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# ***Experimental Aspects on Chronic Whiplash-Associated Pain***



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If you want to make an apple pie from scratch, you must first create the universe.

Carl Sagan

To my son, Rikard

## Linköping 2008-Abstract

**Introduction:** Chronic pain after whiplash trauma (chronic WAD) to the neck is still a common clinical problem in terms of pain management, rehabilitation and insurance claims. In contrast to the increased knowledge concerning mechanisms of chronic pain in general, no clinical guidelines exist concerning assessment, pain control and rehabilitation of patients with chronic WAD.

**Aim:** The general aim of this thesis was to use experimental techniques to better understand the complex mechanisms underlying chronic pain after whiplash trauma. The specific aims of papers I and II were mainly to use analgesic drugs with different target mechanisms alone or in combinations to assess their effects on pain intensity (VAS). Experimental pain techniques were used in all studies to assess deep tissue sensitivity (electrical, mechanical and chemical stimuli). Paper IV aimed at assessing deep tissue sensitivity to mechanical and chemical stimulation. The aim in paper III was to investigate if biochemical changes in interstitial muscle tissue (trapezius muscle) could be detected in WAD patients.

**Materials and Methods:** The thesis is based on three different groups of patients with chronic WAD. In paper III and IV two different groups of healthy controls also participated. All patients were initially assessed in the pain and rehabilitation centre. In paper I (30 patients) and II (20 patients) two different techniques of drug challenges were used. In paper I: morphine, ketamine and lidocaine were used as single drugs. In paper II: remifentanyl, ketamine and placebo were used in combinations and together with experimental pain assessments. Microdialysis technique was used in paper III (22 patients from study IV and 20 controls). In paper IV (25 patients and 10 controls) a new quantitative method, computerized cuff pressure algometry, was used in combination with intramuscular saline. In all papers, experimental pain techniques for deep tissue assessment (except cutaneous electrical stimulation in paper I) were used in different combinations: intramuscular hypertonic saline infusion, intramuscular electrical stimulation and pressure algometry.

**Results and Conclusion:** There are multiple mechanisms behind chronic whiplash-associated pain, opioid sensitive neurons, NMDA-receptors and even sodium channels might play a part. A significant share of the patients were pharmacological non-responders to analgesic drugs targeting the main afferent mechanisms involved in pain transmission, this implies activation of different pain processing mechanisms (i.e. enhanced facilitation or changes in the cortical and subcortical neuromatrix). Experimental pain assessments and drug challenges together indicate a state of central hyperexcitability. Ongoing peripheral nociception (paper III), central sensitization and dysregulation of pain from higher levels in the nervous system may interact. These findings are likely to be present early after a trauma, however it is not possible to say whether they are trauma-induced or actually represents pre-morbid variations. Clinical trials with early assessments of the somatosensory system (i.e., using experimental pain) and re-evaluations, early intervention (i.e. rehabilitation) and intensified pain management could give further knowledge.

**Key words:** Neck injury, whiplash-associated disorders, chronic pain, central sensitization, pain assessment, drug challenges.

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## List of abbreviations

5-HT	5-Hydroxytryptamine (serotonin)
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AUC	Area under curve
CI	Catheter insertion
CON	Control
E	Exercise
FM	Fibromyalgia
Glu	Glutamate
G <sub>z</sub>	Resultant force affecting the body with an axial/vertical vector
ICF	International classification of functioning, disability and health
IL-6	Interleukin-6
IS	Infraspinatus muscle (shoulder)
K <sup>+</sup>	Potassium
KET	Ketamine
LTP	Long term potentiation
MRI	Magnetic resonance imaging
Nav	Voltage-gated sodium channel
NK <sub>1</sub>	Neurokinin-1
NMDA	N-methyl-D-aspartate
NOS	Nitric oxide synthase
NSAID	Non-steroid anti-inflammatory drug
P	Placebo
PCA	Principal component analysis
PDT	Pain detection threshold
PLS	Partial least squares regression
PPT	Pressure pain threshold
PTel	Electrical pain threshold
PTT	Pain tolerance threshold
PVA	Pressure-VAS area
REMI	Remifentanyl (opioid)
RS	Repeated stimulation
RT	Reaction time
SP	Substance P
SS	Single stimulation
TA	Tibialis anterior muscle (leg)
TNF- $\alpha$	Tumour necrosis factor- $\alpha$
TVA	Time-VAS area
WAD	Whiplash associated disorders
VAS	Visual analogue scale

## List of papers

- I.** Lemming D, Sørensen J, Graven-Nielsen T, Arendt-Nielsen L, Gerdle B.  
The responses to pharmacological challenges and experimental pain in patients with chronic whiplash-associated pain.  
Clin J Pain 2005; 21(5): 412-421.
- II.** Lemming D, Sørensen J, Graven-Nielsen T, Lauber R, Arendt-Nielsen L, Gerdle B. Managing chronic whiplash associated pain with a combination of low-dose opioid (remifentanyl) and NMDA-antagonist (ketamine).  
Eur J Pain 2007; 11(7): 719-32.
- III.** Gerdle B, Lemming D, Kristiansen J, Larsson B, Peolsson M, Rosendal L.  
Biochemical alterations in the trapezius muscle of patients with chronic whiplash associated disorders (WAD) – A microdialysis study.  
Eur J Pain 2008; 12(1): 82-93.
- IV.** Lemming D, Graven-Nielsen T, Sørensen J, Arendt-Nielsen L, Gerdle B.  
Facilitated temporal summation and generalized hyperalgesia in whiplash associated disorder. Submitted.

# 1 Introduction

## 1.1 Clinical background

Since the introduction of moving vehicles there has been an awareness that the sudden acceleration-deceleration forces affecting the human body due to impact, especially affect the neck (i.e. whiplash trauma) and might under certain circumstances cause acute symptoms such as stiffness and pain (i.e., acute Whiplash Associated Disorder, WAD). Positive signs from X-rays and MRI are rare. The vast majority of subjects with acute WAD will recover within three months after the trauma (Spitzer et al. 1995), however the risk for developing pain, generally in the head, neck, shoulders and/or upper back regions, which lasts for 6 months or more (i.e., chronic WAD) is still significant. Chronic WAD account for a large share of the traffic injury related impairment and disability (Sterner and Gerdle 2004). In addition to pain the patients with chronic WAD often presents with a variety of other symptoms: neck stiffness, headache, shoulder pain, back pain, numbness in the arms, dizziness, visual and auditory disturbances, sleeping problems, concentration problems, memory disturbances and fatigue (Provinciali et al. 1996; Radanov et al. 1995; Sterner and Gerdle 2004).

## 1.2 Epidemiology

Annual incidences of acute WAD range between 0.8 and 4.2 per 1000 inhabitants (Sterner and Gerdle 2004). The estimated prevalence of subjects with chronic WAD in the population is 1%, and the prevalence of subjects with *severe* chronic WAD is estimated to 0.4% (Barnsley et al. 1994). Approximately 5-10% of patients diagnosed with acute WAD develop long-term disability (after 6 months duration) with major problems regarding daily functioning and work capacity (Barnsley et al. 1994; Sterner and Gerdle 2004). However the proportion of patients with chronic symptoms after 6 months is larger and has been estimated to 25 % (Barnsley et al. 1994).

### **1.3 Trauma**

Traditionally a linear acceleration-deceleration-hyperextension mechanism (typical rear-end car collision) has been favoured, but the physical mechanism of the trauma at the time of impact, is probably far more complex. The relative horizontal velocity between head and torso as well as pressure gradients in the cervical spine may be of importance (Svensson et al. 2000). Similar symptoms can develop under different circumstances (e.g., high onset  $G_z$ -maneuvers during air combat)(Albano and Stanford 1998) and levels of psychological stress (Castro et al. 2001).

