycodone and noroxymorphone.

3.3.3 CR665

CR665, is a tetrapeptide agonist with affinity to the κ -opioid receptor, and very low affinity at other opioid receptor subtypes (Binder et al. 2001; Vanderah et al. 2008). This tetra peptide does not significantly cross the blood-brain barrier and therefore, does not have the same profile regarding central mediated side effect. CR665 has been shown to relieve pain in rodent models including jejunal distension-induced visceral pain (Binder et al. 2001; Vanderah et al. 2008). Based on this pharmacological profile, as well as preclinical studies of other κ -opioid agonists (De Schepper et al. 2004; Riviere 2004), it was believed that CR665 could inhibit visceral pain in humans as several studies have suggested that κ -agonists can attenuate visceral nociception via a peripheral action (Field et al. 1999; Sengupta, Su, & Gebhart 1996; Su, Sengupta, & Gebhart 1997). Study I was designed to explore the effect of CR665 in a multi-modal and multi-tissue experimental model. A selective effect on visceral pain was demonstrated.

3.4 Hyperalgesia and the opioid system

Opioid antinociceptive effects are more pronounced in inflamed tissue for several reasons and several studies have shown involvement of peripheral mechanisms in opioid-induced analgesia in inflammatory and neuropathic pain (Janson & Stein 2003; Obara et al. 2009; Stein, Machelska, & Schafer 2001). After the induction of peripheral inflammation, the axonal transport of opioid receptors in fibres of the sciatic nerve is greatly enhanced. Subsequently, the density of opioid receptors on cutaneous nerve fibres in the inflamed tissue increases (Hassan et al. 1993; Walker 2003). Furthermore, in the inflammatory state opioid agonists have easier access to neuronal opioid receptors because inflammation entails a disruption of the perineurium (a normally rather impermeable barrier sheath encasing peripheral nerve fibres) and because the number of peripheral sensory nerve terminals is increased in inflamed tissue (sprouting). Moreover, opioids can act through the opioid receptors present on immune cells (e.g. macrophages) which migrate to the inflamed/injured tissue (Obara et al. 2009). In addition, previously inactive neuronal opioid receptors may become active in the inflammatory milieu (Stein, Cabot, & Schafer 1999). One study suggests that inflammation induces centrally-mediated neuroplastic changes which enhance μ -opioid receptor- and κ -opioid receptor-mediated antinociception (Schepers, Mahoney, & Shippenberg 2008). It was suggested that an enhanced κ opioidergic control of nociception, behaviour and pathology occurs in response to inflammatory pain (Cesselin et al. 1999). For example, Walker et al. found κ -opioid agonists to be powerfully anti-inflammatory, reducing

disease severity by as much as 80%; attenuating arthritis in a dose-dependent, stereoselective, antagonist-reversible manner in rats. By contrast opioids acting at other receptors were only therapeutic at near toxic doses (Walker 2003).

Moreover, as different opioid receptors are located differently on on-cells and off-cells both pre- and post-synaptically, and inflammation will produce an on-cell state of the pain system, the differential effect of opioids can be more pronounced in inflammation (Fields 2004). Therefore, study III explored the differential effects of morphine and oxycodone in experimental induced hyperalgesia. The results supported the theory that κ -opioid drugs are anti-inflammatory to a higher degree than opioids acting at other receptors, as oxycodone showed better effect than morphine on pain stimulations in skin, muscle and viscera in a human experimental pain model of hyperalgesia.

4. Methods

Experimental pain models have been applied in animal studies. In these experiments, the neuronal nociceptive activity can be recorded or behaviour can be assessed (Sengupta & Gebhart 1994). Nociceptive reflexes or electrophysiological recordings from selected pathways in the animal nervous system are important in basic research and screening of analgesics. However, animal experiments typically suppress central pain mechanisms and associated complex reactions seen in man. Furthermore, the neurobiology of nociceptive systems differs between species, and this limits the extrapolation of findings from animal studies to man even further. These limitations stress the need for experimental human pain models in preclinical studies of new analgesics. Animal models may provide pharmacodynamic information during drug development, but have limitations mimicking human pain conditions as human pain is a net result of complex sensory, affective and cognitive processing (Langley et al. 2008). Pain research with patients offers a mean to explore the actual pain states of interest. On the other hand they suffer from difficulties in assessment of the pain, due to many confounding factors such as general malaise, fever, nausea, psychological status etc. (Langley et al. 2008). Therefore, experimental pain models in healthy volunteers - controlling the pain stimulus and assessment, as illustrated in Figure 2 - can act as a translational bridge from studies in animals to humans (Arendt-Nielsen, Curatolo, & Drewes 2007).

Quantification of the human painful sensory experience is an essential step in the translation of knowledge from animal nociception to human pain. Translational models for assessment of pain are very important, as such models can be used in: 1) basic mechanistic studies in healthy volunteers; 2) clinical studies for diagnostic and monitoring purposes; 3) pharmacological studies to evaluate analgesic efficacy of new and existing compounds (Arendt-Nielsen & Yarnitsky 2009).

Much of what we know about the mechanism of pain derives from experimental studies of somatic and not visceral nociception. Visceral pain is the most common form of pain produced by disease and one of the most frequent reasons for patients to seek medical attention. According to Cervero et al. visceral pain can be characterised by the following (Cervero & Laird 1999):

- a) Pain is not evoked from all viscera as not all viscera are innervated by “sensory” receptors.
- b) As there is an extensive visceral afferent input to the autonomic and enteric nerve system, visceral pain is to a greater degree than somatic pain accompanied by motor and autonomic reflexes, such as nausea, vomiting or muscle tension.

c) Compared to somatic afferents, the proportion of visceral afferents is much lower and their divergence on the spinal and supraspinal level of the central nervous system is much more extensive. This explains to some degree why visceral pain is diffuse with poor localisation.

d) The referral of pain from the viscera to somatic structures contributes to the localisation difficulties as well. The referred pain phenomenon is thought mainly to be caused by convergence of visceral afferents onto spinal (and supraspinal) neurons, which also receive somatic afferent information.

Therefore, it is necessary to develop and apply visceral experimental pain models in order to get a wider understanding of the basic visceral physiology and pathophysiology and, based on this information, develop treatment strategies for visceral pain. Methods related to experimental pain research in man aim at activating different nociceptors, evoking pain from different tissues and activating specific pathways and mechanisms. There are still major problems in exact determination of the activated pathways and pain mechanisms, but the experimental human models provide the possibility to obtain reproducible results in test-retest experiments and hence be useful for evaluation of analgesic effects. Human experimental models have been refined and robust models for superficial and deep activation of the nociceptive nervous system now exist (Arendt-Nielsen & Yarnitsky 2009). Assessment of the output from these pain models can be based on psychophysical or neurophysiological methods. Psychophysical methods are based on the subjective experience of pain, measured on standard scales or as pain thresholds. It has been shown that suprathreshold pain responses are more clinically relevant than responses to threshold-level noxious stimuli (Edwards et al. 2005). Examples of neurophysiological methods are measurement of nociceptive withdrawal reflexes or evoked brain potentials. Only pain stimulations used in the protocols and projects are described in the following. For further detailed description of experimental pain models see for example Staahl et al. 2004 (Staahl & Drewes 2004).

