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Transdermal Opioids in Experimental Pain and Hyperalgesia Evoked in Skin, Muscle and Bone

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The present thesis is partly based on the papers below, which are referred to in the text by Roman numerals. The studies have been carried out at Mech-Sense, Department of Gastroenterology, Aalborg Hospital in cooperation with Center for Sensory-Motor Interaction, Department of Health Science and Technology, Aalborg University.

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Abbreviations

ACC : anterior cingulated cortex
AMPA : α-amino-3-hydroxy 5-methyl-4-isoxazeloproprionic acid
CNS : central nervous system
DLPT : Dorsolateral pontine tegmentum
DNIC : Diffuse noxious inhibitory system
DRN : dorsal reticular nucleus
EEG : electroencephalography
EP : evoked potential
NGF : Nerve growth factor
NMDA : *N*-methyl-D-aspartic acid
NS : Nociceptive-specific
PAG : Periaqueductal gray
PB : Parabrachial
PDT : Pressure detection threshold
PK-PD : Pharmacokinetic/pharmacodynamic
PTT : Pressure tolerance threshold
PTTol : Pressure tolerance threshold
TDS : Transdermal delivery system
RVM : Rostral ventromedial medulla
UVB : Ultra violet B-light
WDR : Wide dynamic range

1. Introduction

Pain is the most common reason why patients seek medical attention [1]. Twenty percent of the adult Europeans suffer from chronic pain and treatment is a major challenge in the clinic. Very few patients are managed by pain specialists and nearly half receives inadequate pain management [2;3]. Hence, there is need for further research and focus on pain physiology and treatment.

1.1 Treatment

Guidelines have been developed for the treatment of pain based on the World Health Organization three-step 'ladder' (figure 1). Nevertheless, there is substantial evidence that pain is inadequately treated [1]. Opioids are widely used in the treatment of moderate to severe non-malignant and malignant pain. The responsiveness, defined as the balance between analgesia and dose-limiting toxicity during dose titration, varies tremendously among individual patients and the different types of pain (chronic, non-malignant, malignant or neuropathic pain) [4].

This thesis focus on two opioids for treatment of moderate to severe pain - buprenorphine and fentanyl administered as transdermal patches. Transdermal delivery systems (TDS) provide steady and constant delivery of a drug thereby maintaining steady state up to several days, permitting less frequent dosing. This leads to improvement in dose control and better compliance and TDS´ are therefore valuable in the treatment of chronic pain conditions [5].

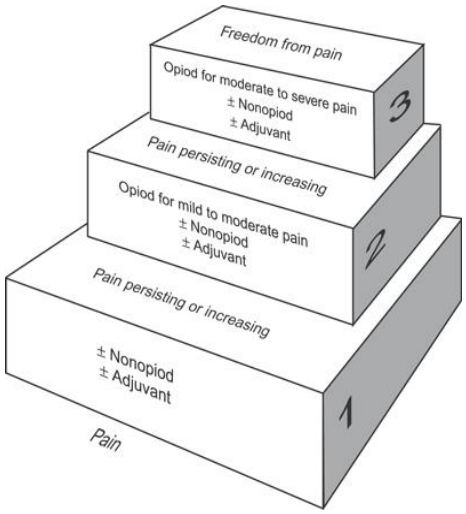

Figure 1 WHO´s pain ladder. If pain occurs, there should be prompt oral administration of drugs in the following order: non-opioids (aspirin and paracetamol); then, as necessary, mild opioids (codeine); then strong opioids such as morphine, until the patient is free of pain. To calm fears and anxiety, additional drugs – "adjuvants" – should be used. This three-step approach of administering the right drug in the right dose at the right time is inexpensive and 80-90% effective.

Opioids have been classified based on their chemical properties, but this has limited usefulness for clinical purposes. Therefore a more functional classification based on their receptor activity at the various receptor types (μ-, κ- and δ-receptors) is now used to ease predictions on clinical effects [6]. Nevertheless, opioids have disadvantages that limit their use in long term treatment, including

potential development of tolerance or unpleasant side effects such as e.g. constipation, sedation or respiratory depression [1;7]. On the other hand, buprenorphine is an opioid with no analgesic ceiling effect, but with a ceiling effect for respiratory depression, reducing the likelihood of this potentially fatal adverse event [8]. Long term treatment with opioids acting as full μ-agonists e.g. morphine may paradoxically even lead to hyperalgesia and thereby exacerbate the pain perception in patients [9;10]. Animal studies suggest that the δ-opioid receptors play an important role in hyperalgesia and in bone associated pain [11-13]. This is of major importance as bone associated pain is frequent in the clinic and is difficult to treat [12]. Osteoarthritis is one of the most common diseases worldwide and a major source of osteoarthritis associated pain derives from nociceptive receptors in the superficial bone and joint structures [14]. Cancer patients with bone metastases also suffer from bone associated pain which is difficult to treat adequately [15-17]. Therefore, new management approaches using opioids with different selectivity to the μ-, κ- and δ-opioid receptors may be important in treatment of hyperalgesia as well as bone associated pain [18] (II). Moreover, pain treatment is often based on a trial and error regimen, but more rational and evidence based approaches should be preferred to obtain effective dosing regimens in the clinic. Pharmacokinetic-pharmacodynamic (PK-PD) modelling can be used to characterize the relationship between drug concentration and drug effect and thus provide a base for a more rational therapy for the individual patient [19] (IV).

1.2 Experimental pain

Regardless of whether an experimental stimulus is electrical, thermal, mechanical or chemical, it is essential that three of its parameters are controlled, namely the intensity, the duration and the surface area to which it is applied. These parameters determine the quantity of nociceptive information from the periphery to the central nervous system [20]. Due to confounders in the clinic e.g. nausea, general malaise, it can be difficult to evaluate the specific pain mechanisms of analgesics in patients with pain, and hence experimental and standardized pain-biomarkers can be used [21]. Experimental pain in healthy volunteers makes is possible to overcome some of the bias (e.g. cognitive, emotional and social aspects) that has an influence on the testing of analgesic compounds [22]. Human experimental pain models therefore appear to be better suited to investigate not only the analgesics effects but also to study pain mechanisms.

In standardized experimental pain studies it is possible to control precisely the localisation, intensity, duration and modality of the stimulus [23]. 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 (I)), 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 (II).

Fundamentally experimental pain models can be divided into acute models and models inducing hyperalgesia [24]. Acute models are short lasting stimuli that activate normal physiological mechanisms and hence limited psychological involvement. Thus, this kind of pain is

believed to be less relevant to mimic pathological pain [21]. Models inducing hyperalgesia can be difficult to control regarding reproducibility compared to acute pain models. Nevertheless, models inducing hyperalgesia is thought to mimic the clinical situation to a higher degree than acute models, as they alter the peripheral and the central pain system [21;25]. The most reliable experimental approach is probably established by multi-modal testing combining acute phasic pain models with models inducing hyperalgesia in the same set-up. This gives the possibility to investigate tissue and modality differentiated as well as anti-hyperalgesic effects of analgesics [24;25] (II).

Acute superficial experimental pain can be induced by stimulating the skin with heat. Heating with 1°C/s preferentially activates C-fibres, which is thought to be important for peripheral opioid receptors [26]. Application of pressure algometry is the most frequently used technique for quantification of pain from deeper tissue (group III (Aδ-fibres) and IV (C-fibres)) [27]. Pressure to the tibia induces bone associated pain, but elements of pain from the skin may contribute to the pain [25] (I). Another model to induce deep pain is the immersion of the hand in cold water (cold pressor test). This model activates the sympathetic nervous system innervating muscle and nerve fascicles and also activates "diffuse noxious inhibitory control" (DNIC) [25;28] (III).

Hyperalgesic pain can be induced experimentally in e.g. the skin by intradermal injection of capsaicin [29]. Other models producing inflammation and hyperalgesia are the burn injury model using ultra-violet B-light (UVB-light) [30] or intramuscular injection of nerve growth factor (NGF), inducing a long lasting soreness and sensitivity to mechanical stimulation [31] (II).

The phasic pain models as well as the models inducing hyperalgesia mentioned above have shown to be sensitive to opioids [25]. Therefore these models were chosen for the studies underlying this thesis.

1.3 Hypothesis

The pathogenesis of bone associated pain is still not fully understood but it is known that the periosteum is innervated by unmyelinated nociceptive afferents [32]. These nociceptors are sensitive to high intensity pressure [32;33], and animal studies have indicated that δ-opioid receptors may play an important role in controlling bone associated nociception [11;13]. As data from animal studies cannot be translated directly to humans, a human model of experimental bone associated pain could be valuable in future studies investigating basic and pharmacological features of bone related pain. Mechanical stimulations using different probe sizes have been used in several studies to evoke experimental pain from deeper tissues such as muscle, nerve and periost [34-38]. However, so far no study has systemically investigated which probe size could be the most optimal to evoke bone associated pain. It was therefore *hypothesized* that it would be possible to evoke bone associated pain using pressure algometry and that a reproducible and valid model could be developed.

Animal studies suggest that δ-receptor-agonists have an antihyperalgesic effect [11]. Animal studies have furthermore showed that δ-receptors play an important role in bone associated

nociception which in clinical practice is difficult to treat [12;13]. Moreover, it has been demonstrated that endogenous κ-agonists (dynorphin) promote hyperalgesic pain states [39]. New management approaches have therefore been taken using opioids with a differential selectivity towards δ- and κ-receptors [40;41]. Buprenorphine is an opioid that exert its effects through μ-, κ- and δ-receptors and may therefore be important in the treatment of hyperalgesia as well as bone associated pain [8;18]. Buprenorphine has been used in clinical practise for more than 30 years using various administration formulations such as intravenous, sublingual and spinal-epidural [42]. However, after the introduction of TDS of the drug, there has been a renaissance of interest in its analgesic profile.

The analgesic effect of transdermal buprenorphine has previously been investigated in experimental pain in healthy volunteers [43]. In this study the analgesic effect of transdermal fentanyl – a μ-opioid agonist – was also investigated. A study by Koppert et al. showed different analgesic – antihyperalgesic ratios between buprenorphine and fentanyl [44]. As changes in the pain and opioid systems occur after sensitization it is of interest 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 of 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 the clinical pain situation [21;24]. It was *hypothesized* that buprenorphine and fentanyl could modulate experimentally induced pain from phasic tissues as well as experimentally induced hyperalgesia differently (II).

Previous findings in clinical trials have suggested that less efficacious DNIC is associated with fibromyalgia, musculoskeletal pain syndromes and irritable bowel syndrome [45-47]. It was speculated that the ongoing chronic pain might “exhaust” DNIC, making it less effective. An alternative explanation for the relationship between chronic pain and decreased DNIC was that less effective DNIC constitutes a predisposition to acquire chronic pain [48]. Willer et al. 1990 further showed that an opioideric link was involved in DNIC both in animals as well as in humans [49]. It was therefore *hypothesized* that the effect of buprenorphine and fentanyl could modulate experimentally induced DNIC (III).

Treatment with opioids is often based on a trial and error regimen. However, a more rational approach can be used to develop effective dosing regimens in the clinic [19]. This approach involves PK-PD modelling which can be used to characterize the relationship between drug concentration and drug effect [19]. Opioid receptors are mainly located in the central nervous system, why the effect of an opioid is likely to be delayed relative to the plasma concentration profile, corresponding to the time needed to pass the blood-brain barrier [50]. PK-PD modelling uses quantitative methods to mathematically characterize the relationship between drug concentration and drug effect [19]. This led to the *hypothesis* of study IV, that it would be possible to quantify the PK-PD relationship for buprenorphine and fentanyl while adjusting for the time-course for the placebo response.

1.4 Aims

The overall purpose of this project was to develop a human experimental bone pain model (Study I), to evaluate the analgesic and anti-hyperalgesic effect of buprenorphine and fentanyl (Study II), to investigate opioids and DNIC (Study III) and finally assess the PK-PD relationship for transdermal opioids (Study IV).

Hence the specific aims were to:

- Develop and evaluate an experimental human bone model
- Evaluate the effects of transdermal buprenorphine and fentanyl in experimental induced skin, muscle, bone and hyperalgesic pain
- Investigate the link between opioids and the endogenous pain inhibiting system
- Describe the PK-PD relationship for transdermal opioids

2. Pain

Pain has a major influence on a patient's quality of life due to decreased mobility, increased risk of depression, risk of social isolation etc (figure 2). Pain is a subjective phenomenon and the healthcare personal is therefore struggling to give the individual patient the most optimal treatment [3].

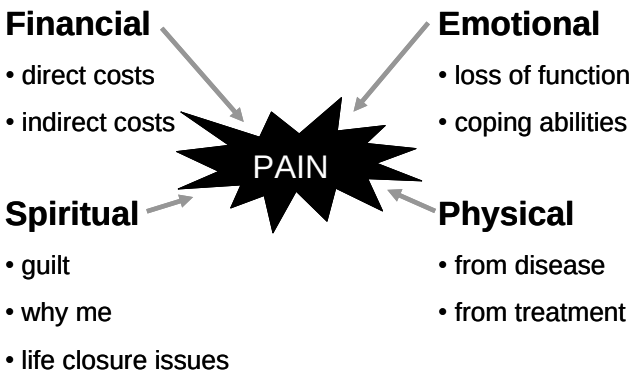

Figure 2 Schematic overview of factors influencing a patient's perception of pain, factors that can also bias the outcome in clinical trials.

Pain is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' by the International Association for the study of Pain. It is considered as a *perception* and not as a *sensation* due to the fact that it involves a psychological component. In contrast to nociception, human pain is a complex network of neurons within the central nervous system and is often referred to as the 'Pain Matrix'. A simplified overview is given in figure 3.

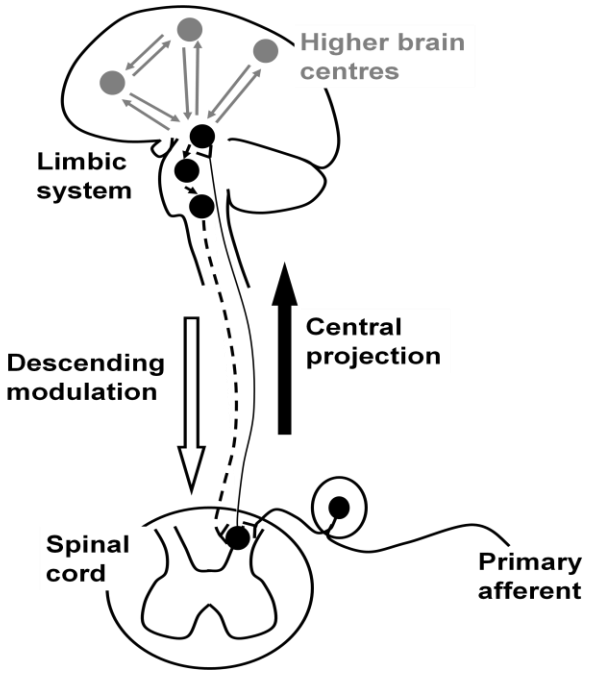

Figure 3 Illustration of the central nervous system involved in pain signalling (inspired by [51-54]. Pain occurs in the periphery and is transmitted through primary afferents to the dorsal horn of the spinal cord (see figure 4 for further details). From here the signal is led via central projection neurones to higher brain centres, where interconnectivity between these centres occurs (see figure 5 for further details). This activates the limbic system leading to descending modulation of the pain signal.

2.1 Sensory transmission

When peripheral sensory fibres (primary afferent fibres) respond to noxious stimuli they transmit this information through the dorsal root ganglion to the dorsal horn of the spinal cord. From here projection neurones activate higher brain centres [52]. There are three main types of primary afferents fibres, having different properties, allowing them to respond to different types of sensory information [52]:

- Aβ-fibres which are large in diameter and myelinated, allowing them to quickly conduct action potentials from the periphery to the central nervous system (CNS). They respond to light touch and convey tactile information
- Aδ-fibres which are small in diameter and thinly myelinated, transmitting a stimulus slower than the Aβ-fibres. They respond to noxious stimuli which can be mechanical, thermal or chemical
- C-fibres which are thin, non-myelinated, transmitting a stimulus slower than both the Aβ- and Aδ-fibres. They respond to the same stimuli as the Aδ-fibres

Aδ- and C-fibres are nociceptors or “pain-fibres” and an activation of these receptors results in a graded potential reflecting the stimuli intensity [55]. Moreover, there are sensory fibres that do not respond to high intensity stimulation under normal conditions but are spontaneously activated and respond to innocuous stimuli under inflammatory conditions. These fibres are termed “silent nociceptors” [56].

2.1.1 Superficial nociceptors

Several types of nociceptors exist in the human skin [57]. The cutaneous Aδ-fibres are associated with the "first pain", which is a characteristic sharp pain felt immediately after noxious stimuli. These fibres are mainly specialized for detection of dangerous mechanical and thermal stress and for triggering a rapid nociceptive response and protective flex [57]. Different subtypes of Aδ-fibres have been identified – type I and type II. Type I respond rapidly to damage and may play an important role in hyperalgesia as they are easily sensitized. Type II reacts slower than type I and seems to be responsible for the "first pain" [58]. In contrast, C-fibres are associated with the "second pain", which is a dull, burning sensation felt after the "fist pain". C-fibres are mainly polymodal but some are specialized for detecting a single stimulus as heat or pinch. These fibres detect strong mechanical, thermal and chemical stimulation [58].