### **1.4 Pathogenesis and prognosis**

The pathogenetic mechanisms behind the variety of symptoms in the different stages of WAD are poorly understood, and seem to differ widely between different subgroups of patients. Remaining nociceptive foci (i.e., structural damage, inflammation etc.) in the tissues might account for chronic symptoms in some patients, while others seem to suffer mainly from pain hypersensitivity without any peripheral pathology when traditional imaging techniques are used. A wide array of etiological mechanisms are suggested and disputed in the range between injuries to the upper cervical ligaments (Johansson 2006; Maak et al. 2006), pathoanatomical disturbances of discs (Taylor and Twomey 1993; Uhrenholt et al. 2002), facet joints (Barnsley et al. 1994; Lord et al. 1996), and psychocultural factors (Obelieniene et al. 1999). These different factors do not necessarily exclude each other but could instead be different aspects of a complex picture of pain; i.e., the bio-psycho-social model that emphasizes an integrated relationship between biological, psychological and social factors (Adams et al. 2006). Several recent studies highlight the central nervous system's ability to modulate pain transmission and perception. A state of hyperexcitability and pain hypersensitivity as a major mechanism in chronic WAD has been suggested (Banic et al. 2004; Curatolo et al. 2001; Koelbaek Johansen et al. 1999; Scott et al. 2005).

Intense acute symptoms and previous neck pain correlate with worsened long term disability prognosis (Berglund et al. 2006; Hendriks et al. 2005; Sterner et al. 2003).

Recent data also suggests that early expectations of recovery affect the prognosis, hence low expectations for full recovery increased the likelihood for high disability (Holm 2007). However, in the individual case the outcome is hard to predict and no biochemical, radiological or neuropsychological markers to assess long-term risk of disability are readily available (Sterner et al. 2003).

### **1.5 Treatment**

Chronic WAD is often associated with poor health and significant socioeconomic impact. Despite this, clearly effective treatments are not supported at this time for the treatment of acute, subacute or chronic symptoms of whiplash associated disorders (Verhagen et al. 2007). However for chronic back pain, there is evidence of intensive multidisciplinary efforts improving function and reducing pain (SBU 2006). Thus no convincing evidence exists concerning effects of rehabilitation in chronic WAD, but it appears that “rest makes rusty” (Peeters et al. 2001). Specific treatments such as zygapophyseal joint blocks (Barnsley et al. 1995), requires identification of patients where zygapophyseal joint damage is the most significant pain generator.

Without evidence, numerous treatments and rehabilitation strategies are used. However from a bio-psycho-social perspective early intervention comprising pain management, normalizing physical activity and cognitive behavioural support seems reasonable.

### **1.6 Classification**

In all papers we used the clinical oriented Quebec Classification of Whiplash Associated Disorders (**Table I**)(Spitzer et al. 1995). The classification is primarily aimed for acute WAD, but has been used in studies of chronic pain. Recently the Swedish whiplash commission suggested a new classification, where Grade 0 and IV are omitted, and indirect trauma is a diagnosis criterion (Rydevik et al. 2005). There is also a symptom based classification suggested by Radanov et al. (Radanov et al. 1992). We have used the

suggested limit of six months or more, as a definition of Chronic Whiplash Associated Pain (Spitzer et al. 1995).

Table I: The Clinical classification of WAD used in this thesis (Spitzer et al. 1995).

<b>The Quebec classification of Whiplash Associated Disorders</b>	
Grade	Clinical presentation
<b>0</b>	No complaint about the neck + no physical sign(s)
<b>I</b>	Neck complaint of pain, stiffness, or tenderness only + no physical sign(s)
<b>II</b>	Neck complaint + decreased range of motion and/or point tenderness
<b>III</b>	Neck complaint + neurological sign(s) (including decreased or absent tendon reflexes, weakness and sensory deficits)
<b>IV</b>	Neck complaint + fracture or dislocation
The following symptoms and disorders can be manifest in all grades: Deafness, dizziness, tinnitus, headache, memory loss, dysphagia and temporomandibular joint pain.	

### **1.7 ICF**

The multidimensional aspect of pain is well described within the new “International Classification of Functioning, disability and health - ICF” (WHO-2001). Bodily functions interact with activity, participation, personal and environmental factors (**Fig. I**). The model represents a contextual framework, which makes it possible to compare complex medical problems (e.g., chronic pain), their natural course and effects of interventions (i.e., rehabilitation). Psychological and physiological functions are included in the category “Bodily functions”. The concept fits well with the biopsychosocial

approach to pain research (Adams et al. 2006). One could argue if chronic pain itself in some cases should be considered a primary disorder. The papers presented in this thesis intentionally focuses mainly on the pain related physiological mechanisms (bodily functions) and not on activity or participation (**Fig. I**).

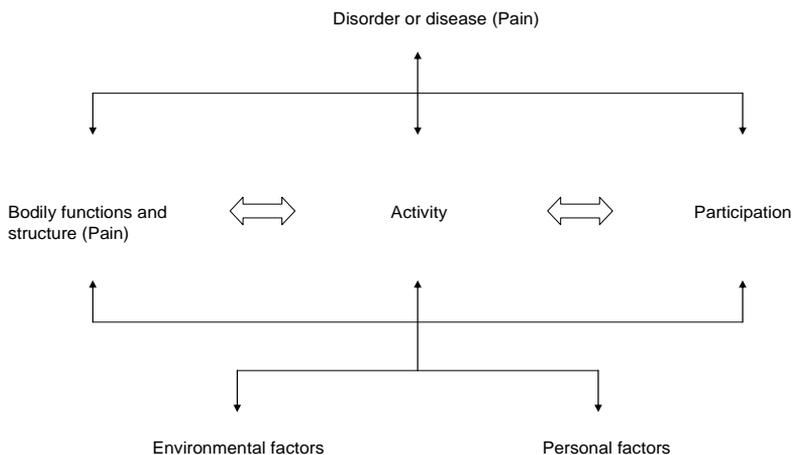


Figure I: The ICF model with respect to pain.

### **1.8 Associated muscle pain**

Local, regional and even, in some cases, widespread pain can be associated with WAD (Buskila et al. 1997; Holm et al. 2007). As earlier discussed the tissue or structure primarily responsible for the pain is not possible to assess by clinical means, but often in a clinical setting muscle tenderness, hyperalgesia and complaints of stiffness, impaired function and muscle soreness are common symptoms. According to clinical examination,

the upper portion of the trapezius muscle is often affected in chronic WAD, but whether this finding is primary or secondary involvement cannot be determined with any certainty. Patients with chronic WAD have unnecessarily increased high muscle tension, which partly can be due to peripheral alterations in the muscle (Elert et al. 2001; Fredin et al. 1997).

Systemic interleukin-6 (IL-6) levels correlate with severity of the injury after major trauma or head injury (Kivioja et al. 2001). In the acute stage (after 3 days) WAD was associated with a systemic dysregulation in the numbers of cells secreting the pro-inflammatory cytokines TNF-  $\alpha$  and IL-6 and the anti-inflammatory IL-10, but these alterations were normalized after 14 days (Kivioja et al. 2001). These systemic changes may be transient indications of local engagement of different tissues – such as muscles – in the neck-shoulder region. In female subjects with chronic work-related trapezius myalgia (TM) increases in lactate, pyruvate, potassium, glutamate and serotonin (5-HT) in the trapezius but with no significant differences in interleukin-6 (IL-6) compared to healthy female controls were found (Rosendal et al. 2005a; Rosendal et al. 2004b).

## **1.9 Neurobiology**

Pain can spread from being a local condition to a regional or general pain condition over time. It has been reported that there is an increased risk for fibromyalgia in patients with acute WAD (Buskila et al. 1997). Persistent/chronic pain is not a simple extension in time of acute pain (DeLeo and Winkelstein 2002), and at least in part it is linked to unique mechanisms in the peripheral and central nervous system (Bennett 2000). Plastic changes can occur at different levels of the pain transmission system, major changes at multiple levels of the somatosensory system have been reported in patients with chronic cervical radicular pain (Tinazzi et al. 2000). The changes induced in the peripheral and central nervous system will probably be less reversible when the stimulus remain and may be related to the pathogenesis of chronic pain (Suzuki and Dickenson 2002). The plasticity responsible for clinical pain hypersensitivity (i.e., allodynia and hyperalgesia) has been

suggested to display two general forms: modulation and modification (Woolf and Salter 2000). *Modulation* represents reversible changes in the excitability of primary sensory (*hetero- or peripheral sensitization*) and central (*central sensitization*) neurons (Woolf and Salter 2000). Low-threshold afferent inputs lead to pain and spread of pain, but innocuous input will lead to amplified responses in pain pathways as well (Salter 2002). Studies of healthy subjects indicate that induction of central sensitisation involves N-methyl D-aspartate (NMDA) receptor mechanisms (Eide 2000). Referred pain and secondary hyperalgesia has been discussed in relation to plasticity of dorsal horn and brain stem neurons and the size of referred pain area is related to the intensity and duration of pain (Arendt-Nielsen et al. 1996a; Arendt-Nielsen et al. 2000). Proximal spread of referred pain is very seldom seen in healthy subjects but often present in patients with chronic pain (Arendt-Nielsen and Graven-Nielsen 2003; Arendt-Nielsen et al. 2000; Arendt-Nielsen and Svensson 2001).