4.1 Experimental pain models

4.1.1 Skin stimulation

Experimental pain models in the skin are highly developed, mainly because of the easy access to the skin. Mechanical, thermal, electrical, and chemical methods are all evaluated methods (Staahl & Drewes 2004). However, as skin pain is a superficial activation of the nociceptive nervous system it can be complicated to translate studies of skin pain into the clinical situation where deeper pain often dominates. In our studies (I and III) we selected the thermal stimulation by a computer driven

“Thermo tester” and the cutaneous pinching pain tolerance threshold was determined by pinching a skin fold with an electronic pressure algometer. The thermal stimulation with slow rate of heating was chosen as it activates C-fibers (Heinricher et al. 2009) and therefore should detect opioid effects.

4.1.2 Muscle stimulation

Muscle pain is a cramp-like, diffuse and aching pain. Referred pain in distant somatic structures and trophic changes in the superficial and deep structures are often associated with muscle pain. Usually, the models are divided into methods without (endogenous) and with (exogenous) external stimuli. Endogenous models include ischemic and exercise induced pain, whereas exogenous models employ mechanical, electrical or chemical stimulation (Graven-Nielsen & Arendt-Nielsen 2003). The model for muscle stimulation in the present studies (I and III) was an acute exogenous pain model excluding induction of inflammation. In study I the pressure pain detection and tolerance thresholds were determined by an electronic pressure algometer with a probe pressed on the supinator muscle on the left forearm. In study I and III muscle stimulation was also performed by the pneumatic tourniquet cuff which was wrapped tightly around the gastrocnemius muscle. The cuff delivers uniformly distributed pressure to deep as well as superficial tissues (Polianskis, Graven-Nielsen, & Arendt-Nielsen 2001). These pain models excluding hyperalgesia was chosen due to ethical and practical limitations in the experimental pain model, as it would be difficult to control inflammation induced in multiple tissues in a human experimental multi-modal pain model.

4.1.3 Visceral stimulation

The effect of analgesics on visceral pain is very difficult to evaluate in the clinic, mainly due to the deep and diffuse nature of the pain (Drewes, Gregersen, & Arendt-Nielsen 2003). Due to the localization of the organs, experimental pain studies in the viscera are more difficult to perform than in the skin or muscles. The risk of perforation and the increased autonomic responses to visceral stimuli also limit the possibilities. However, during recent years, experimental pain has been evoked in most part of the gastrointestinal tract and the uterine cervix (Drewes, Gregersen, & Arendt-Nielsen 2003). It is possible to use reliable pain stimulation with different modalities and hence stimulation of different groups of afferents. Mechanical, thermal, electrical and chemical stimulus modalities have been used for evoking pain in the viscera mainly in the gastrointestinal tract (Drewes, Gregersen, & Arendt-Nielsen 2003).

Figure 12 is an illustration of a probe used for experimental stimulation of oesophagus. Mechanical stimulation is a widely used technique and can be evoked in hollow organs by distension of the organ wall. This mainly activates nociceptors in muscle layers surrounding the organ. Thermal stimulation can be evoked by circulation of hot or cold water in a balloon placed inside a hollow organ (Drewes et al. 2003a). Hereby, nociceptors in the mucosa are selectively stimulated. Recently a new and improved endoscopic method for linear control of heat stimulation of the oesophagus was developed and proved to be reproducible (Olesen et al. 2009).

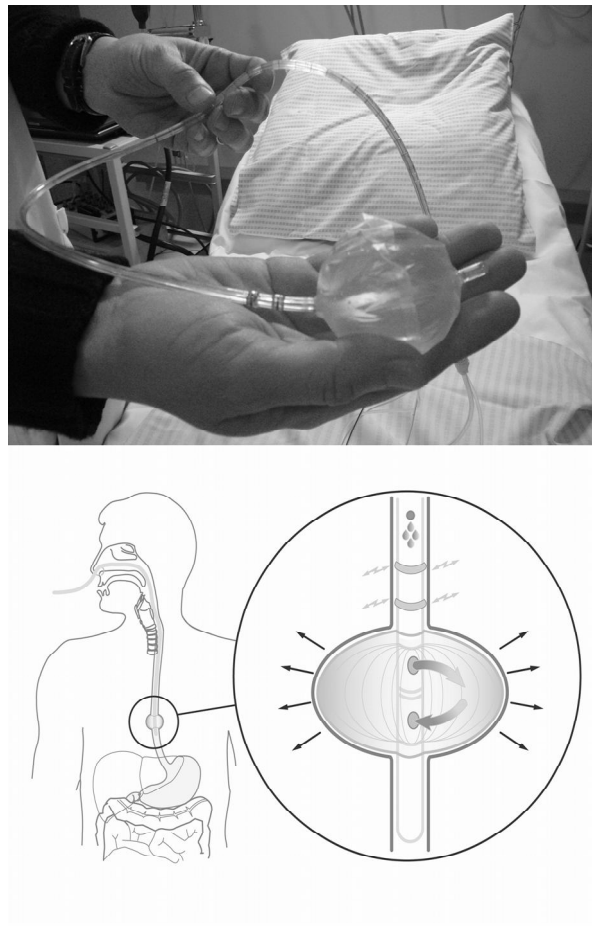


Figure 12. Pain stimulations of the oesophagus as used in I-IV. Upper part showing the size of a water filled balloon and the lower left side illustrates the placement of the probe in the oesophagus. The lower right side of the figure is a schematic illustration of the multimodal oesophageal probe for mechanical, thermal, electrical and chemical stimulations. The probe has a balloon mounted for mechanical and thermal stimulations. The electrodes for electrical stimulations are placed above the balloon and a side hole for chemical perfusion of oesophagus is placed above the electrodes.

Electrical stimulation bypasses the nociceptors and stimulates the nerve fibres directly. Figure 12 illustrates the different modalities available for oesophageal stimulation and Figure 3 gives an overview of the gut layers affected by the different stimulations. The multimodal probe was used in studies (I-IV). One limitation of multimodal pain stimulation in the gut is that it is done without visualisation of the inside of the intestine. Hence, diseases such as oesophageal erosions cannot be excluded. Recently, an attempt to combine the multimodal probe with endoscopies was done in our laboratories (Olesen et al. 2009). Hereby, the probe allows full multimodal stimulation including mucosal inspection and biopsies (albeit small) for histology and specific immunohistochemical staining. The experimental pain methods for visceral stimulation can also be used to unravel central pain mechanisms, such as those involved in allodynia, hyperalgesia and referred pain. Abnormalities in central pain mechanisms are often seen in patients with chronic gut pain and hence methods relying on multimodal pain stimulation of the gut may help to understand the symptoms in these patients and hereby characterise patients with different diseases of the gastro-intestinal tract (Drewes & Gregersen 2006).