2.1.2 Deep muscle and bone nociceptors

Skeletal muscles are innervated by type III (Aδ- fibres) and IV (C-fibres) that terminate as free nerve endings. The term 'free nerve ending' indicates that when seen under a light microscope, the nerve ending lacks a visible receptive structure [59]. The classification into III and IV is only used for muscle afferents and is based on the diameter of the fibres. Substance P, calcitonin-gene-related peptide and NGF are some of the neuropeptides that are present in the nerve free endings. These neuropeptides are synthesized in the dorsal root ganglion and transported to the receptive endings of the primary afferent muscles as well as to the central terminals in the spinal cord [60]. Findings in animal studies indicate that Aδ- and C-fibres innervating skeletal muscles possesses high mechanical thresholds and slowly adapting responses, consistent with a role for these fibres in mechanical nociception. In addition these fibres also respond to a variety of substances, suggesting that they are also chemo sensitive nociceptors. Small subpopulations of the fibres also respond to innocuous and noxious thermal stimuli. These findings suggest that the majority of Aδ- and C-fibres serve as polymodal nociceptors [61]. Inputs from deep tissues produce more robust dorsal horn hyper excitability than inputs from cutaneous tissues [62].

The periosteum of the bone is a frequent site of pain and is innervated by Aδ- and C-fibres. The terminals of these fibres are especially extensive in the periosteum of long bones and mostly contain polymodal receptors [57]. The periosteum is therefore very sensitive to mechanical stimulation [63] (I).

2.2 The spinal level

Transmission of stimuli from the periphery to the brain goes through the spinal cord. The spinal cord is an important site where various incoming sensory and nociceptive signals undergo convergence and modulation [52]. The dorsal horn of the spinal cord is the major zone for primary afferents to transmit information from the periphery to the nervous system. The dorsal horn is organized into different laminae, where the central terminals of primary afferents terminate. Most nociceptive Aδ- and C-fibres terminate superficially in laminae I-II, but also terminate directly (Aδ-

fibres) or poly-synaptic (C-fibres) in lamina V, whereas Aβ-fibres predominantly innervate laminae III-V [52] (see figure 5 in section 2.4).

The spinal cord contains various neuronal cell types which connect with the primary afferents. Some cells are termed nociceptive-specific (NS) cells and are mostly found superficially and synapse with Aδ- and C-fibres only [52]. Cells interacting with Aβ-fibres are proprioceptive and only respond to touch. Another type present in the spinal cord is termed wide dynamic range (WDR) neurones. They receive input from all three types of sensory fibre and therefore respond to a full range of stimulation from light touch to noxious stimuli. WDRs fire action potentials in a graded fashion depending on stimulus intensity and also exhibit 'wind-up', which is a short-term increase in the excitability of dorsal horn neurones [52]. This short term phenomenon is believed to be linked to *N*-methyl-D-aspartic acid (NMDA) receptors [64]. Besides excitatory interneurones there also exist inhibitory interneurones in the spinal cord. The response of NS cells and WDRs can therefore be increased as well as decreased. Hence, the output of the dorsal horn is influenced [52].

Primary afferents release a variety of chemical mediators e.g. substance P, calcitonin gene related protein, but all appear to use glutamate as their principal neurotransmitter to excite second order neurons in the dorsal horn of the spinal cord [54]. These neurotransmitters mediate nociceptive signalling in the spinal cord through activation of various receptors. The most important receptors are α-amino-3-hydroxy 5-methyl-4-isoxazeloproprionic acid (AMPA) and NMDA, but also the kinate receptor plays a role when nociceptive signals are conveyed [53]. A simplified illustration of the pharmacological mechanisms is given in figure 4.

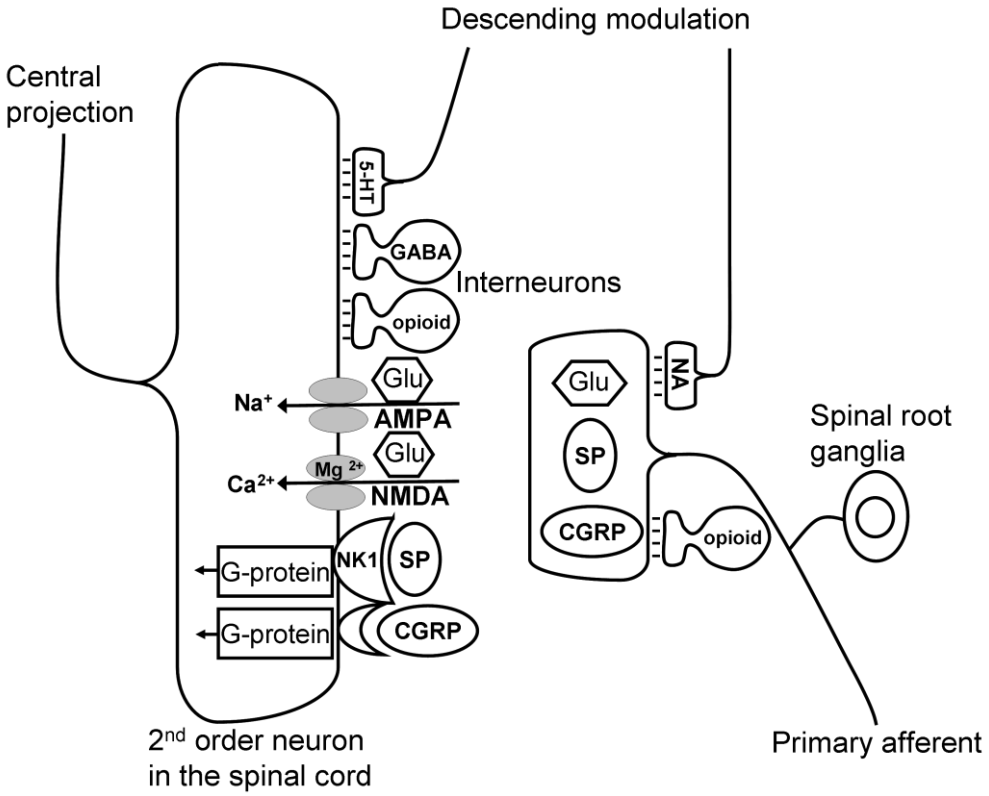

Figure 4 Simplified overview of the most important neurotransmitters that mediate nociceptive signalling in the spinal cord (inspired from [52-54]). Right side of the figure shows the presynaptic terminal of the primary afferent neuron. Left side of the figure shows the postsynaptic terminal of the second order neuron. The predominant presynaptic nociceptive transmission is through release of neurotransmitters e.g. glutamate (Glu), substance P (SP) and calcitonin gene-related peptide (CGRP), which in return act on receptors in the postsynaptic terminal of the second order neuron. Glutamate activates the receptors alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) and *N*-methyl-D-aspartate (NMDA – Mg^{2+} normally block this receptor, but is very likely unblocked by the neurotransmitters co-released with glutamate e.g. SP and CGRP). Activation of AMPA is responsible for the initial response of spinal dorsal horn neurones to both noxious and tactile stimuli. Whereas, if repetitive and high-frequency stimuli occur, there is a prolongation of the response of spinal dorsal horn neurones (wind-up), which is a result of enhanced activity of the NMDA receptors. SP activates the neurokinin-1 (NK1) receptor. It is unknown, at which receptor CGPR exerts its action, but the presence of CGRP extends that of SP in the release zone and therefore it contributes to the nociceptive transmission. These events all lead to mediation of nociceptive signalling to supraspinal sites. In response there is a descending modulation from supraspinal sites. Modulatory inhibition is mainly through noradrenergic (NA) and serotonergic (5-HT) pathways, pre- and postsynaptically. However, it can be facilitatory as well, depending on the receptor subunit classes. Other mechanisms that modulate nociceptive transmission in the spinal cord are the interneurons releasing gamma-aminobutyric acid (GABA) and endogenous opioid peptides.

2.3 The supraspinal level

The output from the dorsal horn in the spinal cord to higher brain centres is carried out by spinal projection neurones along ascending pathways [52]. It has been shown that neurokinin-1 cells in laminae I project to areas in the brain such as the thalamus, the periaqueductal gray (PAG) and the parabrachial (PB) and that these cells respond to noxious stimuli. In addition these neurones also

project into brainstem areas such as the rostral ventromedial medulla (RVM), a region that has descending projections back to the dorsal horn [52]. A large number of projection neurones are also found in the deeper lamina III-V predominantly projecting to the thalamus and primarily provides the sensory component of the pain experience [52]. From the thalamus the nociceptive information is transmitted to several regions of the brain such as primary and secondary somatosensory cortices, insular, anterior cingulate and prefrontal cortices [51] (figure 5). These areas then project to the PAG, which in turn indirectly controls pain transmission in the dorsal horn through the RVM and the dorsolateral pontine tegmentum (DLPT).

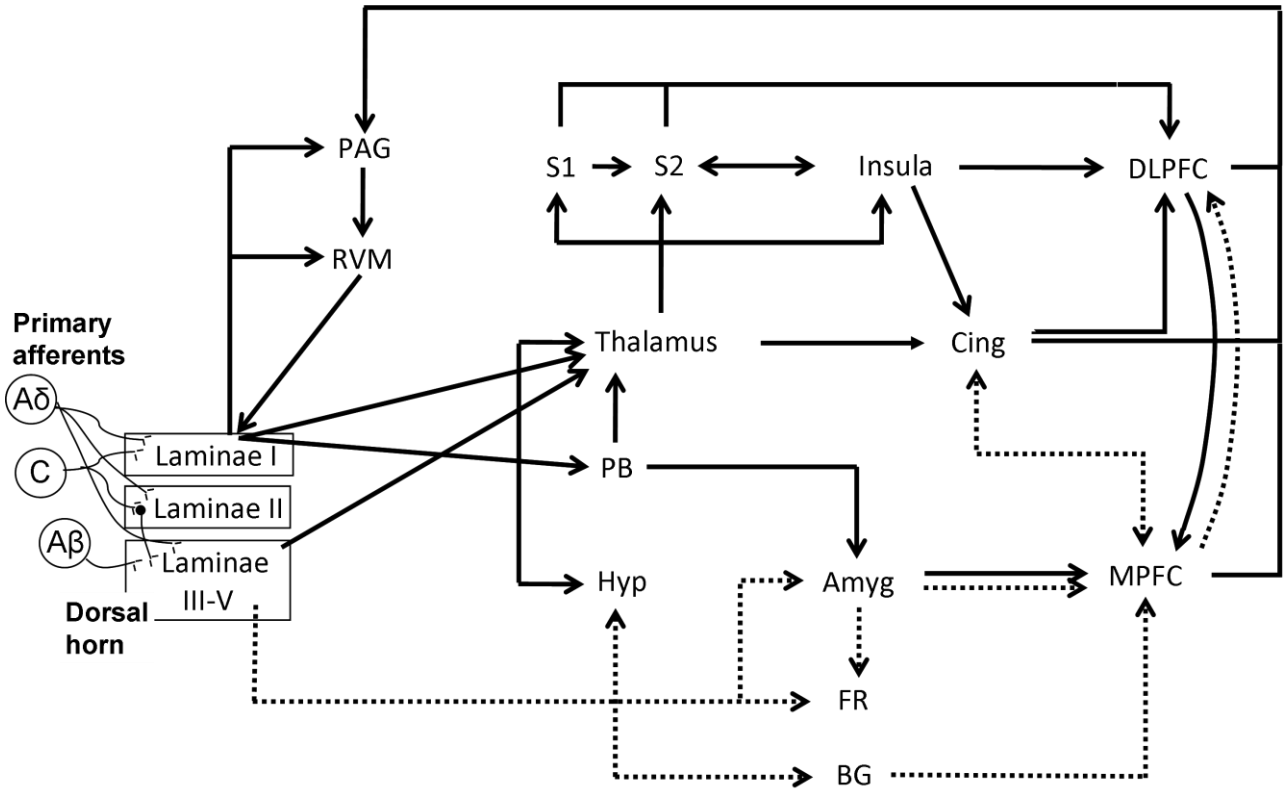

Figure 5 Schematic simplified illustration of the main subcortical and cortical areas involved in pain processing and the connectivity between these areas (modified from [51]). Primary afferents (Aδ-, C and Aβ-fibres) transmit impulses from the periphery through the dorsal root ganglion to the dorsal horn of the spinal cord. Most nociceptive Aδ- and C-fibres terminate superficially in laminae I-II, but also project either directly (Aδ-fibres) or poly-synapticly (C-fibres) to laminae V, whereas Aβ-fibres terminate predominantly in laminae III-V. In the dorsal horn the primary afferents connect with various neuronal cell types which project to different areas of the central nervous system. Neurones from laminea I project mainly to the periaqueductual gray (PAG), rostral ventromedial medulla (RVM), thalamus and parabrachial (PB). Neurones found in the deeper laminea III-V also project to the thalamus as well as to the formatio reticularis (FR) of the brainstem. Dotted lines represent the interaction between basal ganglia (BG), amygdala (amyg), and medial prefrontal cortex (MPFC), which constitutes the emotional, affective and motivational components, hypothesized to play an important role in chronic pain. Solid lines represent the areas involved in sensory-discriminative processing of pain and the connectivity between them – thalamus, cingulate cortex (cing), primary and secondary somatosensory cortices (S1 and S2), insula, and dorsolateral prefrontal cortex (DLPFC), hypothesized to play a role in acute pain. These areas then project to PAG, which in turn indirectly controls pain transmission in the dorsal horn through RVM (see also figure 9 section 3.1).

The pain modulating circuit can as well inhibit as facilitate the nociceptive transmission and it is now clear that this dual control is a result from the activity of the ON- and OFF-cells [65;66]. ON-cells show activity after pain stimulation leading to increased pain activation, whereas OFF-cells pause after pain stimulation leading to decreased pain inhibition. Both cell types project directly to the dorsal horn laminae that relay nociceptive signals [65]. The remaining neurons in the RVM are classified by exclusion and referred to as NEUTRAL-cells. It is clear that the ON-cells facilitate pain and that OFF-cells inhibit pain, whereas whether the NEUTRAL-cells have any role in modulating pain is an unsolved question [67]. However, one possibility is that NEUTRAL-cells are recruited to become ON- and OFF-cells in chronic pain states, but this still remains an open question. In addition to the PAG-RVM system, two other areas of the caudal medulla, the dorsal reticular nucleus and caudal lateral ventrolateral medulla, have been implicated in descending control of dorsal horn nociceptive processing [67]. An illustration of the interconnectivity between brain regions are given in figure 6.

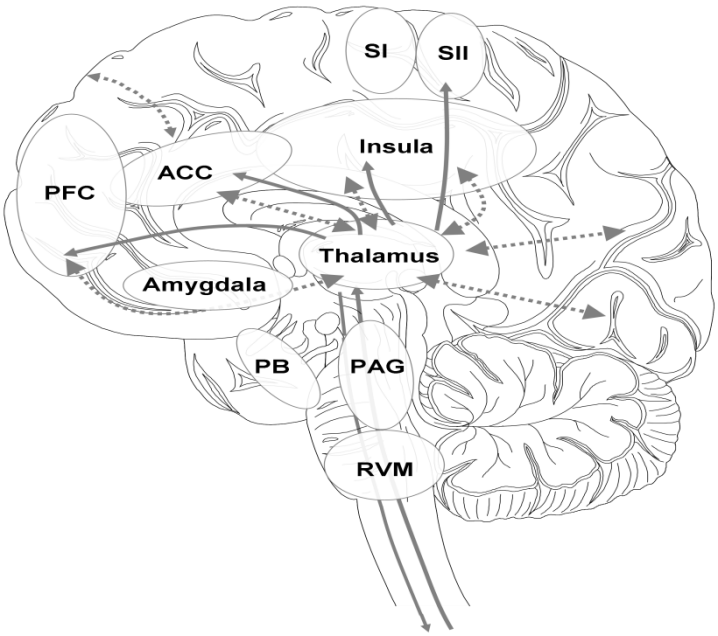

Figure 6 Illustration of cortical and sub-cortical regions involved in pain perception, their interconnectivity, ascending and descending pathways (inspired by [68]). Various region are involved in the pain process: primary and secondary somatosensory cortices (SI and SII), prefrontal cortex (PFC), anterior cingulated cortex (ACC), insula, thalamus, amygdala, parabrachial area (PB), periaqueductal gray (PAG) and rostral ventromedial medulla (RVM).

2.3.1 Control of pain

The brain controls the complex network of the underlying mechanisms behind pain control. As mentioned in section 2.4 pain control involves both descending inhibition and facilitation. Hence, the subjective pain perception is a dynamic balance of bidirectional pain-control mechanisms [62;69].