*Modification* represents long lasting alterations in the expression of transmitters/ receptors/ ion channels or in the structure, connectivity and survival of neurons (Woolf and Salter 2000). Thus, modification is a condition when the pain system is highly distorted.

The events in the dorsal horn are regulated from descending tracts (cortex and hypothalamus) and altered activity in facilitatory and inhibitory tracts can result in long lasting net facilitation. This phenomenon has been suggested to play an important role in fibromyalgia (Arendt-Nielsen and Henriksson 2007; Suzuki et al. 2004)(**Fig. II**).

It is important to emphasize that none of the phenomena discussed so far has a proven causative role for the perception of pain (Sandkuhler 2007) and that most of the “mechanism based” pain research has been done on rodents. The dorsal horn of the spinal cord is one main target for pharmacological modulation of pain (**Fig. II**): opioid-, NMDA-receptors and sodium-channels are discussed in the following section.

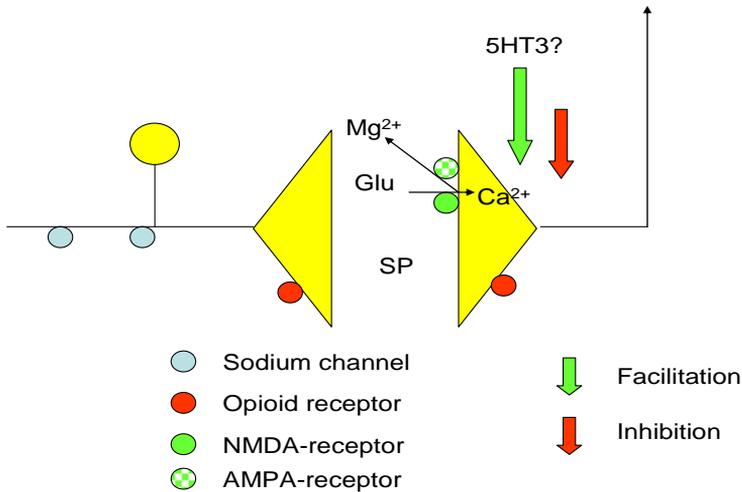


Figure II: Pharmacological target mechanisms and modulation discussed in this thesis (Modified with permission from Hansson)(Hansson 2006).

(5HT3 = 5-Hydroxytryptamine-receptor-3-subtype, AMPA = Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, NMDA = N-methyl-D-aspartate, Glu = Glutamate, SP = Substance P)

### 1.10 Pharmacological challenges

Intravenous drug challenges with single drugs or combinations of drugs with specific target mechanisms (or placebo) can be used clinically to assess pain response or other somatosensory effects of a specific drug or drug combination under controlled conditions. In research, a randomized, placebo-controlled and double-blind design can be used. The technique has been used in neuropathic pain states (Eide et al. 1994; Kvarnstrom et al. 2004), and also in other pain syndromes (i.e., fibromyalgia)(Bennett and Tai 1995; Sorensen et al. 1997). A positive response can indicate that the oral equivalent drug will

be effective for pain treatment, however long-term effects are not possible to predict (Cohen et al. 2004; Cohen et al. 2006).

### **1.11 Pharmacological aspects**

#### **Opioids (morphine and remifentanyl)**

Analgesic opioid effects are mediated by specific receptors (i.e., mu ( $\mu$ ), kappa ( $\kappa$ ) and delta ( $\delta$ )), located on cell membranes, causing neuronal inhibition either by blocking the release of neurotransmitters or by hyperpolarization of the cell. Effect is mediated via changes in calcium and potassium ion channels respectively, the intracellular mechanism is G-protein linked (Tran and Warfield 2003)(**Fig. II**).

Opioids play a role in the treatment of chronic non-malignant pain even though this role is discussed and long-term randomized trials are few. Long-term clinical problems such as addiction and tolerance are not sufficiently assessed (Kalso et al. 2004). Tolerance with attenuated effect develop, sometimes also in early stage (Vinik and Kissin 1998), even opioid induced hyperalgesia may develop (Luginbuhl et al. 2003).

#### **Ketamine**

Ketamine is thought to act primarily as an antagonist to the NMDA (N-methyl-D-aspartate)-receptor, but it may also have actions at sodium channels and at kappa and mu opioid receptors (Baranowski 2003; Mikkelsen et al. 1999). Pre-activation of the postsynaptic AMPA-receptor release a magnesium ion-blockade of the calcium ion-channel associated with the NMDA-receptor. This process makes the NMDA-receptor available for further activation of excitatory amino acids (glutamate) and results in increased excitability of the neurone (Hansson 1998) (**Fig. II**). The NMDA-receptor antagonist ketamine showed inhibition of the responses to repeated nociceptive stimuli (i.e., temporal summation) and a marked hypoalgesic effect on high intensity nociceptive stimuli in humans (Arendt-Nielsen et al. 1995). Administration of ketamine can give analgesia in pain states associated with central hyperexcitability, such as neuropathic pain, phantom limb pain and postoperative pain (Eide et al. 1994; Nikolajsen et al. 1996;

Stubhaug and Breivik 1997; Stubhaug et al. 1997). The dosage of ketamine, when administered as single drug, is associated with psychiatric side effects (Eide et al. 1994) and can be toxic during long-term treatment (Stubhaug and Breivik 1997). In patients with chronic pain, NMDA receptor blockade inhibits abnormal temporal summation and sometimes other characteristics related to central sensitisation (Eide 2000).

Earlier work has tried to explore some aspects of the pain generating mechanisms in fibromyalgia patients using intravenous drug challenges. Using ketamine, lidocaine and morphine, with different pain blocking mechanisms in the central nervous system, fibromyalgia patients were divided into different subgroups (Sorensen et al. 1997). According to this study a significant share (i.e., 8/13) of the responding patients were classified as ketamine responders. These results support the association of central sensitisation with activation of NMDA receptors as part of the pathophysiology of the pain and allodynia in a prominent subgroup of patients with FM.

## **Lidocaine**

It is well accepted that systemic lidocaine can be used for treatment of neuropathic pain (Carroll 2007) at plasma concentrations below those required for block of axonal conduction (Wallace et al. 1996). Lidocaine was used in earlier studies on patients with chronic low back pain and fibromyalgia (Sorensen et al. 1995; Sorensen et al. 1996). Lidocaine is thought to reduce hyperexcitability via unspecific sodium channel block (Nurmikko 2003)(**Fig. II**). Administered systemically this drug can inhibit ectopic activity in the peripheral nerve and the dorsal root ganglia but also exert central modulating effects (Nurmikko 2003), experimental data suggest prolonged central effects (Chaplan et al. 1995). In recent years a subset of voltage-gated sodium channels have been investigated extensively and the channels: Nav 1.3, 1.7, 1.8 and 1.9 have been highlighted in pain modulation (Rogers et al. 2006). In the neuropathic state these channels can be both up- and down-regulated (Rogers et al. 2006). With this growing knowledge it is reasonable to assume that sodium channels can be of importance in different pain conditions, other than neuropathic.