Visceral sensitization: Sensitization of the visceral pain system is also possible by e.g., perfusion of the gut with chemical substances. Thus, peripheral and central mechanisms relating to the clinical situation involving chronic pain syndromes can be evoked, and the effect of pharmacological modulation evaluated.

Previously, visceral hyperalgesia have been induced by e.g., acid, capsaicin and glycerol (Drewes et al. 2003a; Hammer & Vogelsang 2007; van den Elzen, Tytgat, & Boeckxstaens 2008). Acid perfusion of the oesophagus is the most widely used chemical stimulus inducing both peripheral and central sensitization as a generalized hyperalgesia (Figure 13) (Drewes et al. 2003a; Sarkar et al. 2001). Examples of experimentally induced visceral hyperalgesia in healthy volunteers are given in Table 2. A direct comparison between the studies investigating visceral hyperalgesia is difficult, as different doses of chemicals were applied to the different visceral organs. Another major shortcoming is that the tissue area affected by the chemical differs, and it has been demonstrated that duration and magnitude of hypersensitivity is related to exposure or dose of the chemicals (Drewes et al. 2005b; Sarkar et al. 2000; Sarkar et al. 2001). For example, perfusing 8 cm of the oesophagus with acid will affect more receptors than perfusing 3 cm of the oesophagus. Furthermore, as different doses were used in the studies, it is not possible to compare the findings directly. However, most of the studies demonstrated hyperalgesia to one or more modalities after experimentally induced sensitization by chemicals (Brock et al. 2009; Drewes et al. 2003b; Drewes

et al. 2003a;Frokjaer et al. 2005;Hobson et al. 2004;Pedersen et al. 2004;Sami et al. 2006;Sarkar et al. 2001;Willert et al. 2007). Tissue injury generates release of multiple molecules acting synergistic. To mimic this situation it may be necessary to use a mixture of chemical substances with diverse effects on the tissue. Such different cellular interaction sites of acid and capsaicin have been proposed where the acid targets the transient receptor potential vanilloid type 1 (TRPV1) extracellularly, while the capsaicin targets TRPV1 predominantly intracellularly (Welch, Simon, & Reinhart 2000). Therefore, a mixture of acid and capsaicin was used to induce hyperalgesia in healthy volunteers in Study II. The model was able to evoke consistent hyperalgesia to the pain threshold to heat and electrical stimuli. Moreover, an increase of the referred pain areas to mechanical and electrical stimulation was demonstrated after induction of hyperalgesia and later Brock et al. demonstrated central sensitization using this model of experimental hyperalgesia (Brock et al. 2009).

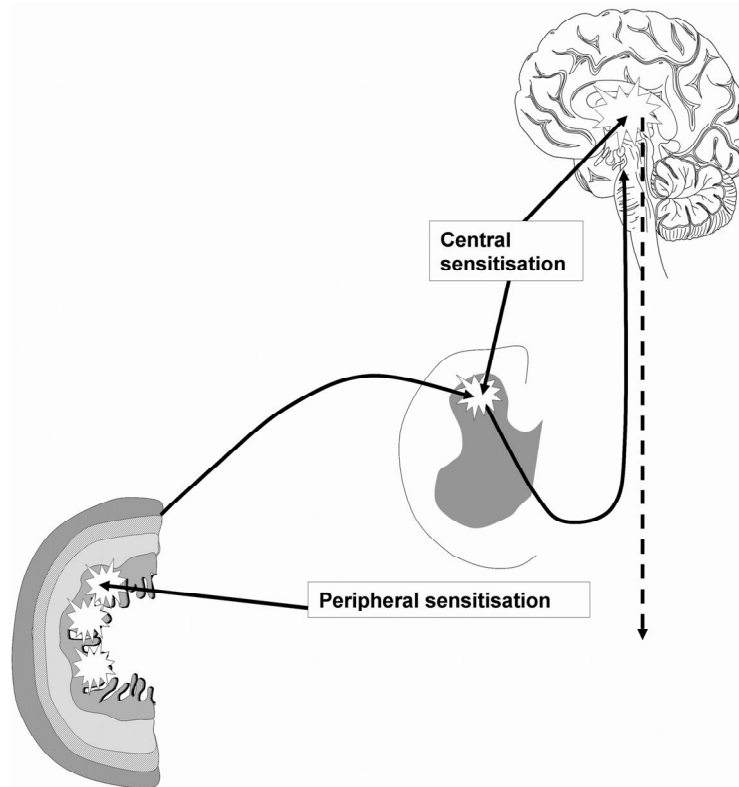


Figure 13. Schematic illustration of how peripheral sensitization can cause central changes and generalised hyperalgesia. Peripheral sensitization is induced by perfusion of the oesophagus with e.g. acid+capsaicin. This gives the possibility to evoke peripheral and central sensitization (illustrated with stars). The central sensitization will affect incomings to the dorsal horn and supraspinal sites (dotted arrow). Referred pain in the somatic tissues is believed to be generated by central mechanisms, where visceral and somatic nerves converge on nerves in the same area of the spinal cord or at supraspinal centres. Hence, an increase in the referred pain after sensitization with acid+capsaicin reflects central neuronal hyperexcitability.

Capsaicin binds to the transient receptor potential cation channel, subfamily V, member 1 (TRPV1), which plays an important role in activation of the pain system and tissue inflammation (Tominaga & Julius 2000). Therefore, in study II it was found interesting to include capsaicin on multiple visceral pain stimuli to mimic the clinical situation including tissue inflammation. Short-term administration of capsaicin to peripheral nerve endings is known to have an excitatory action on thin, unmyelinated primary afferent neurons and simultaneously stimulates the release of substance P, CGRP, and possible other neurotransmitters from the peripheral endings of these sensory neurons.

Acid sensing ion channels are a family of cation channels expressed principally in neurons and that are activated by protons (Dube, Elagoz, & Mangat 2009). Acid-sensitive fibres have been demonstrated in animal studies, and mucosal afferents are often sensitive to different chemical stimuli (Ness & Gebhart 1990; Sengupta & Gebhart 1994). Acid alters the activation of capsaicin responses in vitro, and capsaicin responses has been found to be about 10 fold longer lasting than acid responses in an animal model (Neelands et al. 2005). Increased responses to mechanical, electrical and thermal stimuli after acid perfusion of the oesophagus have also been demonstrated in human beings (see Table 2) (Sarkar et al. 2003). Prior to study II pilot studies were made to investigate the add-on effect of capsaicin to acid. The acid+capsaicin perfusion was better than acid to evoke hyperalgesia to mechanical stimulation, thermal stimulation whereas similarity was seen for electrical stimulation (study II).

4.2 Pharmacokinetic-pharmacodynamic modeling

There are various approaches to the study of opioid kinetics and dynamics.