The existence of a specific pain modulatory system was first clearly articulated by Melzack and Wall in 1965 in the gate control theory of pain. It builds on the theory that large myelinated non-nociceptive Aβ-fibres activates an inhibitory interneuron, which in turn stabilizes the nociceptor and prolongs the period for depolarization of the pain-coding afferent [70]. Other structures involved in pain modulation are PAG and RVM. Animal studies have shown that electrical stimulation of PAG resulted in descending inhibition. PAG projects to RVM, which can be considered the output of the midline pain modulation system. Moreover animal studies have shown that opioid injection in RVM produces behavioural anti-nociception, which is likely to be a shift in the balance between the facilitatory ON-cells and the inhibitory OFF- cells, such that OFF-cells predominate [62;71-73]. Another advanced capacity to modulate pain the so-called diffuse noxious inhibitory control (DNIC) was introduced by Le Bars and his colleagues: ”pain inhibits pain” [74;75]. The ascending limb of the DNIC-loop travel through the ventro-lateral funiculi and the descending limb travel through the dorso-lateral funiculi [76;77]. DNIC result from the physiological activation of brain structures putatively involved in descending inhibition. Nevertheless, it has been debated which structures are involved in mediating the DNIC-like pain inhibition. Based on animal data, lesions of the following structures did not modify DNIC: PAG, cuneiform nucleus, PB area, locus coeruleus/subcoeruleus, RVM including raphe magnus, gigantocellularis and paragigantocellularis nuclei. In contrast, lesions of the dorsal reticular nucleus (DRN) in the caudal medulla strongly reduced DNIC [76-78]. Hence, it has been proposed that DRN is exclusively inhibitory [78]. However, other studies have suggested that DRN is also involved in descending facilitation [79]. An illustration is given in figure 7. Neurons in DRN are believed to respond to high intensity noxious stimuli and may therefore mediate DNIC-induced analgesia. DRN is therefore seen as one of the structures involved in the brainstem-circuit of inhibition and facilitation of pain [78].

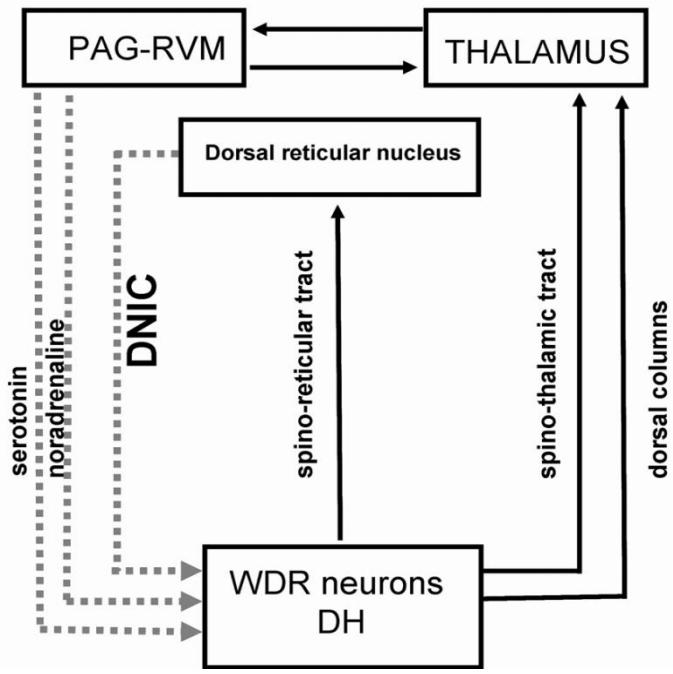

Figure 7 The spinal-reticular-spinal loop consist the mechanisms behind DNIC [76-79]). Nevertheless, DNIC is probably not completely separated from other inhibitory circuits. The loop involves the dorsal reticular nucleus, which is part of the brainstem-circuit, which can either inhibit or facilitate pain. It has been shown that physiological parameters can shape the DNIC response e.g. expectation of hyperalgesia can completely block DNIC. Hence, the loop is likely a part of an integrated inhibitory central control which incolces both DNIC and modulation through thalamus, periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). Wide dynamic range (WDR) neurones respond to stimulation from light touch to noxious stimuli.

It has now become clear that several levels of pain modulation contribute to the descending inhibition in humans which results in direct or indirect inhibition of spinal and supra-spinal neuronal responses [73;76]. For simplicity, four different regions within the CNS are listed which contribute to pain control: 1) Segmental spinal inhibition (modified gate control) involves inter-neurones located in the dorsal horn [80]; 2) Diffuse noxious inhibitory control (DNIC) operates through heterotopic conditioning stimulation. It operates through a spino-bulbo-spinal loop, which includes the dorsal reticular nucleus, characterized by multiceptive neurons with the whole body as receptive field [48;76]; 3) Inhibition through the brainstem network consisting of PAG-RVM, which possesses modulating abilities through “ON-cells” and “OFF-cells”, (pro-nociceptive or anti-nociceptive respectively) [66;67;81] and 4) top-down control, where the cognitive and affective role in the descending inhibition yet has to be addressed [82]. Moont et al. suggested that DNIC acts independently from distraction [82]. So far DNIC-induction is the most robust to induce experimentally by use of a noxious conditioning stimulus (III). The inhibitory effect can be extremely potent sometimes lasting for several minutes after removal of the stimulus [48]. Both the RVM and the SRD are rich in opioid receptors [83;84] and could therefore play an important role in DNIC-analgesia.

2.4 Sensitization

2.4.1 Peripheral level

An injury in the periphery results in a release of numerous inflammatory mediators such as histamine, serotonin, substance P and prostaglandins. They activate and sensitize terminals of primary afferents [85]. Some chemicals can directly activate nociceptors, while others act indirectly via inflammatory cells. Hence, the transduction threshold for these primary afferents is reduced. The same mediators may also activate silent nociceptors resulting in an increased input to the second order neurones and thereby sensitization of spinal neurones [86;87]. Peripheral injury can produce central changes which are maintained even after the inputs from the injury are removed. Therefore, once hyperalgesia is established, it does not need to be maintained by inputs from the injured peripheral tissue [88;89].

2.4.2 Central level

Following prolonged peripheral tissue or nerve injury a *sensitization* of the *central* nociceptive system can occur [88]. *Central sensitization* is believed to result from either a reduction in the threshold of nociceptors or an increase in the excitability of CNS neurons involved in pain transmission [88]. The most prevalent excitatory neurotransmitters are aspartate and glutamate. AMPA and NMDA open and close quickly and are thus responsible for most of the fast excitatory synaptic transmission in the spine. Under normal physiological conditions the transmission is supposed to occur mainly through AMDA receptors. The NMDA receptors are likely to play an important role in mediating the increase in the excitability of CNS neurones [88]. Of clinical importance, there is much evidence to suggest that descending facilitation of spinal nociception also is a major contributor to central sensitization as well as the development of secondary hyperalgesia [67].

2.4.3 Hyperalgesia

Chronic pain is often associated with inflammation and hyperalgesia which can lead to changes in the pain network [85]. Hyperalgesia in the skin can be divided into *primary* and *secondary* hyperalgesia. Primary hyperalgesia is the area immediately developed right around an injury site and is mediated by peripheral mechanisms, whereas secondary hyperalgesia is a central phenomenon and is a much larger area than the site of the injury [88]. Secondary hyperalgesia is seen as a state of hypersensitivity in the CNS that enlarges nociceptive input arising from the periphery [85].

3. Opioids

Opioids have been used for thousands of years for the treatment of pain. They are derived from the plant opium poppy, which was cultivated as early as 3400 BC in Mesopotamia. The term opium refers to a mixture of alkaloids from the poppy seed. In 1975 John Hughes and Hans Kosterlitz discovered 'endogenous morphines' or 'endorphins' and since then a wide variety of receptors and subtypes have been identified [90]. Opioids are today used widely to treat chronic pain conditions.

3.1 Pharmacology

The opioid system plays a major role in regulating pain. The majorities of the clinically relevant opioids have their primary activity at μ-receptors and are therefore considered as μ-agonists [90]. Opioid receptors are widely distributed throughout the central nervous system as well as the periphery. Opioid receptors are also found at the highest centres of the brain, but their contribution to analgesia is not fully understood and may partly relate to cognitive and sedative effects of the drugs [91]. There are currently four well-established members of the opioid receptor family:

- μ-receptors: primarily found in the brainstem and medial thalamus and are responsible for supraspinal analgesia, respiratory depression, euphoria, sedation, decreased gastrointestinal motility and physical dependence
- κ-receptors: found in the limbic and other dienchephalic areas, brain stem and spinal cord and are responsible for spinal analgesia, sedation, dyspnea, dependence, dysphoria and respiratory depression. It is thought to be closely related with nociceptive input from the viscera
- δ-receptors: widespread located in the brain and the periphery, but their effects are not well understood. They may be responsible for psychomimetric and dysphoric effects
- Opioid-receptor-like 1 (ORF): found in hypothalamus, thalamus, the spinal cord and the dorsal root ganglion

The receptors are differentially distributed throughout the central and peripheral nervous systems, why drugs modulating their activity can induce a variety of physiological and behavioural effects [92]. They are located presynaptically on the terminals of primary afferents as well as on the spinal neurones that convey the pain message onto the brain [91]. 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. Activation of a receptor located on a presynaptic terminal of nociceptive C- and Aδ-fibres leads to a release of a portion of the G protein, which diffuses intracellular to the target (either an enzyme or an ion channel). This leads indirectly to inhibition of voltage-dependent calcium channels as well as opening of potassium channels, leaving the inside of the nociceptor more negative and resulting in a decrease in nociceptor activity. These mechanisms leads to a blockade of the release of pain

neurotransmitters such as glutamate, substance P and calcitonin gene-related peptide from the nociceptors, resulting in analgesia [90;93] (figure 8).

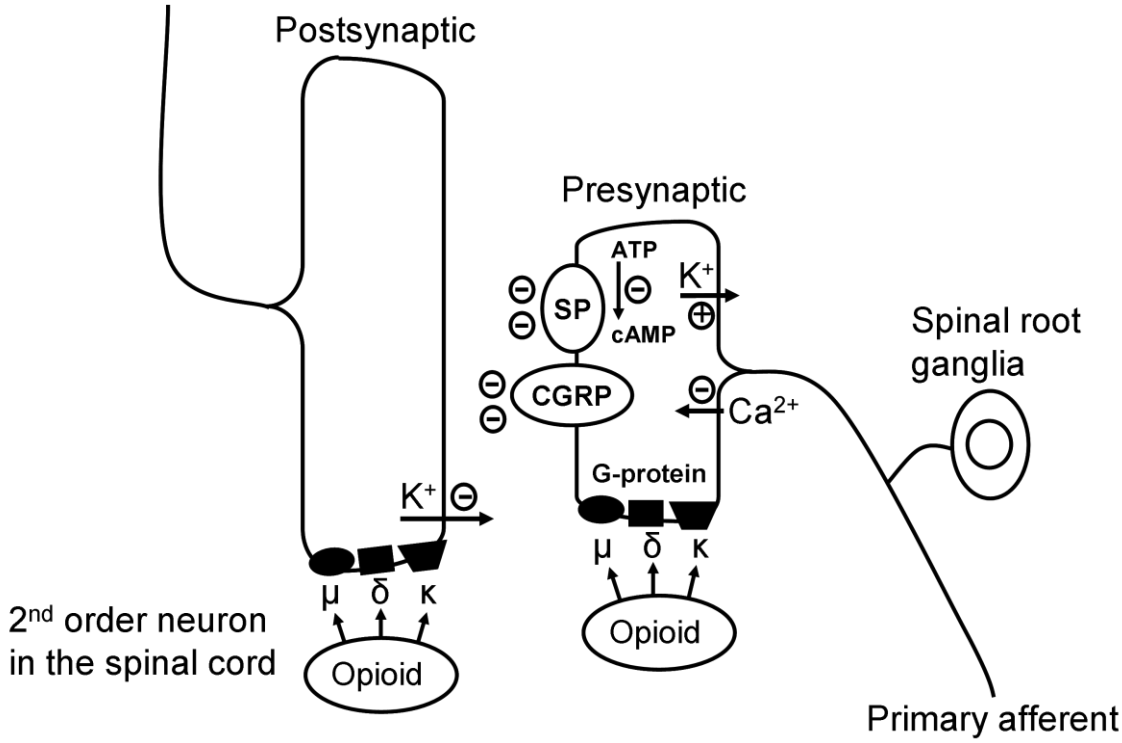

Figure 8 Simplified illustration of the mechanisms of the opioid action in the spinal cord (with inspiration from [90;93]). Activation of the opioid-receptors located presynaptic leads to inhibition of the calcium influx, opening of the potassium channels and inhibition of the release of excitatory neurotransmitters e.g. substance P (SP) and calcitonin gene-related peptide (CGRP). Postsynaptic action involves inhibition of potassium ion efflux, which decreases the neuron excitability. Opioid receptors are predominantly located presynaptic, which implies that the main mechanisms of spinal opioid analgesia are via activation of presynaptic opioids receptors.

Peripheral: The μ-, κ- and δ- receptors have been identified on peripheral sensory nerve fibres. The receptors are synthesized in C-and Aδ-fibres dorsal root ganglion cell bodies and transported centrally and peripherally, where they can be activated by opioids [94]. The relative contribution of peripheral opioid receptors to mediate analgesic effect of opioids has not been examined thoroughly. However, it is known that tissue damage stimulates the expression of peripheral opioid receptors likely leading to an increase in their function [95]. Especially μ- and δ- receptors are accumulated and contribute to the peripheral effect [94].

Spinal: Opioid receptors are mostly located in the superficial laminae I and II of the dorsal horn within the spinal cord. The receptors are predominantly located presynaptic on the central terminals of the primary afferents where opioids exert their main analgetic action, but are also represented postsynaptic at second order neurons and on interneurons. The net effect of opioids in the spinal cord is to decrease the ascending nociceptive signal [91].

Supraspinal: Opioid receptors are distributed throughout the CNS. When an opioid is administered these receptors account for the multiplicity of pharmacological responses such as respiratory

depression, sedation, opioid induced bowel dysfunction and physical dependence [90;96]. A number of brain areas are involved in the analgesic effect of opioids, the most important and best studied being PAG and RVM. Opioids exert their analgesic effect in these areas through increased activity of the inhibitory descending control system terminating in the dorsal horn of the spinal cord. The projection from the PAG does not go directly to the dorsal horn. Instead PAG sends a large projection to the RVM. In addition to PAG and RVM other brain areas such as the amygdala, prefrontal cortex, cingulate cortex, the parabrachial area and the sensory cortex are likely to play a key role in the overall analgesic effect. Areas such as insula, prefrontal cortices and thalamus are also rich in opioid receptors [51]. The areas are connected with opioids as mediators. For example activity between anterior cingulated cortex (ACC) and PAG during pain and opioid analgesia, but not during pain alone has been shown [97]. Nevertheless, whether opioids exert their analgesic effect in these regions through activation of the PAG-RVM axis or through independent pathways is yet to be investigated. Finally, it should be emphasized that the knowledge about these higher brain structures is mainly based on findings in rodents where the brain structure is very different from that in man [91].

The enhanced activity of the inhibitory descending control system by opioids is associated with a pronounced reduction in the activity of ON-cells and increased activity of OFF-cells (described in section 2.3). The main sites of opioids´ actions are illustrated in figure 9.