## **Combinations**

It is well known that opioids reduce the initial spinal nociceptive response whereas NMDA-antagonists inhibits the integration (wind-up)(Chapman and Dickenson 1992; Shimoyama et al. 1996). Animal studies have shown that ketamine can attenuate and reverse opioid tolerance (Shimoyama et al. 1996). Healthy volunteer studies have shown synergy between ketamine and opioid on experimental cutaneous pain, pin prick and hyperalgesia (Sethna et al. 1998). Co-administration of NMDA-antagonist and opioid may result in synergistic or additive analgesic effects (Chapman and Dickenson 1992; Plesan et al. 1998; Sethna et al. 1998; Yamamoto and Yaksh 1992). Even though combination therapy with opioids and NMDA-antagonists could be considered from a mechanism-based point of view in several conditions with suspected central sensitization, the evidence for such treatment is still weak, even when cancer pain is considered (Bell et al. 2003).

### ***1.12 Experimental pain assessments***

Experimental pain can be used to mimic different pain states (e.g., musculoskeletal pain, neuropathic pain, visceral pain), represent different modalities (e.g., electrical, chemical, mechanical) and temporal qualities (e.g., repeated, phasic, tonic). The technique can be used for neurophysiological research, to study effects of drugs and psychophysical assessments of human subjects with or without ongoing pain.

#### **1.12.1 Electrical pain**

*Electrically induced muscle pain* is tissue specific (insulated needles) but receptor-unspecific, it is confounded by concurrent muscle twitches which might be painful. At pain threshold levels, the electrically induced muscle pain is probably mediated by group III afferent fibres (corresponds to cutaneous A $\delta$ -fibres)(Laursen et al. 1999). Electrical stimulation actually bypass the receptor level and depolarize afferent nerve fibres directly. Since thick myelinated fibres are activated at lower intensities, the afferent signals are not nociceptive specific (Graven-Nielsen 2006). Cutaneous electrical

stimulation (skin electrodes) can be applied on the skin, with similar limitations. Electrical pain models have been used to establish central hyperexcitability in both patients with fibromyalgia and whiplash associated disorder (Curatolo et al. 2001; Sorensen et al. 1998).

Repeated stimuli of constant intensity may evoke an increase of perception during the repeated stimulation, so that the last stimuli are perceived as painful (Arendt-Nielsen et al. 1994). This phenomenon is called temporal summation and under normal conditions this is a short-lasting hyperexcitability of the spinal cord. To elicit temporal summation with electrical stimulus, the stimulus burst is delivered 5 times with 2 Hz (Arendt-Nielsen et al. 1994).

### **1.12.2 Chemical pain**

The *intramuscular hypertonic saline model* has been used in several studies to characterize the sensory and motor effects involved in acute muscle pain (Graven-Nielsen 2006). Group III and IV (corresponds to cutaneous C-fibres) nociceptors in the wall of arterioles and in the connective tissue are activated (Graven-Nielsen 2006). The computerized hypertonic saline model (Graven-Nielsen et al. 1997) has been used to assess the processing of acute muscle pain in: WAD, fibromyalgia and osteoarthritis patients (Bajaj et al. 2001; Koelbaek Johansen et al. 1999; Sorensen et al. 1998). The model is safe and the intra-individual variation is reasonable (Graven-Nielsen 2006).

### **1.12.3 Mechanical pain**

*Mechanically induced pain (pressure)* is a non-invasive widely used method to assess cutaneous and deep tissue mechanosensitivity. Group III and IV fibres from deep tissue are strongly involved in the evoked sensation (Graven-Nielsen 2006). Skin sensitivity influenced pressure pain thresholds (PPTs) in healthy subjects (Kosek et al. 1999), however this was not found in patients with FM (Kosek et al. 1995). The pressure sensitivity is not restricted to the muscle tissue, connective tissues underlying the skin seem to contribute (Kosek et al. 1995; Kosek et al. 1999). The short-term, intra-individual and experienced inter-rater repeatability is good (Persson et al. 2004). However the long-

term reliability is limited and the inter-individual variation is large (Kosek et al. 1993). In contrast to the manually operated algometer, a computer-controlled cuff pressure algometer assess the complete stimulus-response function, from pain detection to tolerance thresholds and compress a larger tissue volume (Graven-Nielsen 2006; Jespersen et al. 2007).

### **1.13 Microdialysis**

Microdialysis is an invasive method to monitor the chemistry of the extracellular space in living tissue. The technique is based on diffusion of molecules between the interstitial muscle fluid and a perfusate with physiological solution. One or several microdialysis catheters (depending on molecule size to be studied) are inserted in the muscle tissue. In order to assess the concentration of a substance in the muscle the relative recovery must be established, this is accomplished by the use of radioactive markers. The method has been used both on healthy subjects in relation to muscle exercise (Rosendal et al. 2004a; Rosendal et al. 2005b) and on patients with work-related trapezius myalgia (Rosendal et al. 2005a; Rosendal et al. 2004b).

## **2 Aims of the thesis**

The aim of this thesis is to use experimental techniques to better understand the complex mechanisms underlying chronic pain after whiplash trauma to the neck and highlight the need for a diversified diagnostic and etiological approach. The impact of major mechanisms involved in modulation and modification of pain are emphasized.

### **2.1 Specific aims**

#### **Paper I:**

- Investigate if patients with chronic WAD can be divided in subgroups with respect to their perceived alleviation of pain response when given morphine, lidocaine, ketamine, and placebo intravenously and
- If there is a correlation between the pattern of responses of the pharmacological challenges and pain duration or experimental muscle pain sensitivity.

#### **Paper II:**

- Assess the analgesic effects of four different combinations of ketamine, remifentanyl and placebo on chronic WAD-related pain and on simultaneously applied experimental acute muscle pain of different modalities. We expected KET/REMI to alleviate habitual pain more effectively than P/P, P/REMI and KET/P.
- Check the stability of ketamine concentrations.
- Assess to what extent experimental pain sensitivity correlates with habitual pain effects.

**Paper III:**

- Investigate whether the trapezius muscle in chronic WAD patients was associated with alterations in interstitial muscle concentrations of IL-6, 5-HT, glutamate, lactate, pyruvate, K<sup>+</sup> and alterations in blood flow compared to healthy controls and
- Whether such alterations correlated with pressure pain thresholds and pain intensity.

**Paper IV:**

- Assess the sensitivity to painful deep tissue pressure in patients with chronic WAD (and healthy controls) using a cuff pressure algometer, including aspects related to temporal summation (tonic stimulation).
- Assess the sensitivity to intramuscular hypertonic saline and associated local and referred pain areas.

### **3 Subjects**

This thesis is based on three different groups of patients with chronic WAD. The same group was utilized for paper III and IV. All subjects were recruited from the Pain and Rehabilitation Centre, University Hospital, Linköping, Sweden. Case history established a Whiplash Associated Disorder (WAD), without other serious disease or pain syndrome. All studies were conducted in accordance with the Declaration of Helsinki, approved by the Ethical Committee of Linköping University (94132, 00-364, M88-04 and M89-04), and all participants gave informed written consent.

#### **3.1 Paper I**

Thirty-three WAD patients with grade II (Spitzer et al. 1995), volunteered to participate: 23 women and 10 men (mean age: 41 years, range 22-64 years). The mean duration of pain symptoms among the subjects was  $28 \pm 21$  months (range 5 - 96 months). Thirty patients completed the study (three patients excluded due to incomplete data) and only seventeen patients completed the experimental pain assessments. The drop-out was mainly due to a time span of one to several weeks from pharmacological challenge until experimental pain assessment. The experimental assessment was initially considered as an “add-on” part of the study. Radiological evaluation (X-ray, MRI) was only performed when there was a suspicion of skeletal damage or disc herniation. Most patients were subject to acute cervical X-ray in accordance to guidelines in the emergency department. Some subjects used paracetamol, NSAID and weak opioids regularly. Medication was discontinued during the day of testing.

#### **3.2 Paper II**

All patients had acquired their symptoms after a traffic accident. Inclusion criteria were WAD classified as Grade II-III according to the Quebec classification (Spitzer et al. 1995), ongoing pain for more than one year and eighteen years or older. Subjects with ongoing or planned pregnancy, known allergic reactions to opioids or ketamine, drug or

alcohol abuse, generalized pain drawing (three quadrants or more), psychiatric disorder, known general drug oversensitivity or phobic reactions to needles were excluded.