Animal studies: These studies allow the investigation of fundamental mechanisms (such as cerebral equilibration rates) and allow the collection of both arterial and venous blood concentration data. However, the dynamic information (e.g. tail flick times, changes in the electroencephalograph (EEG) or changes in magnetic resonance imaging (MRI) signal) cannot be readily related to analgesia in man. Representative studies include sheep studies performed at the University of Adelaide where a model to study the relationship between plasma concentration and the CNS concentration has been developed by Upton and coworkers. Upton et al. has previously used a sheep preparation to examine the cerebral kinetics and dynamics of analgesic drugs used in the peri-operative period (Upton et al. 1997; Upton et al. 2003). Physiological PK-PD models developed in

sheep have been adapted to assess the clinical profile of these drugs in man (Upton & Ludbrook 2005).

Surgical patient studies: These studies are typically conducted in patients just before or during surgery where patients have an arterial cannula (often for patient management). As the patients are generally sedated (and can not report pain) and the dose is high, the dynamic information is generally derived from changes in the EEG. A representative study would be that of Pöyhiä et al. where EEG was used to quantify the central nervous system effects of oxycodone during anesthesia for primary coronary artery bypass grafting (Poyhia et al. 2004).

Volunteer and awake patient studies: For ethical reasons these subjects usually only have a venous cannula placed in an arm vein. However, they can report highly relevant dynamic information such as pain and sedation scores, and can be studied using doses and routes that are directly relevant to clinical practice. Representative studies would be study IV, the opioid study by Staahl et al. (Staahl et al. 2008). or the intranasal fentanyl studies of Christrup et al. and Foster et al. (Christrup et al. 2008; Foster et al. 2008). Differences in the site of action for opioids may be reflected in the delay between opioid blood- and opioid CNS concentration and the analgesic effect. These differences might be more pronounced in diseases where both the liver and kidney function could be reduced or affected or due to an up-regulated pain system. Understanding these differences has implications for interpretation of PK-PD opioid-studies, providing insight into optimal clinical analgesic management of for example visceral pain (Staahl et al. 2008). A robust pain assessment is needed to obtain a reliable model of the PK-PD relationships for opioids. Experimental pain models in healthy volunteers provide less variable and less confounded pain measures which are suitable for PK-PD modeling (study IV). A neurophysiologic objective assessment of pain response and analgesic effect could be EEG, which also can support the subjective findings in experimental pain studies. In a study of biophase kinetics within the PK-PD analysis a wide range of opioids, morphine showed profound hysteresis between the blood pharmacokinetics and EEG effect (Groenendaal et al. 2008). Groenendaal et al. concluded that within the wide range of opioids used in their study, only morphine displayed complex biophase distribution kinetics, which can be explained by its relatively low permeability of the blood brain barrier and the interaction with active transporters present at the blood-brain barrier (Groenendaal et al. 2008).

4.3 Healthy volunteers vs. patients

Experimental pain models in healthy volunteers are important tools to investigate analgesic effect in a standardized, controllable setup. However, pain experienced and reported by healthy volunteers is different from clinical pain, and in the laboratory it is not possible to reproduce the full complexity of the pain experience in patients (Arendt-Nielsen, Curatolo, & Drewes 2007; Curatolo, Arendt-Nielsen, & Petersen-Felix 2006). Traditional models are short lasting and have many limitations compared to the complex clinical pain conditions. On the other hand, hyperalgesia, a hallmark of clinical pain, can be evoked experimentally in healthy volunteers by sensitizing the oesophagus with capsaicin or acid (Drewes et al. 2003a; Hammer & Vogelsang 2007; Willert et al. 2007). Local sensitization also can evoke generalized hyperalgesia involving other viscera and somatic tissues (Frokjaer et al. 2005; Sarkar et al. 2006; Willert et al. 2007). Experimental pain models of hyperalgesia are more difficult to control than simple and short lasting pain stimuli, but may initiate some of the inflammatory processes and changes in the pain system that are seen in the clinic (Frokjaer et al. 2005; Sami et al. 2006). Hereby they may reasonably explain many of the abnormal pain responses typical of chronic pain. Therefore, human experimental pain models of hyperalgesia may help to predict analgesic efficacy in a sensitized pain system (Study III). In summary, models evoking controlled hyperalgesia have the advantages of experimental pain and reflect the clinical situation to a higher degree than acute models. Therefore, they may speed up development programs within the industry and provide new fundamental knowledge on analgesics and pain mechanisms (Arendt-Nielsen, Curatolo, & Drewes 2007).

5. Opioids effect in experimental pain and hyperalgesia

Strong opioids are potent analgesics and effects are found both amongst acute models and models evoking hyperalgesia. Assessment of the analgesic effects of opioids has most frequently been done by skin stimulation (Stahl et al. 2009). Several examples can be found where opioids preferentially modulate the higher pain intensities (Stahl et al. 2009). To obtain a good trial design in evaluating drug effect in experimental pain studies, at least three factors need to be considered: 1) A model (including an appropriate induction and assessment method) that activates mechanisms and pain pathways being sensitive to the analgesic in question. For example, in study I it was essential to include visceral stimulation as the opioid CR665 was believed to alleviate visceral pain specifically; 2) Right dose, which ensures sufficient efficacy combined with a limited amount of side effects and 3) Right dosing regime (single dose/multiple dose) and time points of testing for analgesia. To improve the trial design, factor 1) was considered and a new human experimental pain model of hyperalgesia was developed in study II and the effect of opioids was evaluated in this model (Study III). In study III the effect of opioids on the sensitized pain system was comparable to the effect seen on experimental pain stimulation in patients with chronic pancreatitis (Stahl et al. 2007). This could be an indication that models evoking controlled hyperalgesia have advantages of experimental pain and reflect the clinical situation to a higher degree than acute models.

The analgesic effect of morphine has been tested in several experimental pain studies in humans including both acute pain (Table 3) and hyperalgesia (Table 4) (Stahl et al. 2009). As indicated in the tables most studies have been performed in acute pain models. There are some shortcomings regarding the models of hyperalgesia, as three of eight studies could not demonstrate analgesic effect of morphine in experimental pain including hyperalgesia (Schulte, Sollevi, & Segerdahl 2004; Tegeder et al. 2003; Warncke, Stubhaug, & Jorum 1997). It should be noted that six of eight studies induced skin hyperalgesia, and only one study investigated the analgesic effect on muscle sensitization (Tegeder et al. 2003) and one study on visceral sensitization (Study III). As skin pain is very different from deep pain as muscle- and visceral pain, it can be difficult to make strong conclusions on the clinical effect of morphine on visceral pain from studies of skin pain. Warncke et al. showed that in contrast to morphine treatment after injury, morphine treatment starting preinjury significantly reduced the development of secondary hyperalgesia (Warncke, Stubhaug, & Jorum 1997; Warncke, Stubhaug, & Jorum 2000). This supports the benefits of pre-emptive analgesia. However, treating a chronic pain patient, preinjury treatment is not possible.