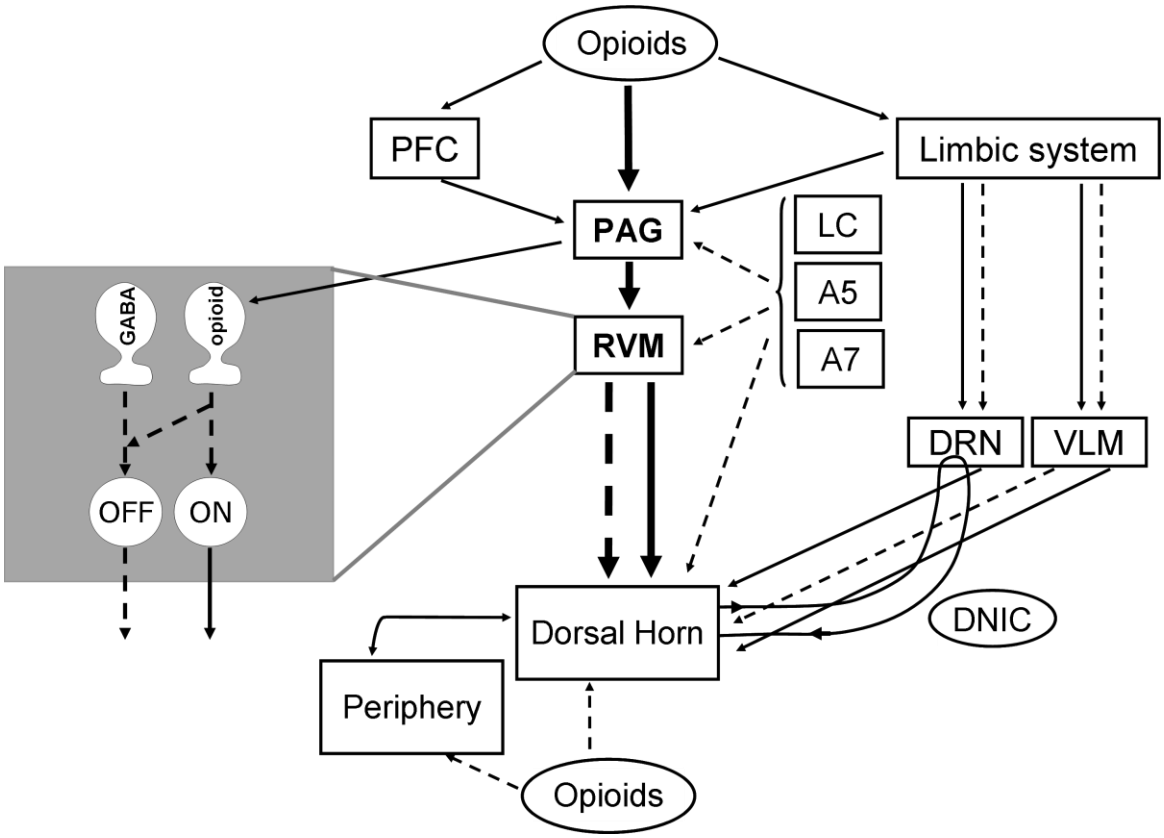

Figure 9 Oversimplified diagram illustrating the main sites of opioids´ actions throughout the central nervous system (dotted lines represents inhibition) (inspired from [90;96]). Opioids excite neurons in the prefrontal cortex (PFC) and several brain areas of the limbic system (hypothalamus, amygdale and cingulated gyrus) and hereby indirectly excite neurons in the periaquadactual gray (PAG). Opioids also directly excite neurons in PAG, which project to the rostral ventromedial medulla (RVM). From the RVM neurons run to the substantia gelatinosa of the dorsal horn and exert and inhibitory or excitatory influence on transmission. In addition to the PAG-RVM system, two areas of the caudal medulla, the dorsal reticular nucleus (DRN) and ventrolateral medulla (VLM), have also implicated in descending control of dorsal horn nociceptive processing. Other areas involved in pain inhibition are the locus coeruleus (LC) and the noradrenergic pathways A5 and A7. One phenomenon of descending inhibition, that can be induced experimentally, is the 'diffuse noxious inhibitory control' (DNIC) system, which is a complex loop of spinal and supraspinal sites, involving DRN. Opioids also excite neurons in these areas and DNIC is also believed to involve an opioideric link (III). Opioids also act directly at the dorsal horn, as well as in the periphery at terminals of nociceptive afferent neurons. The left side of the diagram illustrates how the opioids act on ON and OFF cells in the RVM. Opioids activate OFF cells indirectly through inhibition of gamma-aminobutyric acid (GABA) releasing input and also inhibit ON cells directly, which leads to anti-nociception.

3.1.1 Endogenous opioids

Endogenous opioids are widely distributed throughout the CNS, which allows them to function as neurotransmitters [90]. They may also play a role in hormone secretion, thermoregulation and cardiovascular control. Enkephalins are derived from pro-enkephalin and are relatively selective δ-agonists. Endorphins are derived from pro-opiomelanocortin and are μ and δ-agonists. Dynorphins are derived from pro-dynorphins and are highly selective μ-agonists. Nociceptins have little affinity

for the μ-, κ- and δ-receptors and their receptors are the so called ORF-1. They may have potent hyperalgesic effects and antagonists can be antidepressants and analgesics [90].

3.1.2 Exogenous opioids

Opioids possess the ability to both block spinal inputs and outputs and therefore have a powerful effect on the pain experienced by a patient [91]. Opioids also have actions on some of the descending pathways that run from the midbrain and brainstem and modulate spinal nociceptive function [98]. Most of the exogenous opioids are agonists and exert their effect by stimulating the opioid receptors. Classical μ-opioid receptor agonist such as morphine may paradoxically induce hyperalgesia [99]. Therefore new approaches have been taken to treat chronic pain conditions with opioids possessing different opioid-receptor profiles e.g. buprenorphine.

3.2 Transdermal delivery systems

TDS´ facilitate controlled release of active ingredients through the skin and into the systemic circulation. TDS were used in study II, III and IV. They have inherent benefits over conventional oral administration, providing a more steady and constant delivery of a drug, permitting less frequent dosing and maintaining steady state similar to continuous intravenous infusion up to several days [100;101]. This leads to improvement in dose control, patient acceptance and better compliance, and TDS´ are therefore valuable in the treatment of chronic pain conditions [5]. Drugs administered as TDS´ by-passes first pass metabolism and avoid influence of other variables such as the pH in the gastrointestinal tract as well as gastric emptying time, which can affect the amount of drug absorbed [5]. Nevertheless in order for a drug to be suitable for transdermal delivery the molecule must fulfil several criteria:

- have low molecular weight (<500DA)
- be very lipophilic, but also hydrophilic
- have high efficacy (compensate for slow trans-tissue mobility, ensure that an adequate dose can be absorbed through a small area of skin)

The first TDS was approved by the Food & Drug Administration in 1979 and was a membrane system with the active drug lying loose in a liquid or a gel [5]. However, if such a system is damaged it can lead to dose dumping, which is why newer delivery systems are developed as a matrix (semisolid layer containing a drug solution or suspension) wherein the drug is dissolved (figure 10a, b and c) [100]. Such a design makes the patch small, thin and less irritable to the skin. However, skin reactions may still occur [101].

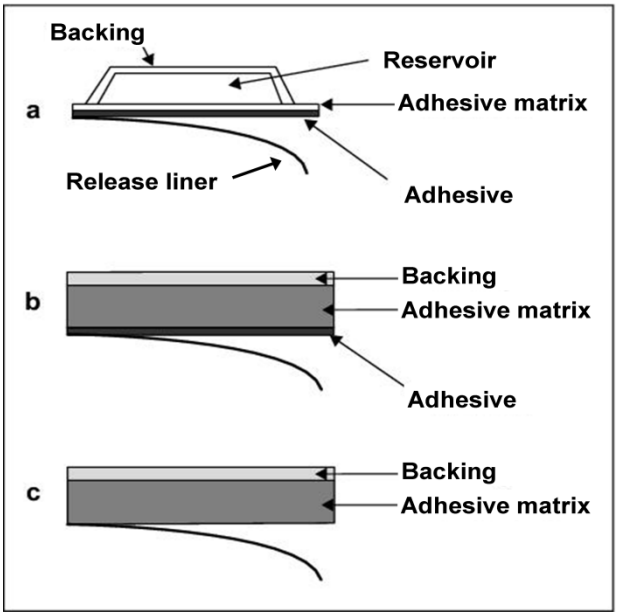

Figure 10a, b and c Schematic illustration of a membrane delivery system (a) and matrix delivery systems (b: with adhesive layer and c: without adhesive layer) (modified from [100]).

Two opioids available as TDS are buprenorphine and fentanyl. They both possess the physiochemical properties essential for TDS. The molecular weight for buprenorphine is 467 Da [102] and 337 Da for fentanyl [103]. Both drugs fulfil the criteria mentioned above and are therefore suitable for transdermal delivery [103;104]. Both drugs are currently used in the treatment of chronic moderate to severe non-malignant and malignant pain [104;105].

3.2.1 Skin permeability and tolerability

The skin is an effective barrier that protects the body from outside agents and in order for a drug to reach the bloodstream it must first pass the hydrophobic outer layer of the skin (stratum corneum) and then the more lipophobic epidermal and dermal layers [100;101]. Hence, a drug must be soluble in both lipid and water to be suitable for dermal application. High lipophilic substances diffuse rapidly into the epidermal tissue with subsequent slow movement into the water-rich dermal tissue. This results in a depot of drug in the epidermis and accounts for the slow onset and prolonged effect of transdermal drugs such as buprenorphine and fentanyl. However, it is important to keep in mind that the rate of transdermal delivery is affected by factors such as skin thickness and application site which can explain the variability observed with TDS [100;101].

3.2.2 Metabolism and cytochrome P450 3A4

The activity of many drugs depends on their interaction with enzymes localized predominantly in the liver, and to a smaller extent in the lungs and small intestine. Metabolism can have major pharmacological and toxicological implications in the use of therapeutic drugs [106]. The most important drug-metabolizing enzymes belong to the cytochrome P450 superfamily, which are divided into families, sub-families and specific enzymes by amino-acid-sequence homology [106].

CYP450 3A4 has the highest abundance in the human liver and metabolizes more than 50% of clinical drugs [107]. Genetic polymorphism in CYP450 3A4 is a well-known phenomenon [106]. It has also known that a person´s genetic makeup can predispose them to adverse effects and reduced efficacy [108]. Polymorphisms have clinical implications for opioids and have been linked to the variability of the analgesic effect of morphine [109] as well as variability in pain relief from alfentanil and levomethadone [110]. Even though polymorphism can have an influence on the analgesic effect of opioids, it is beyond the scope of this thesis to conclude as no urine or blood samples were collected for analysing genetic polymorphisms.

3.2.3 Buprenorphine

Buprenorphine was introduced in Europe more than two decades ago [104]. Its clinical effect results from binding to the opioid receptors. It is classified as a partial μ-opioid receptor agonist as well as a κ-receptors antagonist, and binds to these receptors with high affinity. It also acts as an antagonist at the δ-receptor, binding with low affinity, but the functional significance of this interaction has not been fully elucidated [104]. Recently it has also been proposed that partial binding to the ORL-1 receptor may contribute to the analgesic effect of buprenorphine [111]. Buprenorphine has been reported to be about 25-100 times more potent than morphine [104].

It is a very lipophilic drug and due to extensive first-pass metabolism in the gut wall and liver, it is suitable for parenteral administration such as TDS. It is a centrally acting analgesic and its distribution to the brain is therefore of importance regarding its analgesic effect. Studies have shown that the concentration in the brain exceeds its concentration in plasma within short time after administration, which is an unusual and interesting pharmacokinetic feature that might link to its clinical profile [112]. Approximately two-thirds of a buprenorphine dose is excreted unchanged, whilst the remaining one-third is predominantly metabolized in the liver by glucoronidation to buprenorphine 3-glucuronide and by *N*-dealkylation to norbuprenorphine via enzyme P450 CYP3A4. Therefore, care should be taken when buprenorphine is co-administered with drugs affecting CYP3A4. The only active metabolite is norbuprenorphine, which in animal experiments has shown to act as a strong agonist at the δ-receptors, but is about 40 times less potent than buprenorphine [104] (figure 11). Buprenorphine is mainly (80-90%) excreted by the biliary system and enterohepatic recirculation and is therefore not affected by impaired renal function [104].

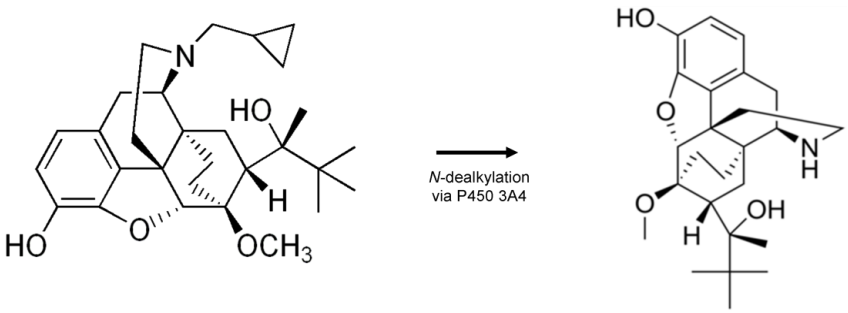

Figure 11 The predominant metabolism of buprenorphine (left) in the liver by *N*-dealkylation to norbuprenorphine (right), the only active metabolite.

For the 144h TDS (Norspan®) buprenorphine is dissolved in a polymer matrix where the rate of drug release is controlled by the concentration gradient across the patch and skin [111]. The concentration released from the patch per hour is proportional to the area of the patch and the time to reach steady state is approximately 24-48 hours. Following removal of the patch, concentrations decreases with one-half the first 12 hours and then declines to a half life of 26h [111;113]. Buprenorphine appears to distribute to two separate kinetic compartments of the brain, at least in the rat. It has therefore been suggested, that the slower half life (26h) could be related to the high affinity and slow dissociation kinetics of buprenorphine at μ-receptors from one of the compartments in the brain [112].

3.2.4 Fentanyl

Fentanyl was introduced in 1960 and is a strong opioid agonist, interacting predominantly with the μ-receptors. It is approximately 75-100 times as potent as morphine [90].

It is a very lipophilic drug and undergoes extensive metabolism in the liver [114] which makes it suitable for parenteral administration such as TDS. Fentanyl is metabolized to hydroxyfentanyl and norfentanyl, both inactive and non-toxic metabolites [90;114] (figure 12). Fentanyl is presumed to be metabolized by the liver enzyme P450 CYP3A4 and might therefore be subject to extensive drug interaction when co-administered with drugs affecting CYP3A4 [114]. Nevertheless, a study performed by Palkama et al. 1998 showed in healthy volunteers that the CYP3A4 inhibitor itracanazole had no influence on the PK of intravenous fentanyl. PK and PD of fentanyl were similar both after itracanazole and placebo [115].

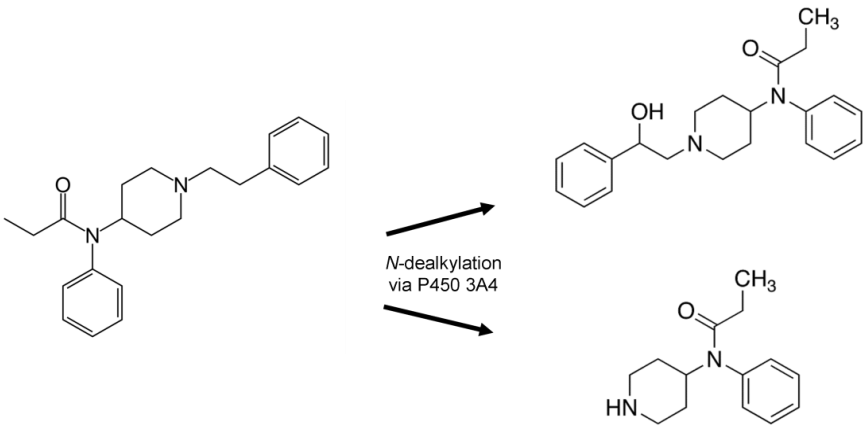

Figure 12 The predominant metabolism of fentanyl (left) in the liver by *N*-dealkylation to hydroxyfentanyl (top, right) and norfentanyl (bottom, right), both in-active and non-toxic metabolites.

The 72h TDS (Durogesic®) consist of four functional layers each providing qualities to continuous release of fentanyl as well as preventing leakage of contents onto the surrounding skin. The system also consists of a rate-controlling membrane reducing the variations in transdermal transport compared to other transdermal delivery systems with Fentanyl [103]. The system allows

2.5µg of fentanyl to diffuse across each cm^2 of the device per hour, meaning that the area varies with different doses. Maximal concentration is achieved after about 36hours, however, with substantial intersubject variability (17-24hours). Following removal the half-life is around 17hours due to continued absorption from the depot in the epidermis during the elimination phase [103]. Elimination half-life after removal of the patch has shown to change with age, elderly having a significantly longer half-life compared to younger patients.

4. Methods

Animals have been widely used to model human pain conditions [116]. Experimental pain models in animal studies have led us to understand some of the pain pathways as well as the role of neurotransmitters and membrane channels. In such studies the neuronal nociceptive activity can be recorded and behaviour can be assessed [116]. Moreover, recordings of selected pathways in the animal nervous system are important in basic research and screening of analgesics. However, as the neurobiology of nociceptive systems differs between species the extrapolation from animal studies to humans is limited. This is especially the case when it comes to more complex mechanisms [117]. Hence, there is need for experimental pain research in humans in preclinical studies of analgesics.

In human pain research comprehensive trials in patients are often used to evaluate pain mechanisms and the effect of analgesics. Although humans suffering from different diseases are the final target of pain research it is often difficult to explore the actual pain state and to assess analgesic mechanisms. The pain quality and intensity are usually only recorded in a subjective manner and often confounded by general malaise, nausea, additional pathophysiological influences or co-medications [117]. For example, anxiolytics without analgesic effects will typically affect general wellbeing and hereby also pain intensity. Therefore, questionnaires and rating scales are not ideally suited to the detailed investigation of pain processing. Experimental pain models in healthy volunteers or patients are therefore valuable in basic studies as well as in studies investigating analgesics, leading to a translational bridge between animals and humans [21]. Human experimental pain models require controlled stimulation and standardized assessment of the evoked response (figure 13). This approach can be applied to healthy volunteers and patients before and after pharmacological interventions [21]. Nevertheless, it is important to keep in mind that pain experienced and reported by healthy volunteers is different from clinical pain conditions [85].