Twenty-one patients fulfilling the criteria volunteered to participate. Eighteen patients corresponded to grade II according to the Quebec classification of WAD and three patients fulfilled grade III criteria (Spitzer et al. 1995). Another 9 patients were screened but did later cancel participation before the first session. Patients came from primary care units in different parts of Östergötland County for clinical evaluation and were not informed of the ongoing study in advance. One patient was withdrawn from the study after one test due to hypertensive reaction and is not included in the results. Hence, 20 subjects (11 women and 9 men) were evaluated (mean age: 34 years, range 19-56 years). The mean duration of pain symptoms among the patients was  $46 \pm 36$  months (range 15-134 months). Some patients used paracetamol, NSAID, tramadol or codeine regularly, medication was discontinued from the evening before the day of testing.

### **3.3 Paper III**

Twenty-five women with chronic WAD were included after examination of medical journals, positive response to information letter and phone call. All patients had acquired their symptoms after a traffic accident. From the history, we determined that all the patients associated their neck pain with the original whiplash trauma and that it was not work-related. Fifty-two percent of the patients were granted some degree of disability pension. Three patients were withdrawn due to technical and methodological problems when inserting catheters. The remaining twenty-two female subjects participated in the experiment (mean age: 36 years, range 24-45 years). Because of technical problems five patients had partially missing data. Inclusion criteria were WAD classified as at least Grade II according to the Quebec classification (Spitzer et al. 1995), with ongoing pain for more than 6 months and eighteen years of age or older. Patients with ongoing or planned pregnancy, drug or alcohol abuse, generalized pain drawing (three quadrants or more) and use of psychotropic drugs or strong opioids were excluded.

All patients were instructed to suspend any pain medication at least from midnight on the day of assessment.

The control group, recruited via advertisements, was comprised of 20 healthy women who were approximately age-matched and who did not identify neck/shoulder pain (mean age: 36 years, range 26-56 years). They were investigated using brief versions of an interview and clinical examination. The data and results concerning these subjects have been published in earlier studies (Rosendal et al. 2005a; Rosendal et al. 2004b).

### ***3.4 Paper IV***

All twenty five women with chronic WAD (mean age: 36 years, range 24-46 years) described in paper III were assessed in this study before they participated in study III. The recruitment process is described in detail above (paper III).

Ten female healthy controls (mean age: 41 years, range 32-50 years) with no clinical pain condition were recruited. No significant differences concerning weight, height, systolic-/diastolic blood pressure or leg size existed between the two groups.

## 4 Methods

The pharmacological tests in Paper I and II were performed according to a randomized, double-blind, cross-over and placebo-controlled design. A summary of the different assessments and pharmacological techniques used are listed in **Table II**. The different experimental techniques as well as pharmacology are discussed separately.

Table II: Summary of experimental methods.

Method/ Paper	I	II	III	IV
30 minute single-drug infusion	X			
65 minute target controlled two-drug infusion		X		
Single & repeated <i>cutaneous</i> electrical pain thresholds	X			
Single & repeated <i>intramuscular</i> electrical pain thresholds	X	X		
Manual pressure algometry (1 cm <sup>2</sup> , 30 kPa/s)	X	X	X	
Intramuscular hypertonic saline infusion	X	X		X
Computerized cuff pressure algometry				X
Microdialysis			X	
Visual Analogue Scale (VAS)	X	X	X	X
Reaction time (auditory)		X		

### 4.1 Drug infusion (Paper I)

Each test session was separated by one week and consisted of a 30 minute period of intravenous administration of morphine hydrochloride (0.3 mg/kg, Morfin®, Pharmacia), lidocaine hydrochloride (5 mg/kg, Xylocain®, Astra), ketamine hydrochloride (0.3

mg/kg, Ketalar®, Pfizer), or isotonic saline (9 mg/ml NaCl). The infusions were accomplished using a syringe pump (Braun Perfusor®, Germany). The hospital pharmacy made the randomisation and delivered the test substances in identical 50ml bottles. All patients received the four drugs (the three active drugs and placebo). The randomisation was made in blocks of 12 patients (i.e., an equal number of patients (3) within each block of 12 patients received the drugs in the same order; for instance: 1) morphine, 2) lidocaine, 3) ketamine, and 4) placebo). Then - within each block of 12 patients - the different combinations with respect to order of the four drugs were randomised.

#### **4.2 Drug infusion (Paper II)**

Each patient was evaluated in four study sessions, at least one week apart. Four different drug combinations, one per study session, were administered in a randomised, cross-over and double-blind fashion. The following four combinations were used: 1) placebo and placebo (denoted P/P), 2) placebo and remifentanyl (denoted P/REMI), 3) ketamine and placebo (denoted KET/P) and 4) ketamine and remifentanyl (denoted KET/REMI).

The sessions were performed in a quiet environment with patients lying supine with the knees slightly flexed and the trunk slightly elevated. Ketamine hydrochloride (Ketalar®, Pfizer) was administered intravenously in the same catheter as remifentanyl/placebo and the target plasma concentration kept constant at 100 ng/ml. This concentration was assumed to be sub-anaesthetic and compared to earlier used concentrations, implied a low likelihood for psychiatric side effects (Arendt-Nielsen et al. 1996b; Stubhaug and Breivik 1997). Equilibration of ketamine effect for 20 minutes was allowed, before start of remifentanyl infusion, to ensure constant plasma and CNS concentrations. Infusion continued for 65 minutes (**Fig. III**). Remifentanyl has rapid pharmacokinetics and equilibration time between effect site (CNS) and plasma (Minto et al. 1997b), we therefore used a different regime for remifentanyl which allowed 1-2 minutes infusion before start of effect assessments. Remifentanyl (Ultiva®, GlaxoSmithKline) was administered intravenously for 15 minutes at target plasma concentration level of 1

ng/ml. After 15 minutes the concentration was raised to 2 ng/ml and the infusion continued for another 30 minutes (**Fig. III**). The level concentration of 2 ng/ml was assumed to be sub-anaesthetic and safe (Egan et al. 1993). Both infusions were ended at the same time. Isotonic saline was used as placebo in both cases. After baseline no measurements or evaluations were made until the calculated target plasma concentrations of the two drugs were reached according to the computer. Plasma concentration was maintained by the use of a target-controlled infusion system. Two infusion pumps (Harvard Apparatus, Kent, UK) were used driven by the Stanpump program which is freely available (S. Shafer, Palo Alto, CA, USA) using the pharmacokinetic parameter set of Minto et al. for remifentanyl and Domino et al. for ketamine (Domino et al. 1984; Minto et al. 1997a; Minto et al. 1997b). Two intravenous catheters were used, one in each arm, for blood sampling and infusion respectively.

Randomization was performed so that an equal number of patients (5) received each treatment during sessions 1-4. This was achieved by drawing different allocation sequences from sealed envelopes at first session (eight sequences were used).

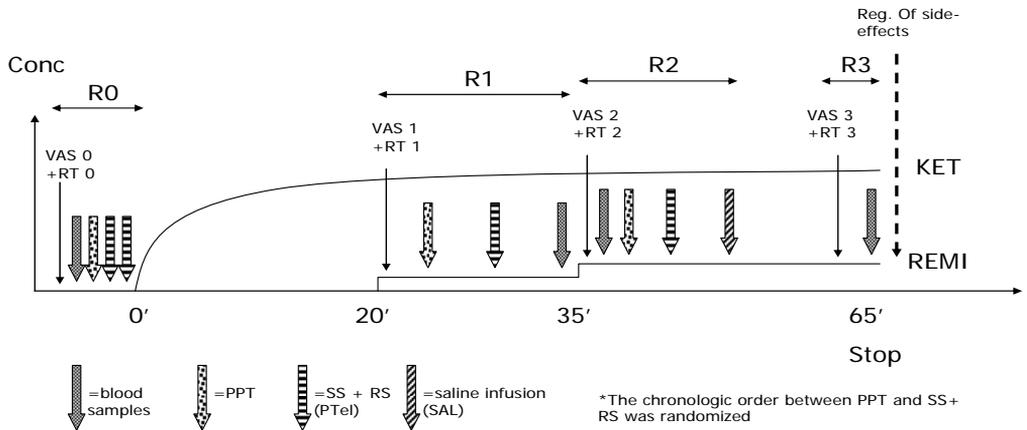


Figure III: Study protocol KET/REMI. Scales for concentration of ketamine and remifentanyl are only relative. R0 denotes baseline assessments; R1 denotes first assessments during REMI/P-infusion (low concentration). R2 denotes first and R3 denotes last assessments during second phase of REMI/P-infusion (high concentration). Abbreviations used; Reaction time (RT), pressure pain thresholds (PPT), electrical pain thresholds (PTel), single stimulation (SS), repeated stimulation (RS), habitual pain intensity assessment (VAS) and saline infusion in TA-muscle (SAL).