The analgesic effect of oxycodone has only been tested in a few human studies (Table 5) and previously only in experimental studies of acute pain. Therefore, investigating the analgesic effect of oxycodone in experimental hyperalgesia in study III and study IV was highly relevant and the results indicated that oxycodone has different effects in hyperalgesia.

5.1 Differential effect of opioids in experimental hyperalgesia

The differentiated effect of morphine and oxycodone on pain in all three tissues in study III provided further support for the notion that oxycodone may interact, at least in part, with a different population of opioid receptors or modulate μ -opioid receptors signalling in inflammation in a different way than other opioids (Nielsen et al. 2007; Virk & Williams 2008). This could be explained by the generalized hyperalgesia leading to changes in the opioid system (Riviere 2004; Stanfa & Dickenson 1995). For example a more pronounced up-regulation of κ -receptors in inflammation has been speculated (Sengupta et al. 1999). Furthermore, animal studies have shown that binding to κ -receptor sites in the spinal cord are increased to a greater degree than binding to μ -receptor during peripheral inflammation (Iadarola et al. 1988). It has been proposed from rodent experiments that oxycodone is a partial κ -agonist (Holtman & Wala 2006; Nielsen et al. 2007; Nozaki et al. 2005; Nozaki, Saitoh, & Kamei 2006; Ross & Smith 1997), but in vitro opioid receptor binding studies have questioned oxycodone's κ -affinity (Kalso 2005). Whether the differentiated effect of oxycodone and morphine is caused by different affinities for κ -opioid receptors cannot be concluded from this or any other human study as it would require a selective κ -antagonist suitable for human administration. However, the present results of differentiated effects support previous clinical studies of opioid rotation from oral morphine to oral oxycodone which in many patients significantly improved pain control (Narabayashi et al. 2008; Riley et al. 2006).

5.2 PK-PD evaluation of oxycodone

The pharmacokinetic (PK) describes the absorption, distribution, metabolism, and excretion of a drug after administration. After oral administration the drug is absorbed from the gastrointestinal tract to the blood. There from the drug enters the portal vein and the liver where many opioids undergo extensive first pass metabolism. Some opioids have low bioavailability due to this first pass metabolism. Blood concentration can be cleared by metabolism, renal excretion and distribution to other organs as the brain. The pharmacodynamic explains what the drug does to the body.

Despite the fact that results from in vitro opioid receptor binding studies have questioned the κ -affinity of oxycodone (Kalso 2005), it has been shown that oxycodone interacts, at least in part, with a different population of opioid receptors or modulate μ -opioid receptors signaling in inflammation in a subtly different way than other opioids (Nielsen et al. 2007; Virk & Williams 2008). When evaluating the PK-PD relationship for oxycodone in study IV no effect delay was found for the analgesic effect compared with the plasma profiles. Figure 14 illustrates The PK-PD relationship of a drug, when no effect delay is found. This indicates a directly correlation of the plasma concentration to the effect, which could be postulated to be effectuated via peripheral receptors.

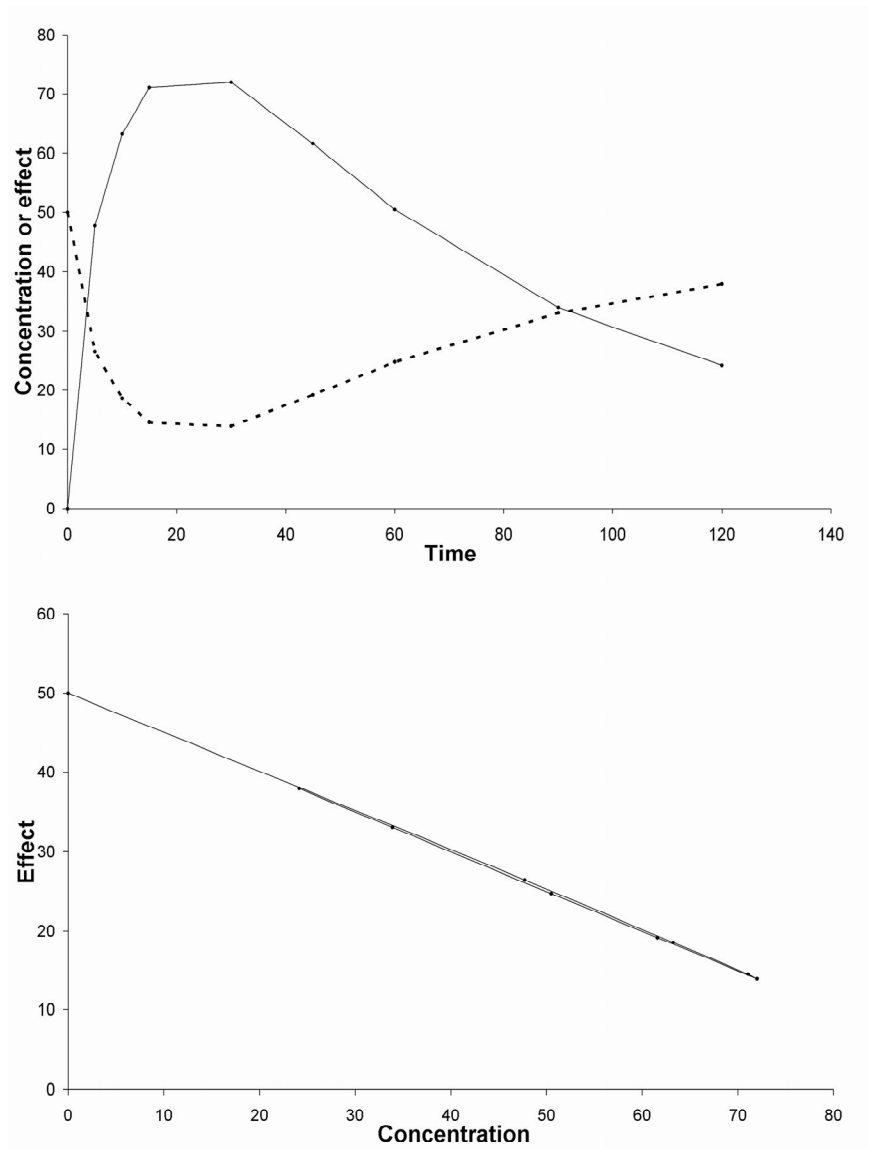


Figure 14. A theoretical illustration of the relationship between concentration and effect over time with no effect delay. The effect is illustrated as pain reduction. Despite the concentrations and effect having curved shapes with time (upper graph, straight line is concentration, dotted line is effect), the underlying concentration-effect relationship is linear (lower graph). This indicates an initial peripheral drug effect.