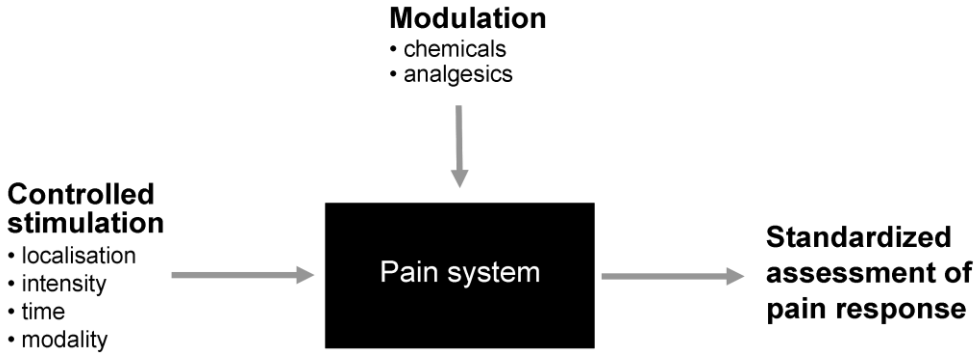

Figure 13 Illustration of the context in experimental pain. The pain system can be considered a black box between the stimulation (input) and the response (output). Experimental pain models provide a situation where it is possible to control the localisation, intensity, time and modality of a given stimuli. Moreover, the pain system can be modulated by e.g. chemicals or analgesics, leading to a standardized assessment of the pain response.

4.1 Experimental pain models

Methods related to experimental pain in humans aim at activating different nociceptors, evoking pain from different tissues and activating specific pathways and mechanisms [24]. There are several standardized experimental phasic pain models as well as models inducing hyperalgesia that can be applied to humans [24] (figure 14) (I, II, III and IV).

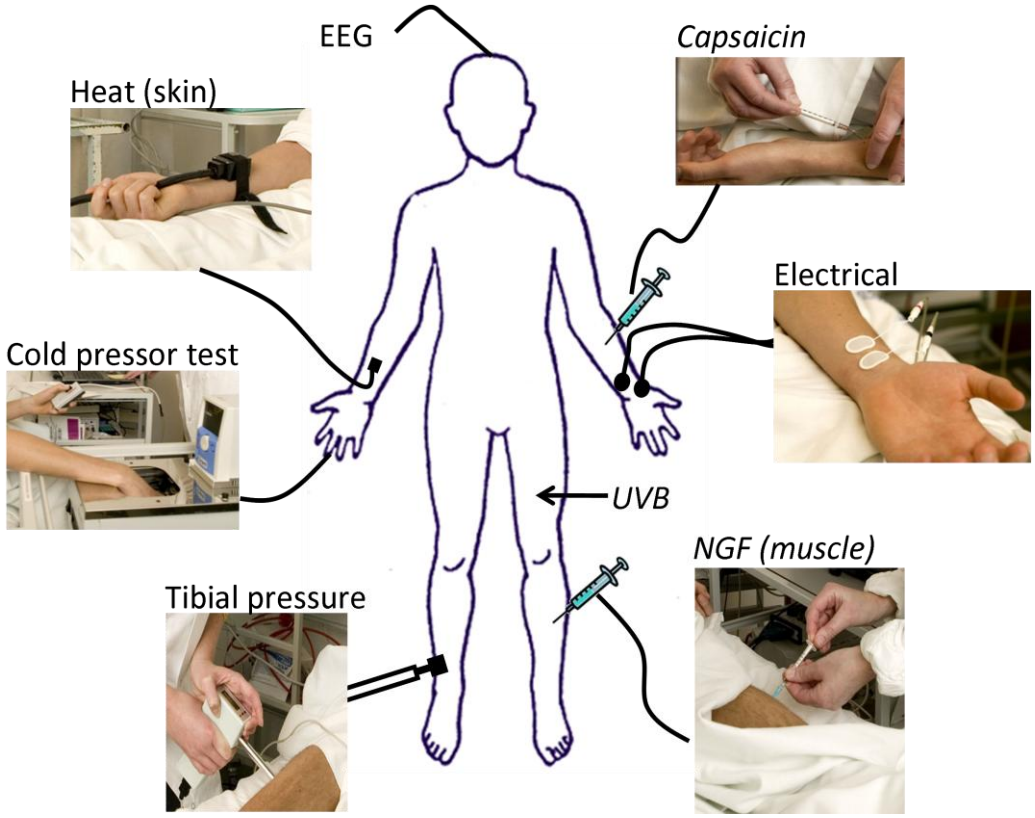

Figure 14 Examples of experimental phasic pain models and models evoking hyperalgesia (italics) that can be applied to humans. The illustrated pain models were used in the studies that this thesis is based on. Abbreviations: EEG=electroencephalography, UVB=ultra violet B-light, NGF=nerve growth factor

Phasic pain models are short lasting and have limitations compared to the complex clinical conditions, only activating a part of the multidimensional mechanisms involved in pain. Models inducing hyperalgesia can act as proxies for clinical manifestations and hence are more clinical relevant than superficial pain models [118;119]. Nevertheless using a combination of both phasic pain models with hyperalgesic pain models gives the possibility to investigate tissue and modality differentiated effects of opioids (study II, IV). The models used in the studies, which this thesis is based on, will be mentioned in the following sections 4.1.1 – 4.1.5.

4.1.1 Bone associated pain

Bone associated pain is very frequent in the clinic. Mechanical stimulation can be used to evoke pain from deeper tissues (see also section 4.1.3) (I). Mechanical pressure has been performed at different bone structures e.g. mastoid processes, external malleolus and the sternum [120], and

Hamilton et al. showed that pressure with a metal probe to the surface of the tibia evoked pain [121]. However, these studies did not systematically investigate which probe size would be the most optimal to evoke bone associated pain. This was therefore performed in study I to develop a model inducing bone associated pain experimentally. In this study we showed that pressure stimulation with probe size 2mm in diameter at the tibia could elicit bone associated pain, and that this size had least influence of the skin component. The tibia was chosen to avoid influence of pain from muscles [122]. Furthermore, to investigate the influence of cutaneous nociceptive input pressure stimulation was performed before and after local anaesthesia. It was possible to show that the skin influence was least with probe size 2mm in diameter. In addition, preliminary data from a study investigating strain and strength of the skin when applying pressure at the tibial bone, suggest that probe size 2mm in diameter is efficient to induce bone associated pain (unpublished data by [123]). Moreover, we showed the model was reliable and reproducible and that the handheld algometer, which in practice is easier to use, was comparable to the more comprehensive computer controlled algometer. The model is a further development of a pain model used by Hamilton et al. who showed that pressure at the tibia could be used experimentally [121].

To investigate whether there could be any clusters of responders/non-responders for buprenorphine or fentanyl in study II to bone pressure pain, individual data were investigated. These data are illustrated in figure 15 for buprenorphine and figure 16 for fentanyl. There were not observed any clusters for either buprenorphine or fentanyl.

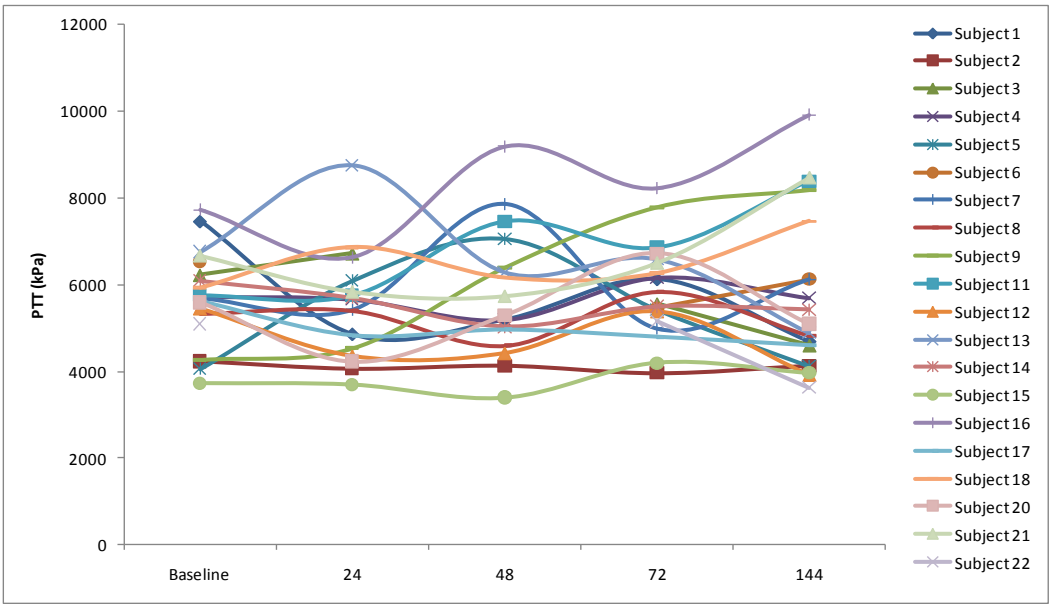

Figure 15 Graph representing individual data for bone pressure pain for the buprenorphine arm. Data are shown as pressure tolerance threshold (PTT) measured at baseline, 24, 48, 72 and 144hours after treatment. No clusters of "responders/non-responders" were observed. Subject 19 and 10 were excluded from the study and data are therefore not shown.

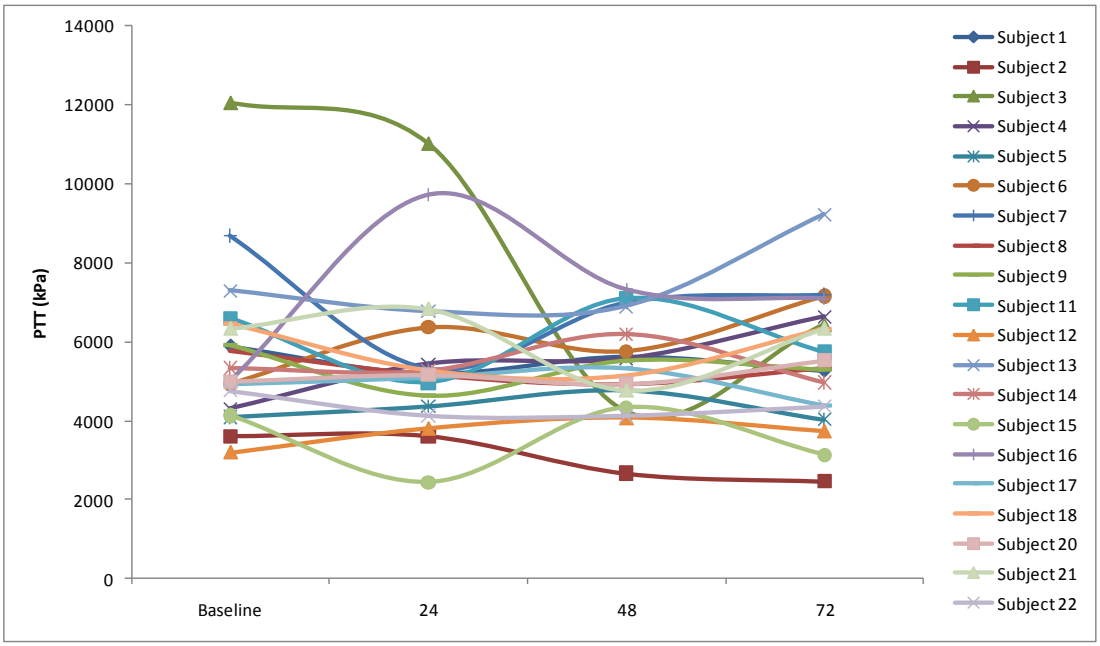

Figure 16 Graph representing individual data for bone pressure pain for the fentanyl arm. Data are shown as pressure tolerance threshold (PTT) measured at baseline, 24, 48, and 72hours after treatment. No clusters of "responders/non-responders" were observed.

In addition, looking into the individual plots for the other pain models in study II, no clusters of "responders/non-responders" were observed. In study II, data were baseline corrected to make sure that any possible variation in the baseline recordings did not influence the statistical analysis [22]. In both study II and IV it was possible to show a significant analgesic effect of buprenorphine to bone pressure pain (Table 1).

4.1.2 Cutaneous pain

Experimental pain models in the skin are highly developed, mainly because of the easy access to the skin. Mechanical, thermal, electrical and chemical models are all evaluated methods [24] (I, II, III and IV). However, as skin stimulation is a superficial activation of the pain system it can be complicated to translate this kind of pain into the clinical situation where deeper pain often dominates [21]. On the other hand, this kind of pain can be used to detect analgesic effects of opioids. This was done in study II and IV using a computerized 'Thermo tester', where a slow rate of heating activates the C-fibres [67] (figure 17). To cutaneous heat it was possible to show similar analgesic efficacy of buprenorphine and fentanyl, supporting equi-analgesic doses used in study II and IV (figure 16).

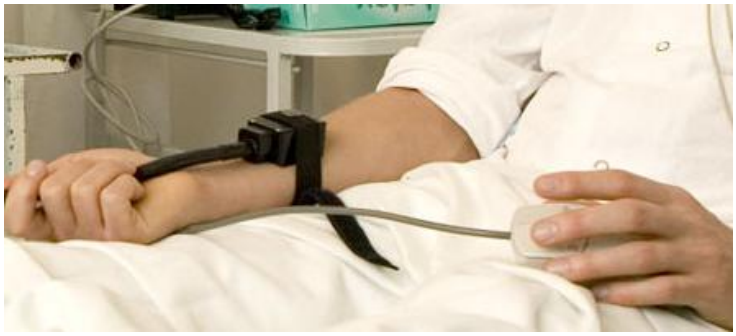

Figure 17 Picture showing the cutaneous heat pain stimulation. The subjects pressed a mouse button when reaching the heat tolerance threshold.

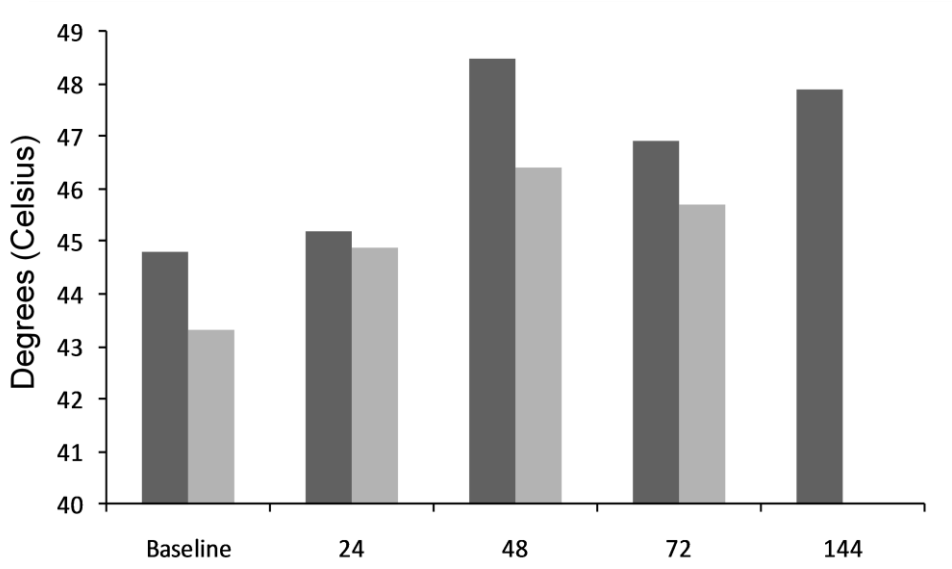

Figure 18 The graph shows an illustration of the analgesic effect of buprenorphine (dark gray) and fentanyl (light gray) to cutaneous heat stimulation before (baseline) and 24, 48, 72 and 144hours after treatment for one subject. Both drugs attenuated heat pain, illustrating that the choice of doses for buprenorphine and fentanyl were similar regarding analgesic effect to cutaneous heat pain.

In study II and IV it was possible to show significantly analgesic effect of buprenorphine to cutaneous heat pain (Table 1). However, for fentanyl it was not possible to show a significant analgesic effect in study IV, which could be explained by the fact using analysis of variance (study II) treats the data categorical whereas PK-PD modelling (study IV) recognises the time-dependent contribution of the concentration of a drug as well as can account for several factors e.g. weight, height etc. (Table 1) [124;125].