### 4.3 Ketamine concentration (Paper II)

Two venous blood samples were taken at baseline (R0), at the first phase of remifentanyl infusion (R1) and two times at the second phase of remifentanyl infusion (R2+R3). A total of 4 samples (i.e., 2x4 tubes) were taken from each patient at each session (**Fig. IV**). The blood samples were centrifuged at 3500 rpm for 30 minutes and the plasma frozen at -20°C for later analysis.

Prior to extraction, plasma samples were allowed to thaw at room temperature, vortexed and then centrifuged at 4°C (2000g, 5 min). To 1 ml of plasma, 1 ml 0.1 M sodium phosphate buffer, pH 6.0 and internal standard was added and vortexed. Extraction and

chromatographic condition were adapted from Feng et al (Feng et al. 1995). Clean Screen solid phase extraction columns (CSDAU-203, World Wide Monitoring, United Chemical Technologies, Inc., Bristol, PA, USA) were preconditioned with 3 ml of methanol, 3 ml of deionised water and 3 ml of 0.1 M sodium phosphate buffer before the plasma samples were loaded (about 0.5 ml min<sup>-1</sup>). The cartridges were rinsed with 3 ml of deionised water, 2 ml of 1 M acetic acid, 3 ml of methanol and then dried under vacuum for at least 5 min. The extracts were then eluted from the cartridge with 3 ml of freshly prepared dichloromethane–isopropanol–sodium hydroxide (39:10:1; vol:vol:vol, prepared with sonication) by gravity filtration. The eluate was evaporated to dryness under nitrogen stream in a water bath at about 40°C. The residues were redissolved in 25 µl butanol, briefly mixed in a ultrasonic bath and loaded into autosampler vials with deactivated inserts. Samples were analyzed by GC-MSD (Hewlett-Packard Model 6890 with a 5972A mass selective detector and automatic injector). Aliquots of 1 µl were injected with pulsed pressure (482 kPa, 1 min.) splitless mode onto a Varian FactorFour VF-Xms, 12 m, 0.2 mm ID capillary column with a 0.33 µm film. The helium flow rate was 1.0 ml min<sup>-1</sup>. Operating temperatures of the GC were: injector 250°C, MSD transfer line 280°C, oven 100°C for 0.5 min rising (25°C min<sup>-1</sup>) to 220°C, rising (45°C min<sup>-1</sup>) to 300°C, hold 2 min. The MSD was operated in the electron impact mode (70 eV) with selected ion monitoring (SIM) with a dwell time of 100 ms each. The data were processed with proprietary mass spectrometer control software (HP G1701AA).

In ketamine (Inselspital, Bern, CH) analysis lidocaine (Sigma Chemical, St Louis, MO, USA) was used as internal standard. Retention times of ketamine and internal standard were 5.18 and 5.14 min, respectively, and for quantitation with SIM the fragment ions m/z 180 and m/z 86 (IS) were used. The intraday and interday coefficients of variation were 4.1% and 6.1% for quality control samples containing 50.8 ng ml<sup>-1</sup> and 4.9% and 6.2% in samples containing 177.8 ng ml<sup>-1</sup> ketamine, respectively. The limit of quantitation (S/N = 10) for ketamine was 8.0 ng ml<sup>-1</sup> for a 1 µl injection. Correlation coefficient values were  $r^2 \geq 0.99$  in the calibration range from 50.8 to 177.8 ng ml<sup>-1</sup>. The recovery of ketamine was  $\geq 65\%$ .

#### ***4.4 Electrical stimulation and pain thresholds***

##### **Cutaneous (Paper I)**

Two surface electrodes (13L20, Dantec, Skovlunde, Denmark) were used to test the cutaneous sensitivity to electrical stimulation. A computer-controlled constant current stimulator (Aalborg University, Denmark) was used. Each stimulus consisted of a train of five 1-msec rectangular pulses repeated at 200 Hz. The pain threshold (PT) was determined with a computer-controlled version of a modified staircase principle, the PT was defined as the mean of the five stimulation intensities evoking the sensations just exceeding the PT. The PT to single stimulation and the summation PT to repeated (2 Hz) stimulations were determined. The summation PT was defined as the stimulus intensity causing the 5th stimulus to be painful (Arendt-Nielsen et al. 1996a; Arendt-Nielsen et al. 1997). A summation ratio (in percent) was calculated as the difference between the PT to single and repeated stimuli divided by the PT to single stimulus (i.e., the relative decrease). A low summation ratio indicated minor efficacy of temporal summation compared with a high value of the ratio.

##### **Intramuscular (Papers I and II)**

Two insulated needle electrodes (Medtronic 9013R0252) with a 3 mm uninsulated tip, inserted into the TA muscle, and separated by 10 mm were used to test the intramuscular (i.m.) sensitivity to electrical stimulation. The rest of the procedure was identical to the cutaneous regime described above.

#### ***4.5 Manual pressure algometry (Papers I, II and III)***

Pressure pain threshold (PPT) was determined using an electronic algometer (Somedic AB, Sweden) mounted with a probe (with a contact area of 1 cm<sup>2</sup>) on the muscle belly of the tibialis anterior (TA) muscle (Paper I, II and III), the central part of the infraspinatus muscle (Paper II) or three points in the trapezius muscles bilaterally (Paper III)(Persson et al. 2000). The pressure was applied perpendicularly to the skin at an increase rate of 30 kPa/s until the subject perceived pain (i.e., sensation of pressure changed to pain) and

pushed a connected stop-button. The PPT is defined as the mean of three trials (one minute between measurements).

#### **4.6 Saline-induced muscle and referred pain (Papers I, II and IV)**

Infusion of hypertonic saline was accomplished with a computer-controlled syringe pump (IVAC, model 770) and a 10 ml plastic syringe. A tube (IVAC G30303, extension set with polyethylene inner line) was connected from the syringe to a 27G hypodermic needle (Braun) (Graven-Nielsen et al. 1997). Infusion of sterile hypertonic saline (6 %) into the tibialis anterior (TA) muscle was performed with a bolus infusion of 0.5 ml over 20 seconds (90 ml/h). The pain intensity of the saline-induced muscle pain was scored continuously on the 100 mm electronic VAS (0 mm indicated “no pain” and 100 mm “most intense pain”). The pain intensities were sampled every 5th second by the computer and recorded for maximum 20 minutes including the infusion time (Graven-Nielsen et al. 1997); the area under the VAS-time curve (AUC), mean VAS (VAS mean), maximum VAS (VAS peak) and the duration (VAS duration) of the saline-induced pain were determined from this registration. In papers I and II the right TA was assessed, in paper IV we assessed the TA muscle on the most painful (according to pain chart), or randomized side.

The patients drew the distribution of the perceived experimental muscle pain on an anatomical map after the pain had ceased. The circumference was then digitised (ACECAD D9000 + digitizer), and the area (in the following labelled pain area) was calculated using Sigma-Scan (Graven-Nielsen et al. 1997). Pain around the infusion site was defined as local pain, as long as the area was continuous. Pain areas separated from “local pain” were defined as referred pain areas (often located at the ankle level). Pain spread “distal to malleol” could represent either local or referred pain as long as it covered an area distal to a line between the malleoli (**Fig. IV**). Pain spread proximal to the knee joint was defined as “proximal pain”.