6. Conclusion and perspectives

In conclusion, the analgesic effect a peripheral restricted κ -agonist (CR665) on visceral pain was demonstrated (study I). Hence, this may provide new opportunities in the management of clinical visceral pain. Even though experimentally induced hyperalgesia in healthy volunteer mimics the clinical situation, limitations exists. Therefore, a new method of evoked human hyperalgesia was developed and proved able to evoke consistent and reproducible hyperalgesia (study II). The evoked phenomenon mimicked clinical diseases of the oesophagus, and was applied in a pharmacological study to test the effect of the two opioids morphine and oxycodone (study III). Oxycodone was superior to morphine attenuating pain from skin, muscle and visceral pain in this experimental pain model of hyperalgesia. This could reflect a central up-regulation of the opioid system and that oxycodone had better analgesic effect in the up-regulated state compared to morphine. Such differences are difficult to show in clinical studies due to the many confounders.

Furthermore, the PK-PD relationship for oxycodone was evaluated in this experimental pain model of visceral hyperalgesia (study IV), supporting the hypothesis of an initial peripheral effect of oxycodone in hyperalgesia.

6.1 Perspectives

As experimental pain models are suitable for showing differentiated effects of analgesics and as there is substantial preclinical evidence that peripheral opioid analgesia is enhanced in the presence of inflammation, the rational next step could be to investigate the effect of peripheral restricted opioid. This could for example be done using the κ -opioid agonists CR665 investigated in study I in a human experimental pain study including hyperalgesia. It could be hypothesized that CR665 would have more beneficial effect in such a model mimicking the clinical situation to a higher degree.

Despite the closer clinical approximation of human experimental pain models of hyperalgesia in healthy volunteers it is not possible to mimic the total clinical pain as both up-regulation of the pain system and previous pain experiences has an effect on the total pain experienced by patients. Therefore, it would be of major value to study the differentiated effect of opioids in patients. However, a crossover study will not be feasible in patients, but a parallel study in a selective and homogenous patient group could give further insight to a differentiated effect. Furthermore, the central effect of different opioids could be studied and compared by brain imaging studies in both

patients and healthy volunteers a give even more knowledge on the different effects of analgesics as well as different pain perceptions and modulation in patients and healthy volunteers.

7. Summary in Danish

Det er vanskeligt at undersøge og vurdere analgetikas effekter i patienter. Dette skyldes blandt andet at patienters smerteopfattelse er påvirket af faktorer, som angst, generel sygdom, social påvirkning m.v. Påvirkning fra sådanne faktorer kan mindskes når analgetika afprøves i humane eksperimentelle smertemodeller. Her kan input i smertesystemet kontrolleres med hensyn til intensitet, modalitet og lokalitet. Endvidere kan smerteresponset bestemmes kvantitativt med standardiserede psykofysiske eller neurofysiologiske metoder. Det er imidlertid vigtigt at sådanne modeller inkluderer flere forskellige stimulerings modaliteter, så flest mulige smertemekanismer aktiveres.

Det overordnede formål med dette studie var at undersøge effekten af en perifer selektiv κ -opioid receptor agonist og sammenligne effekten med oxycodons effekt og placebo i et multi-modalt, multi-tissue eksperimentelt smertestudie i raske frivillige forsøgspersoner (studie I); derefter at udvikle og teste en eksperimentel smertemodel for visceral hyperalgesi (studie II) og teste effekten af de to opioider morfin og oxycodon (studie III); samt at evaluere farmakokinetik-farmakodynamik forholdet for oxycodon i denne eksperimentelle smertemodel for visceral hyperalgesi.

I det første studie (I) blev et nyt stof CR665 med en forventet perifer selektiv visceral analgetisk effekt undersøgt. CR665 er et tetrapeptide og selektiv κ -opioid receptor agonist. Resultaterne viste at CR665 havde analgetisk effekt på viscerale stimuleringer, men ikke på somatiske, hvilket kunne skyldes binding til perifere viscerale κ -receptorer. Dette studie viste således, at det er vigtigt at stimulere forskellige væv, når en vævs-specifik effekt skal påvises.

Eksperimentelle smertemodeller der inkluderer hyperalgesia har fordelene fra de eksperimentelle smerte modeller og reflekterer samtidig den kliniske situation i højere grad end de akutte smertemodeller. Derfor, kan de viderebringe ny viden om analgetika og smertemekanismer. Derfor blev en ny human smertemodel af eksperimental hyperalgesi udviklet og testet i studie II, hvor vi fandt en god reproducerbarhed.

Derfor blev denne model videre brugt til at undersøge opioiders analgetiske effekt på eksperimentel generaliseret hyperalgesi i raske forsøgspersoner (studie III). Forskellige effekter af oxycodon og morfin blev fundet på hud, muskel og viscerale smertestimuli hvor oxycodon viste bedre effekt. Dette indikerede at oxycodon virker anderledes end morfin, muligvis grundet en fordelagtig effekt i et opreguleret smertesystem. Der er stor forskel på smertesystemet og opioidernes virkning før og efter induktion af hyperalgesi. Derfor er det vigtigt at eksperimentelle

smertemodeller i raske forsøgspersoner også undersøger smerter ved inflammation og hyperalgesi. Dette sikrer, at man ved undersøgelser af analgetika kan opnå viden om stoffets varierende effekt ved hyperalgesi. Studiet forbandt fund omkring opioiders mulige differentierede virkninger fra eksperimentelle studier i raske frivillige forsøgspersoner til patienter og udgjorde et vigtigt translationelt led i smerteforskningen.

Endeligt blev oxycodons farmakokinetiske-farmakodynamiske egenskaber undersøgt i studie IV. Da et lineært koncentration-effekt forhold blev fundet, var det ikke muligt at demonstrere en forsinket dynamisk effekt i forhold til plasmakoncentrationen, som man ville forvente, hvis virkningen af opioidet var central. Dette kunne muligvis skyldes en initial perifer effekt af oxycodon ved hyperalgesi, hvor smertesystemet forventes at være opreguleret.

Eksperimentelle smertemodeller kan vise forskellige effekter af analgetika, og der er studier, der viser at perifer opioid analgesi øges ved hyperalgesia og inflammation. Derfor kunne næste skridt i smerteforskningen være at undersøge effekten af perifert virkende opioider yderligere i en eksperimentel smertemodel med hyperalgesi.

En anden mulighed er at kæde de eksperimentelle resultater yderligere sammen med den kliniske virkelighed. Dette kan gøres i veldegnede kliniske studier, der sammenligner effekten af morfin og oxycodon samt andre opioider yderligere i det opregulerede smertesystem. Endvidere er studier af opioiders effekter i hjernen med billeddannende og neurofysiologiske metoder stadig et relativt uudforsket område og vil muligvis kunne bidrage yderligere til forståelsen af opioiders differentierede effekter. Herved vil det samtidig være muligt at belyse forskelle i smertesystemet hos raske forsøgspersoner, og patienter.