4.1.3 Muscle pain

In comparison to cutaneous pain, which is more localized, muscle pain is more diffuse. Experimental pain models applied to healthy volunteers – inducing muscle pain - are usually divided into endogenous (natural stimuli e.g. ischemic or exercise induced pain) and exogenous (external interventions e.g. mechanical, electrical or chemical induced pain (I, II, III, IV)) models

[126]. Mechanical painful stimulation can be achieved with pressure algometry – handheld or computer-controlled (I). The most widely used technique is the manual pressure algometer (II, III, IV). An alternative to pressure application is computer-controlled pressure algometry, where the rate and peak pressure can be predefined and automatically controlled. Although there could be concerns about (reproducibility on pain threshold, inter-examiner variability) using a handheld algometer, these concerns have been addressed carefully and the method has proven to be valid and reproducible [127]. This was also shown in study I where the handheld algometer was comparable to the more comprehensive computer controlled algometer regarding validity and reproducibility. Both algometers were used in study I, whereas only the handheld algometer was used in study II, III and IV. Pressure stimulation with probe size $1cm^2$ obviously also excites cutaneous receptors. Nevertheless, it has been demonstrated that mechanically elicited pain mainly originates from deep tissue and only to a minor degree from the skin [27].

4.1.4 Inflammatory induced pain and hyperalgesia

Experimental models inducing inflammation and hyperalgesia are thought to mimic the clinical situation to a higher degree than phasic pain models [21;25]. By evoking different central phenomena like allodynia and hyperalgesia in experimental pain studies, it is possible to study central pain mechanisms in humans. This is of major importance since many disorders associated with pain is characterized by abnormal central pain processing [25]. Chronic pain is often associated with peripheral and central hypersensitivity conditions and experimentally induced secondary hyperalgesia is mainly seen as a manifestation of hypersensitivity of the central nervous system [85;128]. Experimental pain models using irradiation with UVB light, injection of capsaicin or NGF has been used to induce tissue specific primary and secondary hyperalgesia, which makes them valuable in studies investigating anti-hyperlagesic effects of analgesics [31;129;130].

Intradermal injection of capsaicin (potent algesic substance of chilli) leads to alterations of sensory processing in the CNS and has been used to study the mechanisms of allodynia and hyperalgesia. Allodynia is thought to be mediated by Aβ-fibres and the area of allodynia can be assessed with a brush. Hyperalgesia is on the other hand thought to be mediated by Aδ-fibres and the area of hyperalgesia can be assessed with a von Frey filament [29] (figure 19) (II, IV).

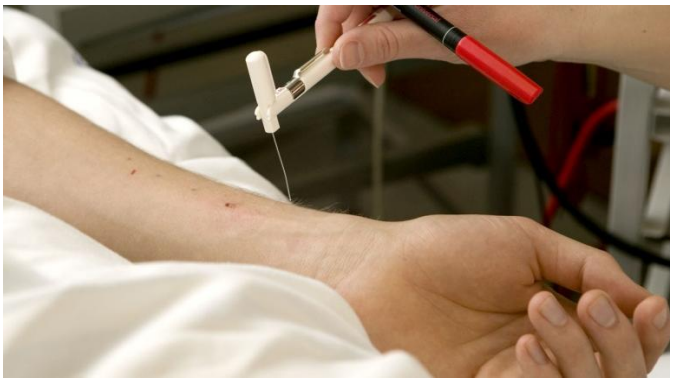

Figure 19 Picture showing assessment of sensitivity to capsaicin induced hyperalgesia. The secondary hyperalgesic area was assessed with a von Frey filament. The stimulation started in normal skin away from the injection site and continued towards the injection site until the subject reported a clear change in sensation. This was performed in 8 radial directions. The borders were marked with a pen and drawn on a transparency film and the area was calculated with a specialized computerized program.

The capsaicin model has shown to be valid for testing of central anti-hyperalgesic effects of analgesics [131]. This method was therefore used in study II and IV, where the secondary hyperalgesic area was assessed with the von Frey filament and the allodynic area was assessed with a brush. After placebo the areas of secondary hyperalgesia or the allodynic area did not change significantly over the entire treatment period (figure 20). Injections were performed on the volar surface of the left arm: 8, 10 and 12cm proximal from the wrist. Hughes et al. suggest that the non-dominant arm appears to be more sensitive to pain than the dominant arm, which is why the same arm was chosen in our studies [29]. In addition, the three injection sites are innervated by the same two nerves – medial and lateral antibrachial cutaneous nerves [132], influencing the results as little as possible.

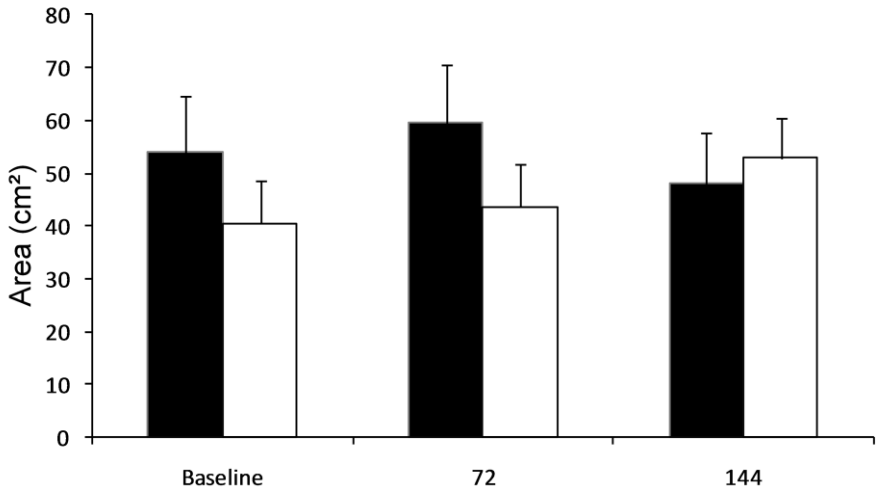

Figure 20 Illustration of the secondary hyperalgesic area assessed with the von Frey filament (gray) and the allodynic area assessed with brush (white) evoked by intradermal injection of capsaicin. Data are shown from the placebo arm. Injections were performed before (baseline) and 72, 144h after application of the patches. Injections were performed on the volar surface of the arm, 8cm (baseline), 10cm (72h) and 12cm (144h) proximal from the hand wrist.

UVB-light (290-320nm) is largely absorbed in the epidermis and leads to hyperaemia and hyperalgesia. UVB irradiation is a suitable experimental model of thermal and mechanical hyperalgesia and its advantage is that it produces prolonged primary hyperalgesia between 20 and 30h. Furthermore, this model also results in secondary hyperalgesia around the light-exposed area, which is a central phenomenon, and is therefore particularly interesting in studies investigating centrally-acting analgesics [133] (II). Nevertheless, in study II the signs of inflammation and hyperalgesia were variable between and within subjects, suggesting that the model might not be reproducible with the set-up used in our study. On the other hand the minimal erythema dose was determined according to each subjects skin type and a 1st degree sunburn was achieved in the primary area in each treatment period where sensitivity to mechanical stimulation was shown (figure 21).

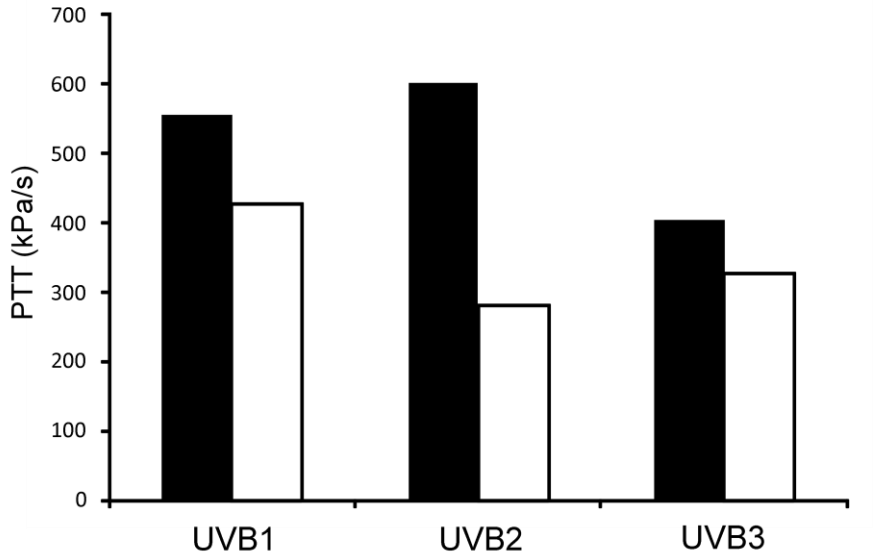

Figure 21 The graphs show an illustration of the sensitivity to mechanical stimulation after the 1st degree sunburn was induced by UVB-light. Mechanical stimulation was performed with pressure algometry and was determined before (baseline, represented by black) and after irradiation with UVB-light (represented in white), inducing 1st degree sunburn. The measurements were performed 24h (UVB1), 72h (UVB2) and 144h (UVB3) in each treatment period. Data shown is for the placebo arm for one subject.

NGF is a neurothrophic protein with a pivotal role in development and maintenance of the nervous system. It is an important modulator of the processing of nociceptive information both in the periphery and the central nervous system and plays a major role in inflammatory and neuropathic pain states. NGF binds to the receptor tyrosine kinase A which then leads to a cascade of mechanisms for survival of sensory neurones [134]. NGF may also act directly on nociceptors to provoke its hyperalgesic effects [135]. In fact, elevated NGF levels in synovial fluid were found in arthritis patients [136]. Among the substances released in inflamed muscle, NGF is of particularly interest as it is synthesized in the muscle and represents a major sensitizing substance for nociceptors in pathologically altered tissue. When injected intramuscularly in humans it induces

sensitization without causing acute pain during injection (II, IV). The sensitization is expressed as a long-lasting muscle allodynia and hyperalgesia and could involve both peripheral and central mechanisms [31]. In study II we found NGF induced sensitization in the placebo arm within the first 72 hours after injection being most prominent after 24 hours, which is in accordance with previous studies [31;137] (figure 22).

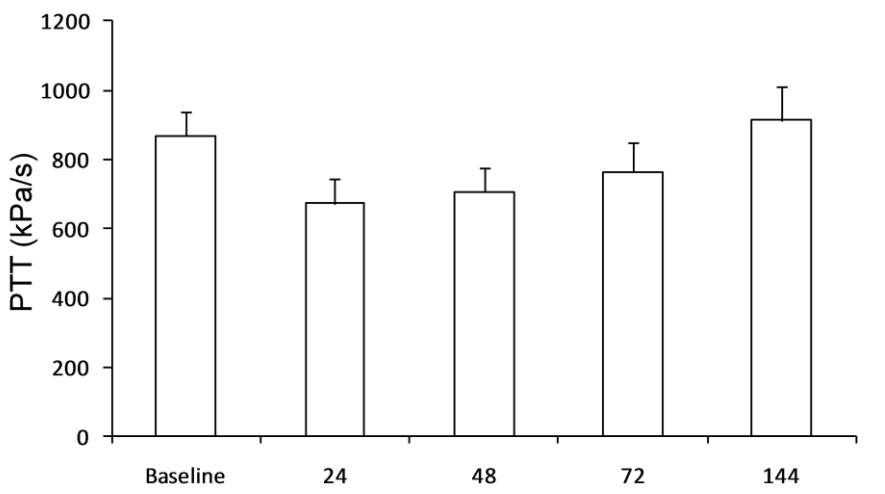

Figure 22 The histogram illustrates NGF-induced sensitization to mechanical stimulation. Mechanical stimulation was performed with pressure algometry before (baseline) and 24, 48, 72 and 144hour after intramuscular injection of NGF into the extensor digitor longus muscle.

4.1.5 Induction of DNIC

Various techniques such as tonic heat, tonic ischemic pain by a tourniquet, tonic cold pain (cold pressor test) can be used to experimentally induce DNIC. Recently a recommendation on terminology and practice of DNIC testing has been published, suggesting to use the following terms: a) the painful stimulus upon which the conditioning effect is tested should be termed *test-stimulus*, b) the stimulus used to induce the change in pain perception should be termed *conditioning stimulus* and c) the phenomenon through which the conditioning stimulus affects the pain stimulus should be termed *Conditioned Pain Modulation (CPM)* [138]. For simplification the term CPM was not used in this thesis or in study III.

The most commonly used paradigm to induce DNIC is the cold pressor test. The outcome of the cold pressor test is dependent on several factors such as accuracy of water-temperature, circulation of water as well as the length of the test [48]. Nevertheless, it is reliable, cheap and easy to work with [139] and was therefore used in study III. In our study the hand was immersed in water as it has been shown that greater pain reduction is induced compared to the foot [140]. Moreover, it was more pleasant for the subjects and easier in the experimental set-up of study III. It has been shown in man, that the loop subserving DNIC involves an opioideric link. This was observed using the antagonist naloxone [49]. In study III it was possible to show that the induced

DNIC effect did not change significantly over the entire treatment period illustrated in the placebo arm (figure 23).

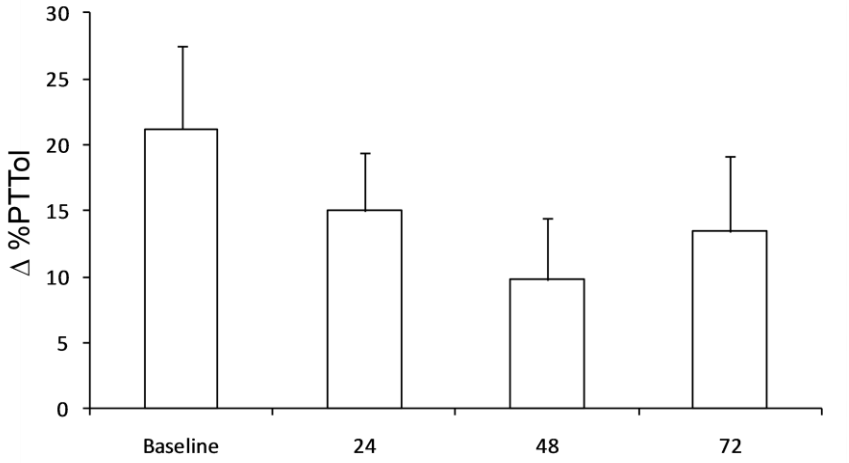

Figure 23 Graphic illustration of the experimentally induced DNIC effect in the place arm. The cold pressor test was used as the *conditioning stimulus* and pressure algometry was used as the *test stimulus*. Data is represented as % increase in pressure tolerance threshold (PTTol) after the *conditioning stimulus*. There was no significant difference in the % increase of PPTol over the 72 hour treatment period.

The average increase in pressure pain tolerance threshold (PPTol) did not change significantly over the 72 hour treatment period. However, the responsiveness of DNIC could change from one session to another, meaning that there were cases where a subject was a DNIC responder in one session but a DNIC non-responder in another (III). To investigate if the order of the treatments (whether placebo, fentanyl or buprenorphine was administered first) could have an influence on being a DNIC-responder or a DNIC-non-responder, an individual plot was drawn for the DNIC response 24hours after treatment with either placebo, fentanyl or buprenorphine (figure 24).

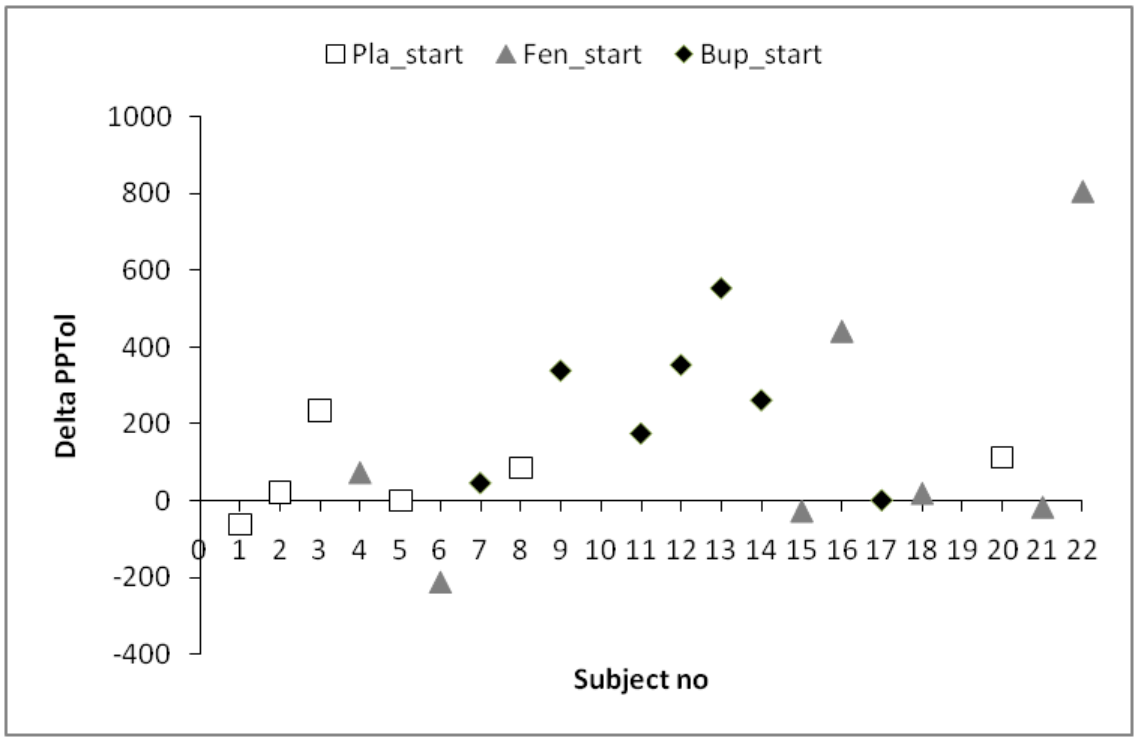

Figure 24 Illustration of the DNIC response 24hours after treatment of either starting a treatment period with placebo (Pla-start), fentanyl (Fen_start) or buprenorphine (Bup_start). Data are represented as evoked changes in pressure pain tolerance threshold (PTTol). The order of treatment (whether placebo, fentanyl or buprenorphine was administered first), had no influence on being a DNIC-repsonder or DNIC-non-responder.