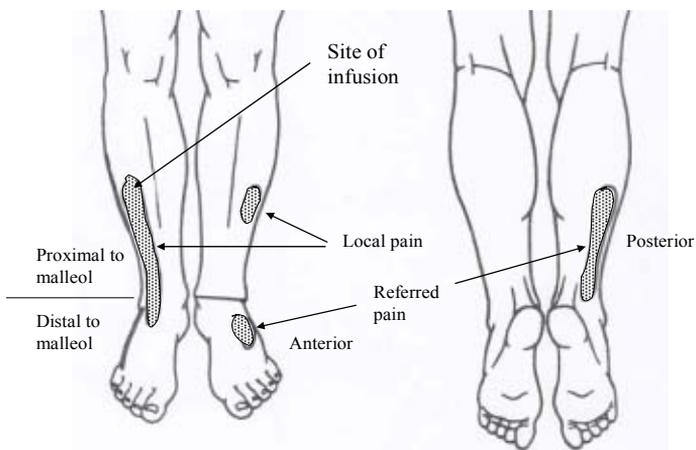


Figure IV: Definition of local- and referred pain areas during intramuscular saline infusion.

#### **4.7 Computerized cuff pressure algometry (Paper IV)**

The experimental setup consisted of a double chamber 13-cm wide tourniquet cuff (a silicone high-pressure cuff, separated lengthwise into two equal-size chambers, VBM Medizintechnik GmbH, Sulz, Germany), a computer-controlled air compressor and an electronic visual analogue scale (VAS; Aalborg University, Denmark). The pain intensity was recorded with the electronic VAS and sampled at 10 Hz. Zero and 100 mm extremes

on the VAS were defined as “no pain” and as “the strongest pain imaginable”, respectively (Polianskis et al. 2002a; Polianskis et al. 2002b).

The compression rate of the compressor was preset and controlled by the computer. The cuff was connected to the compressor and wrapped around the mid-portion of the gastrocnemius-soleus muscles in the leg and around the heads of biceps and triceps muscles in the arm. The maximum pressure limit used was 100 kPa (760 mmHg). The stimulation could be aborted at any time by the subject (push button) or the experimenter (via computer or pressure release button).

Each session (except temporal profile) started with a constant rate of cuff inflation at 1 kPa/s, generating a VAS-pressure curve from which the area under curve (Pressure-VAS Area = PVA) was calculated. Pain detection threshold (PDT) and pain tolerance threshold (PTT) were extracted together with the pain tolerance intensity (i.e., VAS corresponding to PTT). PDT was defined as the pressure equivalent to the moment of transition from strong to painful pressure (i.e., VAS > 0 first time) (**Fig. Va**). PTT was defined as the pressure level corresponding to a pain sensation strong enough to make one feel like interrupting or stopping the session, at which subjects were instructed to press the stop button (Polianskis et al. 2002a).

The temporal profiles were performed measuring VAS as a function of time (TVA) during a constant preset pressure with double cuff inflation for 10 min followed by a 2 min recording with zero pressure (**Fig. Vb**). The time of cuff inflation was momentary. Two successive recordings were made, separated by 5 minutes, with the first pressure level set to 25 kPa and the following level calculated by the formula:  $PDT_{mean} + 0.5(PTT_{mean} - PDT_{mean})$ . The formula was designed in order to achieve a pressure level related to the individual pain sensitivity to pressure. The time-VAS area (TVA) and Temporal Summation Index (VAS peak/time to VAS peak) were calculated and the VAS peak recorded. If a subject aborted the temporal profile in advance, the remaining time with pain was recorded.

# Cuff Pressure Algometry

(constant pressure increase-rate = 1 kPa/s)

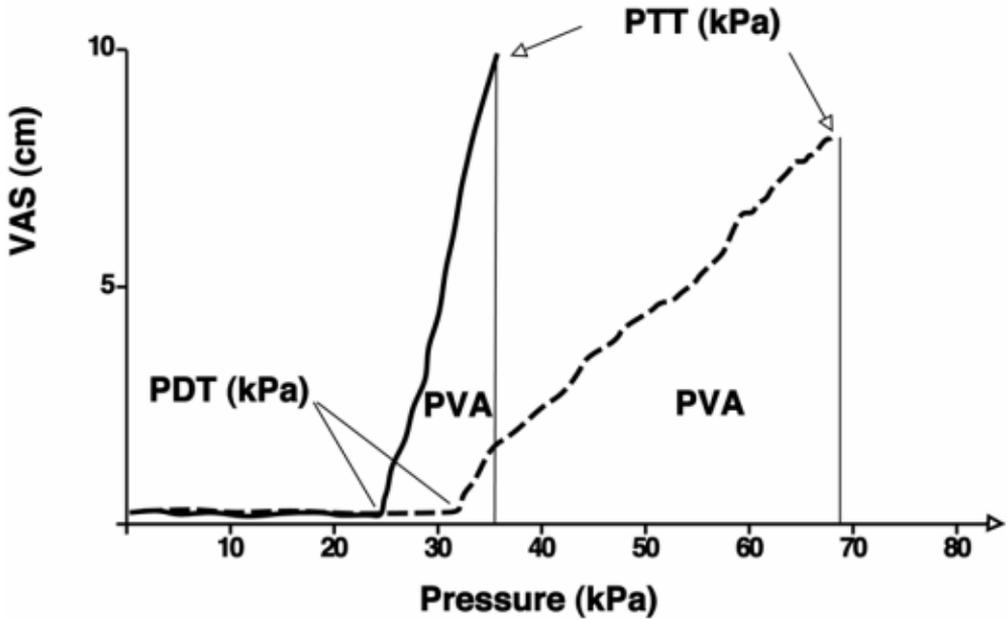


Figure Va: Schematic figure explaining the outcome measures during the constant pressure inflation-rate (1 kPa/s). PTT=Pain Tolerance Threshold, PDT=Pain Detection Threshold and PVA=Pressure-VAS Area. The figure shows two different outcomes (bold and broken lines) resulting in different thresholds and areas under the VAS curve.

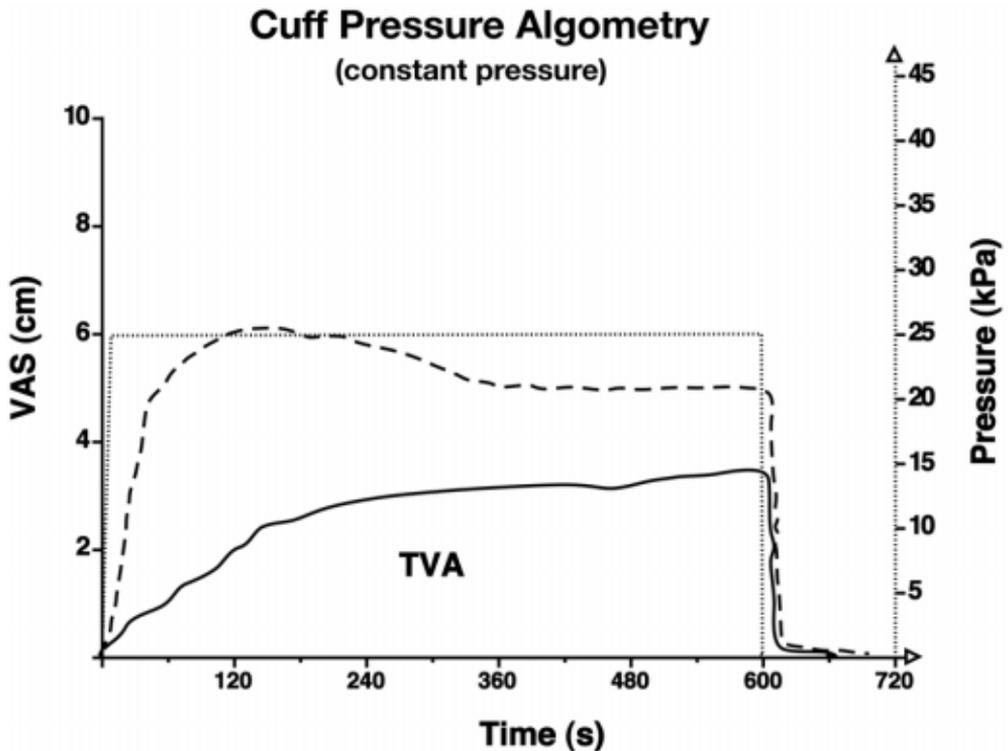


Figure Vb: Schematic figure explaining the outcome measures during the constant pressure stimulation. The figure shows two different patterns of adaptation (bold and broken lines). Dotted line and axis shows the cuff pressure. TVA=Time-VAS Area, represents the area under the VAS-curve.