Tables

Table 2. Visceral experimentally induced hyperalgesia in healthy human volunteers

Author, year	Model	Results	Central sensitization
(Bouin et al. 2001)	10 ml glycerol in the rectum	Intraluminal injection of glycerol increased rectal tone and sensitized healthy volunteers to rectal distension, since they showed significantly lower thresholds after glycerol.	-
(Brock et al. 2009)	Distal oesophageal acid+capsaicin infusion (7 cm above LOS). 180 ml HCL 0.1M and 2 mg capsaicin in 20 ml solvent at a rate of 10 ml/min.	Rectal hyperalgesia to heat and mechanical stimulations were demonstrated after chemical perfusion of the oesophagus	Yes
(Drewes et al. 2003a)	Distal oesophageal acid infusion (7 cm above LOS). 100 ml 0.1 M HCl, 4 ml/min for 30 min	Hyperalgesia to electrical and mechanical stimuli and allodynia to cold and warmth stimuli. Referred pain area to mechanical stimuli increased indicating the presence of central hyperexcitability.	Yes
(Drewes et al. 2003b)	Increasing volumes of capsaicin 50 µg/ml (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3 ml), glycerol (2.5, 5, and 10 ml) applied to the ileum via the stomal opening	Hyperalgesia was found to distension of the gut after capsaicin. No difference after application of glycerol. Application of capsaicin to the human ileum induced pain and mechanical hyperalgesia.	Yes
(Frokjaer et al. 2005)	Distal oesophageal acid infusion (5 cm above LOS). 200 ml 0.1 M HCl	11 of 12 volunteers showed increased sensitivity to one or more stimulation modalities. Central mechanisms could explain remote hyperalgesia	Yes
(Gonzalez et al. 1998)	Intraoesophageal application of a capsaicin-containing red pepper sauce (Tabasco) suspension.	Decreased perception and discomfort threshold of intraoesophageal balloon distension	-
(Hammer & Vogelsang 2007)	Intraluminal capsaicin in different regions of the upper gastrointestinal tract (200 µg/mL) capsaicin. Infusion rate was 2.5 mL/min, duration of perfusion was 60 min or until discomfort.	Balloon pressures needed to induce perception were unaffected, except that first sensation in the jejunum was reached at a lower barostat pressure after capsaicin. In contrast jejunum balloon volumes were significantly lower after capsaicin.	-
(Hobson et al. 2004)	Duodenal acid infusion. 0.15 M HCl, 30 min	After acid infusion, there were reproducible reductions in esophageal pain threshold to electrical stimulation. Comparison of the latencies of EEP components prior to and following acid and saline infusion revealed a reduction in the N1 and P2 components.	Yes
(Hu, Martin, & Talley 2000)	Distal oesophageal acid infusion (8.5 cm above LOS). 0.1 M HCl, 7 ml/min for 20 min.	Reduced perception threshold to mechanical distension of oesophagus	-
(Pedersen et al. 2004)	Distal oesophageal acid perfusion. 0.1 M hydrochloride acid was infused (7 cm above LOS) at a rate of 7 ml/min for 30 min.	Following acid perfusion there was a selective sensitization to the heat pain stimuli whereas the sensation to the cold stimuli was unchanged. After acid perfusion, the referred pain area to the heat pain stimulation increased but did not change to cold stimulation	Yes

Table 2 (continued).

Author, year	Model	Results	Central sensitization
(Sami et al. 2006)	Distal oesophageal acid infusion (7 cm above LOS). 200 ml 0.1 M HCl, 8 ml/min	The sensitization resulted in decreased pain threshold to electrical stimulation.	Yes
(Sarkar et al. 2001)	Distal oesophageal acid infusion (3 cm above LOS). 0.15 M HCl	Reduced pain threshold to electrical stimulation of the non-acid-exposed proximal oesophagus.	Yes
(Schmidt et al. 2004)	Perfusion site at the ligament of Treitz and, 7 cm distally, a barostat balloon. Capsaicin solutions (40, 200, and 400 µg /ml) 2.5 ml/min were perfused for 60 minutes or until severe discomfort occurred.	Pain thresholds during distensions were not different before and after capsaicin perfusion. Results ruled out the fact that abdominal discomfort evoked by capsaicin involves sensitization of mechanoreceptors	-
Study II	Distal oesophageal acid+capsaicin infusion (8 cm above LOS). 180 ml HCL 0.1M and 2 mg capsaicin in 10 ml solvent	Reduction of the pain threshold to heat and electrical stimuli. Increase of the referred pain areas to mechanical and electrical stimulation. All volunteers were sensitized to one or more modalities by acid+capsaicin.	Yes
(van den Elzen, Tytgat, & Boeckxstaens 2008)	Distal oesophageal acid infusion (3 cm above LOS). 30 min HCL 0.15 M	Induction of visceral hypersensitivity without affecting somatic sensitivity to electrical stimulation.	No
(Willert et al. 2007)	Distal oesophageal acid infusion (3 cm above LOS). HCl 0.15 M, 8 mL/min for 30 min.)	Reduced pain threshold to electrical stimulation on the anterior chest and proximal oesophagus but not the foot.	Yes

LOS = Lower oesophageal sphincter, HCl = Hydrochloric acid

Table 3. Morphine and experimental pain in acute experimental pain models in healthy volunteers

Author, year	Dose	Model	Results
(Arendt-Nielsen, Oberg, & Bjerring 1991)	4 mg injected perineurally to ulnar nerve and epidurally	Short lasting radiant heat PDT and warmth detection threshold), evoked brain potentials (argon laser, 200 ms)	PDT and warmth detection threshold increased Amplitude of evoked brain potentials decreased Latencies of evoked brain potentials increased after perineural administration
(Brennum et al. 1993)	4 mg epidural	Heat and cold skin stimulation detection threshold, PDT, PTT (1 and 2°C/s) Electrical skin stimulation PDT, VAS to 1 ms stimulation Pressure algometry PDT, PTT, moderate pain to 20 ms stimulation (toe nail) Short lasting radiant heat VAS	Cold detection threshold unaffected Warmth detection thresholds, PDT, PTT to heat, pressure, and electrical stimulation increased VAS after short lasting radiant pain decreased Short lasting pain to mechanical and electrical stimulation unaffected
(Chapman et al. 1990)	0.142 mg/kg IV	Electrical tooth stimulation evoked brain potential, subjective pain report	Amplitude of evoked potentials and pain score decreased
(Eckhardt et al. 2000)	60 mg extended release p.o.	Cold pressor test PTT	PTT increased
(Fillingim et al. 2005)	0.08 mg/kg IV	Heat skin stimulation PDT, PTT (0.5°C/s) Deep pressure PDT Ischemic pain PDT, PTT	All thresholds were increased
(Grach et al. 2004) (Naef et al. 2003)	0.5 mg/kg p.o. 30 mg p.o.	Cold pressor test PDT, PTT, VAS Heat skin stimulation PDT, PTT (2°C/s), cold, Pressure algometry PTT (finger pulp) Electrical skin stimulation Cold pressor test VAS	Latency to onset of pain and PTT increased Pain to pressure, cold pressor test and electrical stimulation were decreased. Heat pain unaffected
(Plesan, Sollevi, & Segerdahl 2000) (Poulsen et al. 1996)	0.1 mg/kg IV 20 mg or 30 mg single dose p.o.	Ischemic pain VAS Cold pressor test AUC _{VAS} , peak pain, discomfort (VAS) Heat skin stimulation and deep pressure PDT	VAS decreased as morphine reduced the pain rating by 27% Peak pain and discomfort in cold pressor test decreased AUC _{VAS} and heat PDT and PTT unaffected Pressure PTT decreased whereas PDT unaffected
(Price et al. 1985)	0.04 – 0.08 mg/kg IV	Heat skin stimulation VAS (sensory and affective dimension) to graded temperatures and VAS to brief pulses (first and second pain)	Dose<0.06: VAS (affective) decreased, VAS (sensory) unaffected Dose>0.06: VAS (affective and sensory and first and second pain) decreased
(Pud et al. 2006)	0.5 mg/kg p.o.	Cold pressor test PDT, PTT, VAS	PDT and PTT increased
(Quante et al. 2004)	10 mg IV	Intra cutaneous electrical stimulation VAS, evoked brain potentials and frequency analysis	Amplitude of evoked potentials and VAS decreased EEG frequency spectrum unaffected

Table 3 (continued).