Even though figure 24 presents a trend in the DNIC response, being higher for most of the subjects treated with fentanyl or buprenorphine compared to the placebo, this only supports the results achieved in this study, where a significant effect of both fentanyl and buprenorphine of the DNIC response could be shown (study III).

4.1.6 Electroencephalography

Recording of evoked potentials (EP), which is the electrophysiological response of the nervous system to a stimulus, e.g. a painful stimulus of the median nerve, have shown that the peak-to-peak amplitude of the EP diminish following administration of the opioids morphine, fentanyl and alfentanil [141;142]. In addition, recording of resting electroencephalography (EEG) has been used as a measure of the narcotic effect of opioids [50]. In study II, we recorded EP during painful stimulation at the median nerve followed by 2 minutes of resting EEG with closed eyes. The EP´s were recorded by use of a single monophasic square pulse of 2 milliseconds duration. This short pulse minimizes the stimulus-artefact and is superior to e.g. stimulation trains. A simple four-channel system, consisting of two recording electrodes at Cz and Fcz was used. Reference was placed on the right earlobe. To minimize artefacts all other electronic equipment was switched off during recording and subjects were instructed to focus on a spot on the wall and blink and swallow only between stimulations. A stimulation paradigm of 60 sweeps of a stimulus intensity

corresponding to a subjects pain threshold applied at 2Hz was used. Recordings were performed at baseline and 4, 24, 28, 48, 72, 78 and 144h after application of the patches (figure 25).

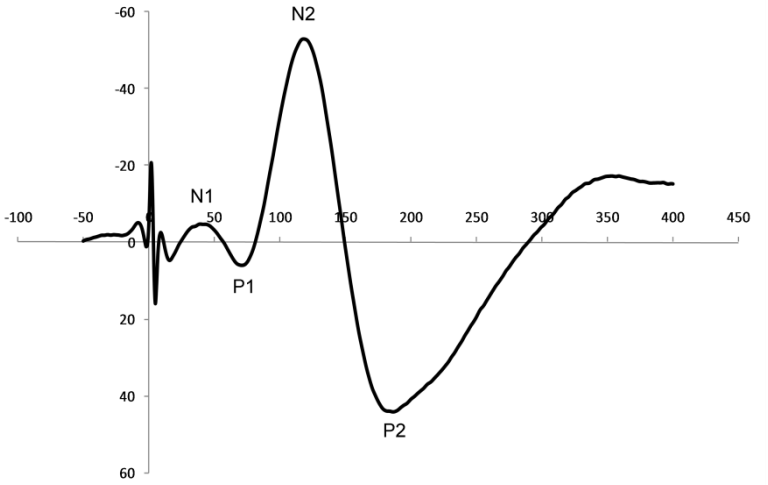

Figure 25 Evoked potential (EP) recorded at Cz electrode at baseline (before drug administration). The x-axis represents latency on millisecond scale and the y-axis represents amplitude on microvolt scale. N1: first negative component, N2: second negative component, P1: first positive component and P2: second positive component of the EP. Buprenorphine prolonged the latency of N1, whereas fentanyl prolonged the latency of N2 and P2 as well as enhanced the amplitude of N2

So far preliminary data have shown that buprenorphine prolonged the latency of N1 (defined as the first negative component around 35 milliseconds), whereas fentanyl prolonged the latency of N2 (defined as the second negative component around 120 milliseconds) and P2 (defined as the second positive component around 180 milliseconds) when compared to placebo. Fentanyl furthermore enhanced the amplitude at N2 compared to placebo. This is in contrast to what has been observed for opioids, where the amplitude was diminished [141;142]. On the other hand, a study with remifentanil demonstrated an increase in the amplitude [143], which has also been shown in a study with morphine by Staahl et al. [144], suggesting conflicting results depending on the opioid investigated.

4.1.7 Adverse events

Although buprenorphine and fentanyl has been used in the clinic for several years, there has been an interest in the mechanistic profile of the drugs after the introduction of transdermal delivery systems. In human pain research comprehensive trials in patients are often used to evaluate pain mechanisms and the effect of analgesics. However, pain in patients is often confounded by general malaise, nausea, additional pathophysiological influences or co-medications (Drewes et al. 2003). The present study as well as experimental pain studies in healthy volunteers in general provides the opportunity to investigate new mechanisms and effects of different opioids as well as determine tissue differentiated effects, providing more rational treatment of pain. The main focus of study II was therefore to investigate effects and mechanisms of buprenorphine and fentanyl

and not the adverse effect profile of the drugs. On the other hand, in clinical trials and in the treatment of chronic pain both analgesic efficacy and tolerability is relevant. In study II we were able to demonstrate that there were no significant differences in the *frequency* of adverse effects between buprenorphine and fentanyl (*P*=0.1). However, regarding *severity* of the adverse effects a difference was observed at 48h for dizziness, where the score was higher for buprenorphine than fentanyl (*P*=0.03)". Even though higher doses of buprenorphine and fentanyl are used in the clinic to treat chronic pain seen in comparison with the doses used in this study, it is problematic to use much higher doses in opioid-naïve healthy volunteers due to the amount and intensity of adverse effects.

4.2 Pharmacokinetic-Pharmacodynamic modelling

PK is the study of what the body does to a drug after administration, including absorption, distribution, metabolism and excretion. PD on the other hand is the study of what the drug does to the body after administration (figure 26).

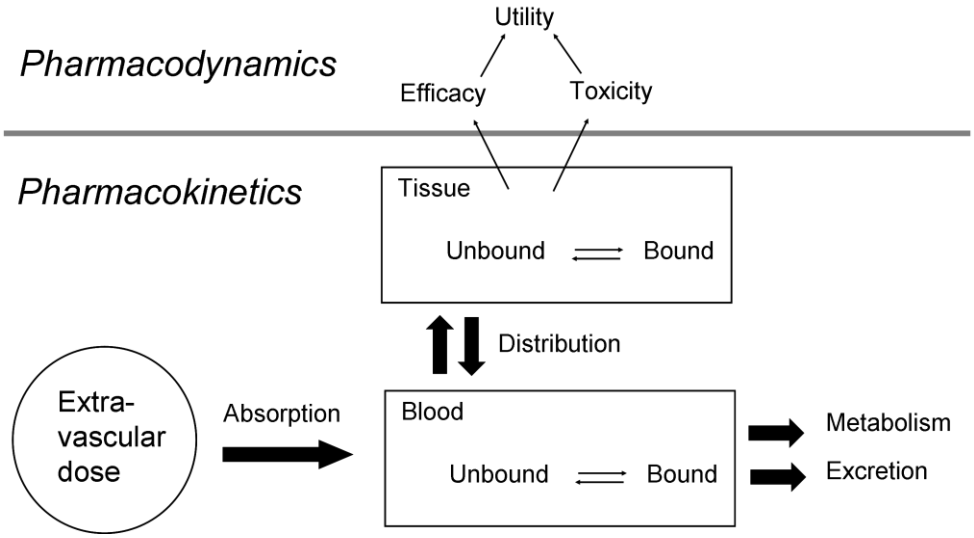

Figure 26 Simplified illustration of what happens to a drug after administration. Pharmacokinetics includes absorption, distribution, metabolism and excretion, whereas pharmacodynamics includes the utility - balance between efficacy and toxicity.

The analysis of kinetic/dynamic data can be complex and time consuming. *PK-PD modelling* can be done using data for 1) individuals; 2) a number of individuals where they are analysed separately and then pooled (a two way stage approach) or 3) a number of individuals where they are analysed together (population approach also known as mixed effect model)). For details the reader is referred to Minto & Schnider 1998 [124]. Data from multiple measurements in multiple individuals consist of two distinct types of variability: Between individual variability and within individual variability [145]. *Nonlinear mixed effect modelling* is a type of modelling where some parameters are the same for all individuals ("fixed" - known observable properties of individuals that cause the descriptors to vary across the population) or some vary between individuals

("random" – cannot be predicted in advance). It is thereby possible to analyse between and inter-individual variability [146]. In the end, the goals of model building are among others to estimate the model parameters and their precision and compare competing [147]. Ideally, concentrations of a drug should be measured at the effect site, the site-of-action or biophase, where the interaction with the respective biological receptor system takes place, but this is in most cases not possible. Hence, concentrations in easily accessible body fluids like plasma are used for PK-PD modelling (IV). Under non-steady state conditions, drug concentrations in plasma undergo time-dependent changes and different PK-PD models can be used to describe the concentration-effect relationship for a specific drug [148]. PK-PD modelling uses quantitative methods to mathematically characterize the relationship between drug concentration and drug effect [19] (figure 27).

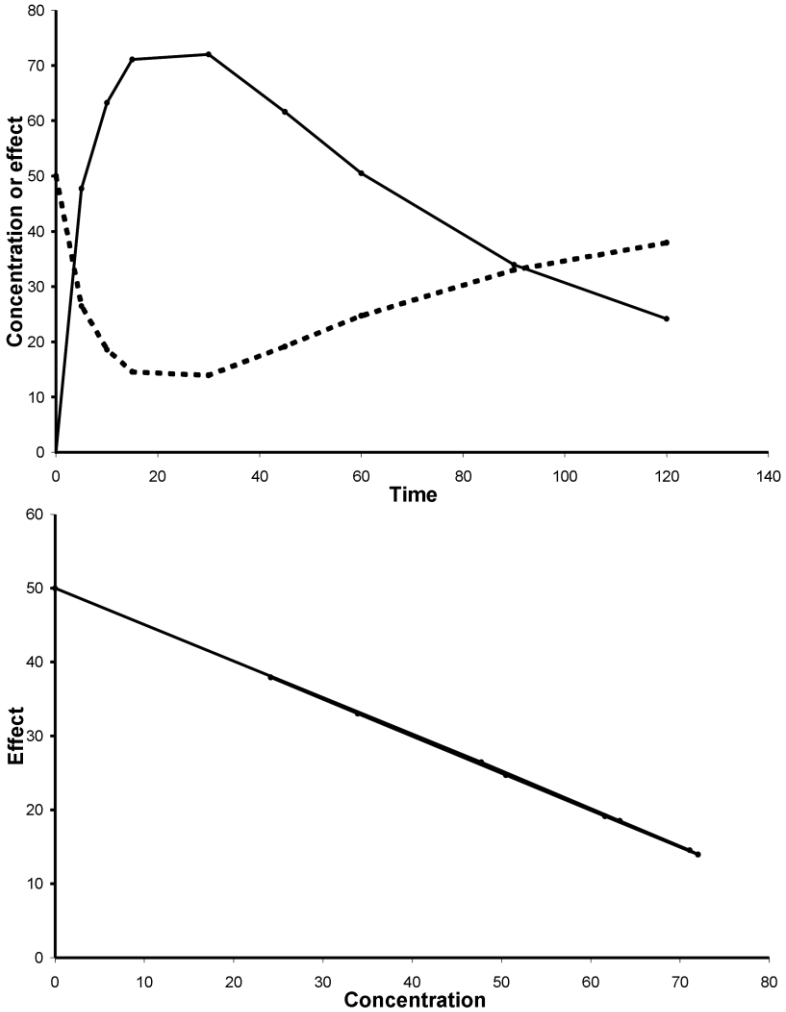

Figure 27 Theoretically illustration of the concentration and effect relationship over time with no effect delay. The effect is represented as pain reduction. In the upper graph the straight line represents the concentration-time relationship and the dotted line the effect-time relationship. Despite the curved shapes of these graphs, the underlying concentration-effect relationship is linear (lower graph).

Linking PK with PD may directly equate the concentration in plasma or another body fluid with the effect site concentration – also termed linear direct concentration-effect relationship (IV). Nevertheless, this straight forward approach is often not the most appropriate. A plot of effect versus plasma concentration frequently results in a hysteresis loop, which may be caused by delays that intervene between plasma concentration and effect.

Delays in response measures can result from the time-lag required for the drug to transit from the plasma to the actual effect site, from the time necessary for the conversion of the drug-receptor binding into a functional response, a rate-limiting receptor dissociation process or from the time needed for the biotransformation of a parent compound into effective concentrations of active metabolites [149]. The most common models to describe drug concentration-effect relationships are the fixed effect model, the linear model and the E_{max} model [19] (IV). To get a good as possible result, it is important to get an adequate number of blood samples to achieve a sound description of PK, as poor fit of the PK will carry over to the PK-PD and result in a poor PK-PD estimate. In some situations where the focus is on PD, a PK model is not required or cannot be developed. In these cases, to maximize the information contained in PD data, cubic spline interpolation of concentrations can be used. By definition, cubic splines pass through every observed data point and interpolate the interval between points with a smooth curve. This empirical approach is suitable for defining the concentration-effect relationship (IV), but does not produce a model that can be used to predict the consequences of altered dose regimens. An important aspect in conducting any PD study is to establish baseline values prior to drug administration (IV). If there are time-dependent changes in the base-line values, then a placebo arm for the drug study is needed to properly characterise the time-course of the PD values in the absence of drug. This time-course needs to be accounted for in any statistical or modelling analysis of the data.

In selection of drug candidates, interspecies extrapolation is a valuable tool, especially for dose selection for the first usage in human studies. Animal studies allow the investigation of fundamental mechanisms (e.g. cerebral equilibration rates) and allow collecting drug concentration from both arterial and venous blood. Nevertheless, the dynamic information (e.g. tail flick time, changes in the EEG or changes in magnetic resonance imaging (MRI) signal) cannot be readily related to analgesia in man. On the other hand PK-PD models developed in sheep have been adapted to assess the clinical profile of analgesic drugs in man [150;151]. However, studies with healthy volunteers or patients can be used to gather highly relevant dynamic information (e.g. pain scores). Moreover such studies can be used to study doses and administration routes that are directly representative for clinical practice. Examples of such representative studies would be study IV, studies with morphine and/or oxycodone [152;153] or studies with intranasal fentanyl [154;155]. Even though similar models described the PK-PD relationship for both drugs, study IV stressed the point that opioids with various receptor affinity can lead to tissue differentiated effects. This may reflect what is observed in the clinic, where some opioids are more effective in individual patients.

In study II and IV the same data set for the pain measurements bone pressure; cutaneous heat stimulation; cold pressor test; NGF induced soreness of the muscle and capsaicin induced hyperalgesia was used for the analytical approach. In study II statistical methods e.g. two-way ANOVA was used, whereas in study IV PK-PD modelling was applied. It is important to keep in mind that using analysis of variance treats the data categorical whereas PK-PD modelling recognises the time-dependent contribution of the concentration of a drug as well as can account for several factors e.g. weight, height etc. This could explain the differences observed between study II and IV (Table 1) [124].

5. Opioids in experimental pain

Strong opioids are potent analgesics and suitable pain models for detecting opioid analgesia are found amongst both acute pain models (phasic pain models) as well as models inducing hyperalgesia [25;119]. To obtain a good trial design, when evaluating a drugs effect in an experimental pain study, the following three factors are important: 1) A pain model – including appropriate induction and assessment method – that activates mechanisms and pain pathways sensitive to the analgesic in question, 2) Right dose, ensuring sufficient efficacy combined with a limited amount of side effects and 3) Correct dosing regimen and time points of testing analgesia. Study I shows the importance of evaluating a new model with regard to reproducibility and thereby validity of a model to be able to test analgesic effects of opioids (II).

Using phasic pain models gives the possibility to investigate tissue and modality differentiated effects of opioids [22;118;119]. Tissue differentiated effect was illustrated in study II, where buprenorphine attenuated bone associated pain, which was not the case for fentanyl. In contrast, both buprenorphine and fentanyl significantly attenuated cutaneous heat pain stimulation, supporting equi-analgesic doses of the two drugs. Furthermore no differences were observed in the recorded adverse effects between buprenorphine and fentanyl.

Models inducing hyperalgesia can act as proxies for clinical manifestations and are more clinical relevant than the phasic pain models [118]. Therefore, standardized experimental pain models in healthy volunteers can act as a translational bridge from studies in animals and to humans [21]. This has previously been demonstrated in our laboratory, where different effects of opioids to hyperalgesia was shown, reflecting the clinical situation [119]. Such models are therefore valuable in studies investigating anti-hyperalgesic effects of drugs [31;129;130]. Several opioids have shown sensitivity to a variety of experimentally induced hyperalgesic pain models [25]. Nevertheless, these studies show conflicting results. For example Eisenach et al. and Wallace et al. showed effect of alfentanil to pin-prick hyperalgesia induced by intradermal capsaicin [156;157], which was not confirmed by Sethna et al. [158]. These studies illustrate that difference in pain assessment methods, populations and dosing regimens play an important role [25]. In study II it was not possible to show an anti- hyperalgesic effect of either buprenorphine or fentanyl to capsaicin induced hyperalgesia and allodynia. Although similar areas of secondary hyperalgesia and allodynia was assessed in the placebo arm, we did not pre-screen the subjects to define a population of responders with a sufficiently large area of hyperalgesia and allodynia to minimize measurement error [159], which might have influenced the results.

Numerous studies have demonstrated sensitivity of analgesics in the primary hyperalgesic area induced by UVB irradiation [130;133;160]. A recent study performed by Bishop et al. showed sensitivity to mechanical stimulation in the primary lesion site [30]. In study II buprenorphine showed anti-hyperalgesic effect to mechanical stimulation in the primary hyperalgesic area. This indicates a peripheral effect of the drug. Nevertheless, Draxler et al. did not demonstrate a peripheral effect of topical applied buprenorphine in the UVB burn injury model [161] and a central effect can therefore not be totally excluded. In this model, it was also possible to show

different anti-hyperalgesic effects between buprenorphine and fentanyl, as fentanyl did not attenuate the mechanical stimulation in the primary hyperalgesic area, which was the case for buprenorphine.

Only few studies have investigated the analgesic and anti-hyperalgesic effect of buprenorphine (Table 2) and fentanyl (See Table 3 in [25]) in human pain models. Koltzenburg et al. showed analgesic effect of transdermal buprenorphine and fentanyl to the cold pressor test, but failed to show a significant effect to heat [43]. It is well known that opioids are sensitive to both the cold pressor test and the heat pain model [25]. Pain reduction by opioids largely results from the activation of opioid-receptors in the central nervous system at both spinal and supra-spinal levels, but a lack of anti-hyperalgesic properties in clinical studies suggests that opioids have only a limited ability to prevent central sensitization of the pain pathways [8]. In support of this hypothesis, Koppert et al. have demonstrated differences between the analgesic and anti-hyperalgesic effects of opioids, showing that buprenorphine had a more pronounced anti-hyperalgesic effect than fentanyl [44]. However, the mechanism for the pronounced anti-hyperalgesic effect of buprenorphine is yet not understood, but the differential affinity to μ-, κ-, and δ-opioid receptors may play an important role. It has also been postulated that the anti-hyperalgesic action is mediated via blockage of voltage-gated sodium channels. In the clinical situation, this particular property of buprenorphine is beneficial for patients suffering from hyperalgesia [8]. The effect of fentanyl has been studied in a few experimental pain studies, where the analgesic effect to phasic pain models was demonstrated and no anti-hyperalgesic effect has been shown [25].

The most commonly used paradigm to induce DNIC is the cold pressor test [48]. Therefore, we used this test in the study III. While animal data suggest that DNIC involves numerous opioidergic links, human data are sparse. Willer et al. showed that naloxone blocked the DNIC effect induced by painful thermal conditioning stimuli in humans, indicating that the loop subserving DNIC involves an opioideric link [49]. Both drugs enhanced the DNIC effect significantly when compared to placebo. As mentioned in chapter 3, opioids exert their analgesic effect from the periphery to the central nervous system and DNIC is a part of the endogenous controlling system of pain that is possible to induce experimentally. It has been suggested that a dysfunction of DNIC may play an important role in the etiology of chronic widespread pain [162]. Jensen et al. demonstrated a specific brain region (rostral anterior cingulated cortex) in fibromyalgia patients, where impairment of pain inhibition was expressed, linking previous results of dysfunctional endogenous pain inhibition in this patient group with the understanding of central mechanisms [163]. Evidence for less efficient endogenous pain modulation has also been demonstrated in patients with functional pain syndromes such as e.g., temporomandibular disorder and irritable pain syndrome [46], fibromyalgia [45;47], chronic tension-type headaches [164] and irritable bowel syndrome [165]. However, it has also been implicated to play a role in patients with pain from more well defined diseases such as osteoarthritis [166;167] and chronic pancreatitis [168]. Therefore, treatment with buprenorphine or fentanyl might be beneficial in the above mentioned patient groups and there is therefore need for further clinical studies investigating this situation.

Study IV is to our knowledge the first to describe the PK-PD relationships for transdermal buprenorphine and fentanyl in humans. PK-PD modelling recognises the time-dependent contribution of the concentration of a drug as well and can at the same time control for several factors such as weight, height etc. [124]. This could explain the differences observed between study II and IV (Table 1).

Opioids exert their main effects in the central nervous system. Hence, after administration of opioids a delay in the time course of the PD effect with respect to the time course of the drug concentration in the blood have been observed [50;149]. Contradictory, Gourlay et al. found a direct relationship between blood concentration and analgesic effect for fentanyl when administered as an intravenous infusion [169]. Similarly, in the present study no delay was observed for the induced experimental pain for either of the drugs. This could indicate a peripheral analgesic effect followed by a central effect or may be a consequence of the long time scale of the study relative to the typical delay for opioids (< 30 min). For all phasic pain measurements linear direct effect models provided the best description of the data for both drugs, suggesting that analgesic effects could directly be related to plasma concentrations for these pain tests. These results imply that there is no upper limit for the analgesic effect of the drugs, at least in these experimental pain models. However, it is important to keep in mind that our study investigated a single dose, and thus a limited concentration range. Both drugs are very lipophilic, meaning that they cross the blood brain barrier easily, suggesting a fast onset of analgesic effect. However, previous studies have shown that slow biophase equilibration kinetics is a major determinant of the time course of the analgesic effect of buprenorphine [42;170]. This might explain the differences observed between the two drugs to bone associated pain and thermal stimulation. Nevertheless, both drugs showed analgesic effects in the cold pressor test (pain scored while immersing the hand in cold water), which is a tonic conditioning stimuli that induces the endogenous central pain inhibiting system and also involves an opioidergic link [171;172].

Even though similar models described the PK-PD relationship for both drugs, the present study stressed the point that opioids with various receptor affinity can lead to tissue differentiated effect. This may reflect what is observed in the clinic, where some opioids are more effective in individual patients.

6. Conclusion

In conclusion, it was possible to develop a valid and reproducible model to evoke bone associated pain using pressure algometry (I). This model could furthermore be used to show tissue differentiated effects of buprenorphine and fentanyl in an experimental study with healthy volunteers (II). In this study it was also shown that buprenorphine had a more pronounced anti-hyperalgesic effect to UVB-induced primary hyperalgesia than placebo, which was not the case for fentanyl. In the same study it was also possible to support equipotent doses, as both drugs attenuated cutaneous thermal pain better than placebo. Study III showed that both buprenorphine and fentanyl enhanced the diffiuse noxious control system (DNIC) which is one of several endogenous pain inhibitory systems that can be evoked experimentally. Using PK-PD modelling it was possible to describe the concentration-effect relationship for both buprenorphine and fentanyl. This was described with similar models but different effects of the two drugs was observed, stressing the point that opioids with various affinity to the opioid receptors may result in tissue-differentiated effects – maybe reflecting what can be observed in the clinic, where different opioids show various effect in individual patients.

Perspectives

As it was possible to develop a model to evoke bone associated pain as well as show tissue differentiated effects of buprenorphine and fentanyl to this model, the next rational step would be to investigate whether this could benefit patients suffering from bone associated pain in patients. It is of value to study differentiated effects of opioids in patients as experimental pain studies in healthy volunteers does not total mimic the clinical situation, as both up-regulation of the pain system and previous pain experiences influence patient´s pain perception.

In future studies it will also be important to investigate if e.g. differentiated DNIC effects of opioids can be evoked in chronic pain populations as this would be an important feature to utilise in pain management. The possible effect on DNIC should also be evaluated for other drugs used in management of chronic pain such as anti-convulsants and anti-depressants, where it would be expected that at least the anti-depressant compounds would enhance the descending drive. In addition new analgesic compounds under development should be screened in mechanism based proof-of-concept studies to profile and compare the DNIC enhancing effect. Besides this it would be possible to investigate PK-PD relationship in patients and thereby see whether some patients may benefit more from one opioid than another. PK-PD relationships provide dose response curves for onset, magnitude and duration of effect, whereby a more rational approach could be used to develop effective dosing regimens in clinical practice.

There is also need for further knowledge about the mechanisms of pain and opioids, leading to more specific mechanisms based treatment in the future. This could be done using a combination of objective measurements e.g. electroencephalography and functional imaging with subjective measurements e.g. experimental pain models in both healthy volunteers and patients,

which in the end could lead to broader insight into the different areas of pain modulation and better treatment of pain.

7. Danish summary

Det kan være vanskeligt at undersøge og vurdere analgetikas effekter i patienter, da deres smerteopfattelse kan være påvirket af faktorer såsom angst, generel sygdom, social påvirkning etc. Påvirkning fra disse faktorer kan mindskes ved at anvende eksperimentelle smertemodeller i raske frivillige forsøgsdeltagere således at både effekten samt smertemekanismer af analgetika kan undersøges. Ved at anvende eksperimentelle smertemodeller kan ”input” (inducere smerte) af intensitet, modalitet og lokalitet kontrolleres og ligeledes kan ”output” (smerteoplevelsen) kvantitativt bestemmes vha. standardiserede psykofysiske eller neurofysiologiske metoder. Fundamentalt kan eksperimentelt induceret smerte inddeles i akutte smertemodeller og modeller som inducerer hyperalgesi. Det menes at modeller som inducerer hyperalgesi efterligner den kliniske situation bedre end de akutte modeller. For at kunne undersøge væv og modalitets forskelle mellem analgetika er det imidlertid vigtigt at kombinere akutte smertemodeller med modeller som inducerer hyperalgesi, således at flest mulige smertemekanismer aktiveres.

Formålene med undersøgelserne der ligger til grund for denne afhandling var at udvikle en model til at inducere knogle associerede smerte (studie I), evaluere forskellige vævs differentierede effekter af transdermal buprenorfin og fentanyl i akut og hyperalgesisk induceret smerte i raske frivillige (studie II), undersøge sammenspillet mellem opioider og det endogene smerte kontrollerende system også kendt som ”diffuse noxious inhibitory control” (DNIC) system (studie III) samt beskrive det farmakokinetiske-dynamiske forhold for de to opioider (studie IV).

Knogle associeret smerte ses ofte i klinikken og sådanne smerter kan være svære at diagnosticere samt behandle. Knoglerne er innerveret af C-fibre, som er sensitive overfor smertefuldt tryk. Det er muligt at inducere knoglesmerte via mekaniske smertestimuli, hvilket tidligere har været anvendt til at inducere smerte i dybe væv. Imidlertid er der ikke nogen studier som systematisk har undersøgt, hvilken probe størrelse der kunne være den mest optimale til at inducere knogle associeret smerte samt hvilken størrelse ville medføre mindst mulig indflydelse af hud komponent. Dette blev undersøgt i studie I, hvor det var muligt at udvikle samt evaluere en reproducerbar smertemodel til undersøgelse af knogleassocierede smerte.

Studier med dyr indikerer at δ-agonister og κ-antagonister besidder anti-hyperalgesisk effekt. Ligeledes viser sɛdanne studier at δ-receptorer spiller en vigtig rolle i knogle associerede smerter. Der søges derfor nye behandlingsmåder, hvor opioider som besidder forskellig selektivitet for μ-, δ-, κ-receptorerne anvendes, således at behandlingen af smerter kan specificeres og dermed optimeres i fremtiden. Buprenorfin har været anvendt i klinikken i over 30år, men først efter introduktionen af transdermal plaster er interessen for dets analgetiske profil igen blevet vækket. I studie II blev vævs forskelle mellem opioider undersøgt, hvorfor både eksperimentel akutte og hyperalgesisk inducerede smertemodeller blev anvendt. I dette studie blev det vist at buprenorfin kunne mindske knogle associeret smerte samt hyperalgesi mere end fentanyl, hvilket illustrerer vævs-forskelle mellem opioider med anderledes affinitet til opioid-receptorerne.

Kliniske studier har vist at sygdomme som fibromyalgi, muskuskeletalt smerte-syndrom og irritabel tyktarm er associeret med nedsat funktion af DNIC. Desuden har det vist sig, at der kan

være tale om et link mellem opioider og DNIC. Dette blev vist i studie III, hvor effekten af DNIC blev øget af både buprenorfin og fentanyl i forhold til placebo.

Behandling med opioider bygger ofte på at et såkaldt 'trial and error' regime, hvor der afprøves en række forskellige opioider for at opnå den bedst mulige behandling med samtidig færrest bivirkninger. Imidlertid kan et mere rationelt regime til optimering af behandlingen anvendes. Dette regime baseres på farmakokinetik-dynamik modellering, som karakteriserer forholdet mellem lægemiddel koncentration og lægemidlets effekt. På denne måde kan indflydelse af effekten af evt. aktive metabolitter og/eller en evt. forsinket effekt af lægemidlet klarlægges. I studie IV blev der vist, at til trods for at buprenorfin og fentanyl begge er lipofile stoffer og begge kan beskrives med lignende PK-PD forhold, så er der tydeligvis forskel mellem de to stoffers analgetiske profil. Dette blev illustreret ved at buprenorfin havde significant analgestisk effekt overfor knogle-associeret smerte, varme, nerve growth factor (NGF) induceret smerte samt tonisk kulde, hvorimod fentanyl kun havde effekt overfor tonisk kulde stimulering samt capsaicin induceret allodyni. Disse vævs - og effektforskelle mellem opioider kan reflektere det som observeres i klinikken, hvor nogle opioider ofte er mere effektive i individuelle patienter.

Table 1 Overview of the results achieved with PK-PD modelling and statistical analysis for the experimental pain tests.

	Bone pain		*Heat pain*		*Cold pressor test (AUC)*		*NGF-induced soreness*		*Capsaicin (sec HA)*		*Capsaicin (allodynia)*	
	PK-PD	Stat	PK-PD	Stat	PK-PD	Stat	PK-PD	Stat	PK-PD	Stat	PK-PD	Stat
BUP	√	√	√	√	√		√					
FEN				√	√							

Abbreviations: AUC=area under the curve (assessed with an electronically visual analogue scale while immersing the hand in cold water), NGF=nerve growth factor, sec HA=secondary hyperalgesia (assessed with von Frey), allodynia=assessed with brush, PK-PD=pharmacokinetic-pharmacokinetic modelling, stat=statistic analysis (two-way analysis of variance), BUP=buprenorphine, FEN=fentanyl. √ represents analgesic effect, whereas empty cells illustrate no analgesic effect of the drug in the experimental induced pain test. Nevertheless, for both buprenorphine and fentanyl analgesic effect to capsaicin induced hyperalgesia and allodynia could not be determined as the best described models for this pain measurements were models with a fixed slope set to zero. This was also the case for fentanyl to NGF-induced soreness.

Table 2 Overview of studies investigating the analgesic and anti-hyperalgesic effect of buprenorphine and fentanyl in healthy human experimental pain studies

		Acute pain		***Chronic pain***	
Author	**Drugs**	**Skin**	**Muscle**	**Hyperalgesia**	**Results**
Koppert et al. Different profiles of buprenorphine-induced analgesia and hyperalgesia in a human pain model Pain 2005; 118: 15-22.	Buprenorphine (0.15mg i.v., 0.2mg s.l.)	- *electrical stimulation*		- *Intradermal electrical stimulation:* - induce secondary mechanical hyperalgesia. Von Frey filament was used to determine the hyperalgesia area.	I.v. and s.l. buprenorphine: - significant analgesic effect compared to the control Buprenorphine: - reductions in the hyperalgesia areas - significantly longer $t_{½}$ for the hyperalgesic effect than for the analgesic effect - better hyperalgesic effect than fentanyl and alfentanil (results from 2 other studies)
Troester et al. abstract from http://		- *electrical stimulation*		- *Transcutan electrical stimulation:* - induce mechanical	Buprenorphine and fentanyl: - supra-additive analgesic effect and an additive hyperalgesic effect of

conferenties.palliatief.nl/fe/abstract.asp? Abstractmainid=553			hyperalgesia	both substances - no antagonistic effect, both drugs seemed to reinforce each other
<u>Koltzenburg et al.</u> Differential sensitivity of three experimental pain models in detecting the analgesic effects of transdermal fentanyl and buprenorphine Pain 2006; 126: 165-174	Buprenorphine (35 µg/h) Fentanyl (12 µg/h or 25 µg/h) (transdermal)	- *cold pressor* - *heat* - *electrical stimulation*		Buprenorphine and fentanyl at 24h: - significant decreased area under the curve for cold pressor - increase in heat pain threshold for TDF 25µg/h and TDB 35 µg/h - no effect towards electrical stimulation - opioid-typical AE´s as dizziness, nausea and vomiting

Abbreviations: i.v.=intravenous, s.l.=sublingual

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