Author, year	Dose	Model	Results
(Roberts, Gennings, & Shih 2006)	0.02 mg/kg IV	Heat skin stimulation VAS (affective and sensory) (37, 49 and 51 °C)	Affective or sensory responses unaffected
(Schulte et al. 2003)	0.1 mg/kg IV	Cutaneous and intramuscular electrical stimulation PDT, AUC _{VAS} to suprathreshold stimulation for 10 s. Intramuscular injection of hypertonic saline AUC _{VAS}	PDT to intramuscular electrical stimulation increased PDT to electrical skin stimulation, AUC _{VAS} after suprathreshold stimulation and hypertonic saline unaffected
(Schulte et al. 2006)	0.14 and 0.28 mg/kg IV	Intramuscular injection of hypertonic saline AUC _{VAS} Intramuscular electrical stimulation PDT, AUC _{VAS} to suprathreshold stimulation for 10 s.	High dose: All parameters affected. Low dose: All parameters unaffected
(Segerdahl, Ekblom, & Sollevi 1994)	0.1mg/kg IV	Ischemic pain AUC _{VAS}	AUC _{VAS} decreased
(Smith et al. 1966)	10mg/70kg IV	Ischemic pain PTT	PTT increased
(Staahl et al. 2006a)	30 mg p.o.	Heat skin stimulation PTT (2°C/s) Deep pressure PTT Pinching PTT Cutaneous and intramuscular electrical stimulation PTT Oesophageal distension and electrical pain PTT Oesophageal heat pain PDT	Oesophageal heat pain unaffected The remaining pain thresholds parameters decreased
(Tegeuder et al. 2003)	100 ng/ml IV	Electrical skin stimulation PDT, PTT	PDT and PTT increased
(van der Burght et al. 1994)	0.15 mg/kg IV	Short lasting radiant heat warmth detection threshold, pinprick PDT	Warmth detection or pinprick PDT unaffected

PDT = pain detection threshold, PTT = pain tolerance threshold, VAS = visual analogue scale, AUC = area under the curve.

Table 4. Morphine and experimental pain in experimental pain models including hyperalgesia in healthy volunteers

Author, year	Dose	Model	Results
(Koppert et al. 1999)	40 ml 0.01 % IV	Ultraviolet (UV-B) skin radiation PDT to heat VAS to mechanical impact	Heat PDT increased VAS to mechanical impact unaffected
(Møiniche, Dahl, & Kehlet 1993)	2 mg subcutaneously	Burn injury, PDT to heat (1°C/s) and deep pressure	Heat PDT increased Mechanical PDT increased
(Schulte, Sollevi, & Segerdahl 2004)	10 µg/kg/min for 45 min. IV	Burn injury PDT to pinprick in injured skin, area of secondary HA to pinprick Repeated pinprick AUC _{VAS}	No parameters were affected
(Schulte, Sollevi, & Segerdahl 2005)	15 and 30 ng/ml measured at steady state	Burn injury in the skin. PDT to pinprick in injured skin, area of secondary HA to pinprick Repeated pinprick AUC _{VAS} Continuous electrical skin stimulation PDT to pinprick, area of secondary HA to pinprick, repeated pinprick (AUC _{VAS}),	Only the high dose had significant effect. Continuous electrical skin stimulation: PDT increased and area of HA decreased Burn injury and repeated pinprick: no parameters were significantly affected
(Tegeder et al. 2003)	100 ng/ml IV	Freeze lesion PDT to pinprick Concentric and eccentric muscle contraction VAS	PDT to pinprick decreased Muscle pain intensity unaffected
(Warncke, Stubhaug, & Jorum 1997)	0.15 mg/kg IV	Burn injury in skin heat and cold detection threshold and PDT in primary and secondary area of hyperalgesia (1°C/s), area of secondary hyperalgesia to pinprick, detection threshold to pinprick, appearance of wind-up like pain to repeated pinprick stimulation	No parameters were affected
(Warncke, Stubhaug, & Jorum 2000)	150 µg /kg + infusion 1 µg /kg per min and 0.5 µg /kg per min	Hyperalgesia induced by burn injury in the skin. The area of secondary hyperalgesia was quantitated using punctate (von Frey filaments) and brush stimuli (electric brush)	Morphine treatment starting preinjury significantly reduced the development of secondary hyperalgesia
Study III	30 mg p.o.	Chemical sensitization of oesophagus Skin heat Muscle pressure (cuff) Oesophageal stimulation: heat, pressure, electrical	Skin heat PTT unaffected, muscle pressure PTT increased. Oesophageal parameters unaffected

PDT = pain detection threshold, PTT = pain tolerance threshold, VAS = visual analogue scale, AUC = area under the curve.

Table 5. Oxycodone and experimental pain models in healthy volunteers

Author, year	Dose	Model	Results
(Stahl et al. 2006a)	15 mg, oral solution	Heat skin stimulation PTT (2°C/s) Deep pressure PTT Pinching PTT Cutaneous and intramuscular electrical stimulation PTT Oesophageal distension and electrical pain PTT Oesophageal heat pain PDT	Pain thresholds parameters decreased
(Zwisler et al. 2009)	20 mg oxycodone, 2 capsules of 10 mg each	pain detection and tolerance thresholds to single electrical sural nerve stimulation, pain summation threshold to repetitive electrical sural nerve stimulation and the cold pressor test with rating of discomfort and pain-time area under curve	For single sural nerve stimulation, increase in thresholds on oxycodone in pain detection and pain tolerance thresholds. In the cold pressor test, there was a reduction in pain AUC.
Study III	15 mg, oral solution	Chemical sensitization of oesophagus Skin heat Muscle pressure (cuff) Oesophageal stimulation: heat, pressure, electrical	Heat skin PTT increased Muscle pressure PTT increased Referred pain area to heat stimulation of the oesophagus decreased PDT to electrical stimulation of the oesophagus increased

PDT = pain detection threshold, PTT = pain tolerance threshold, VAS = visual analogue scale, AUC = area under the curve.

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