#### 4.8 Methods of microdialysis (Paper III)

##### Technique

Microdialysis was performed as described by Lönnroth et al., (Lonnroth et al. 1987). As guidance the insertion of *two* microdialysis catheters were preceded by ultrasound investigation of distance between the skin and the trapezius muscle and the width of the muscle. Two custom-made microdialysis catheters (molecular cut-off: 5 kDa and 3000 kDa) were inserted, using the ultrasound investigation as guidance, into the pars descendens of the trapezius muscle using a spinal needle with a inner steering pin (Yale 18G (1.2 x 90 mm), Becton-Dickinson). The microdialysis catheters were placed in the

trapezius muscle in parallel to the muscle fibres. The insertion point was located on the centre of the descending upper part of the trapezius muscle, midway between the processus spinosus of the seventh cervical vertebra and the lateral end of acromion (**Fig. VI**). In the patient group the side with most pain symptoms was chosen, if symmetric pain conditions prevailed (and in the control group) the dominant side (e.g., right handed) was assessed. The skin and the subcutaneous tissues above, where the

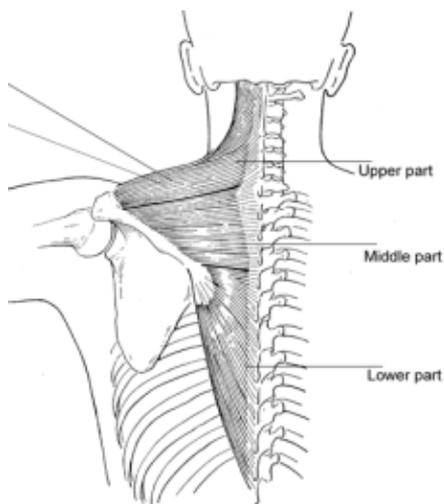


Figure VI: Anatomy of the trapezius muscle.

catheter entered and exited the trapezius muscle, were anaesthetized with a local injection (0.2-0.5 ml) of Xylocaine (20 mg/ml) without adrenaline. Care was taken not to anaesthetize the underlying muscle. The distance between the entrance and exit sites of the catheters in the skin was ~ 7 cm with at least 5 cm of the catheter in the trapezius muscle, ensuring that the entire 30 mm membrane was within the muscle. The inter-catheter distance was approximately 2 cm. In order to facilitate the positioning of the microdialysis catheters the skin of the entrance point was carefully inserted with a needle. The insertion was done in order to eliminate the toughness of the skin. Thus a high

control of the tip of the spinal needle was possible as well as to pass slow through skin, subcutaneous tissue, fascia and entering the muscle with the spinal needle. Typically a brief involuntary contraction and change of resistance were perceived when the tip of the spinal needle entered the fascia and muscle. When the spinal needle was in correct place the inner steering pin was removed and the microdialysis catheter was inserted, then the needle was carefully removed.

To determine interstitial IL-6, a microdialysis catheter was constructed from a single plasmaphoresis hollow fibre (0.2 mm in diameter, molecular mass cut-off 3000 kDa; Asahi Medicals, Japan). It was glued to gas-tight nylon inlet and outlet tubing (Portex Autoclavable Nylon Tubing, Portex Limited, Smiths Industries, Kent, England) and an inner wire (100  $\mu\text{m}$  stainless steel wire) was attached to improve the mechanical stability of the catheter. The length of the dialysis membrane available for diffusion was 30 mm. To determine interstitial lactate, pyruvate, glutamate, 5-HT and  $\text{K}^+$  a 5 kDa microdialysis catheter was constructed. This catheter was made from single plasmaphoresis hollow fibres (0.4 mm in diameter) (Alwall, GFE 11, Gambro Dialysatoren, Hechingen, Germany) glued to a gas-tight nylon inlet tubing (Portex Autoclavable Nylon Tubing, Portex Limited, Smiths Industries, Kent, England) and with a suture thread (Johnson & Johnson, Brussels, Belgium) glued to the membrane to improve the mechanical stability of the fiber. The length of the microdialysis membrane was 30 mm. As a thumb-rule the manufactures guarantee that if cut off for a membrane is e.g., 5 kDa the heaviest molecule to pass the membrane has the weight of 5 kDa. Furthermore 80-90% of the 5 kDa molecules are retained.

Before use, the catheters were sterilized with ethylene oxide. The microdialysis catheters were perfused with a high-precision syringe pump (CMA 100, Carnegie Medicine, Solna, Sweden) at a rate of  $5 \mu\text{l min}^{-1}$  with a Ringer acetate solution (Pharmacia & Upjohn, Copenhagen, Denmark) containing 3 mM glucose and 0.5 mM lactate to minimize the risk of draining the interstitial space (Lonroth et al. 1987).  $0.25 \mu\text{g ml}^{-1}$  [ $^3\text{H}$ ] human type IV collagen (130 kDa, specific activity  $5.9 \text{ MBq mg}^{-1}$ , NEN, Boston, USA) was added to the perfusate to mimic the *in-vivo* relative recovery (RR) of IL-6 using the internal

reference method (Scheller and Kolb 1991). 1.0  $\mu\text{M}$  [ $^{14}\text{C}$ ]- lactate (specific activity: 2.22 GBq  $\text{mmol}^{-1}$ ; Amersham, Bucks, UK) was added to the perfusate used in the microdialysis catheters to determine the *in-vivo* relative recovery (RR) of lactate, pyruvate, glutamate, and serotonin (approximately similar molecular size and weight) using the internal reference method (Scheller and Kolb 1991). Furthermore, nutritive trapezius muscle blood flow was estimated by the microdialysis ethanol technique (Hickner et al. 1994) using  $^3\text{H}_2\text{O}$  instead of ethanol (Stallknecht et al. 1999). 0.3  $\mu\text{l ml}^{-1}$   $^3\text{H}_2\text{O}$  (specific activity, 37 MBq  $\text{g}^{-1}$ , PerkinElmer Life Sciences, Boston, USA) was added to the perfusate, the ratio of  $^3\text{H}_2\text{O}$  in the dialysate and the perfusate (the outflow-to-inflow ratio) varies inversely with the local blood flow in the tissue (Hickner et al. 1994; Stallknecht et al. 1999). In the present study, a separate labelled internal reference substance for  $\text{K}^+$  was not used in order to minimise the radioactive dose. Because the RR of  $\text{K}^+$  was not measured, the dialysate concentrations are presented in accordance with previous published studies (MacLean et al. 2000; Rosendal et al. 2004a). To determine RR of each sample from the catheters, 3  $\mu\text{l}$  dialysate was pipetted into a counting vial and 3 ml of scintillation fluid were added (High-flash Point LSC Cocktail UCH maGold Packard Bioscience B.V., Groningen, The Netherlands) before the samples were counted in a beta counter. RR was calculated as  $\text{RR} = (\text{cpm}_p - \text{cpm}_d) / \text{cpm}_p$ , where  $\text{cpm}_p$  was counts per minute in the perfusate and  $\text{cpm}_d$  in the dialysate. It was assumed that the RR from the interstitial fluid to the perfusate of an unlabelled metabolite equals relative loss from the perfusate to the interstitial fluid of a labelled metabolite. The interstitial concentrations ( $C_i$ ) were calculated (Scheller and Kolb 1991) as  $C_i = (C_d - C_p) / \text{RR} + C_p$ , where  $C_d$  was dialysate concentration and  $C_p$  perfusate concentration (**Fig. VII**).

The distal exteriorized tip of the microdialysis catheter was placed in a 200  $\mu\text{l}$  capped microvial for dialysate collection. Dialysate collection was delayed by 1 minute to adjust for the transition time of the dialysate in the non-permeable outlet part of the catheter. To ensure that the actual flow within the catheter was 5  $\mu\text{l min}^{-1}$ , each microvial was weighed on a precision electronic scale before and immediately after each collection (Sartorius BP 211 D, Bie & Berntsen, Copenhagen, Denmark). Deviation from the aimed

dialysate volume by more than  $\pm 15\%$  resulted in discarding the sample. In the present study, no samples were discarded. Dialysate samples were collected and the samples were immediately frozen and stored at  $-80^{\circ}\text{C}$  until analyses were performed.

## Microdialysis

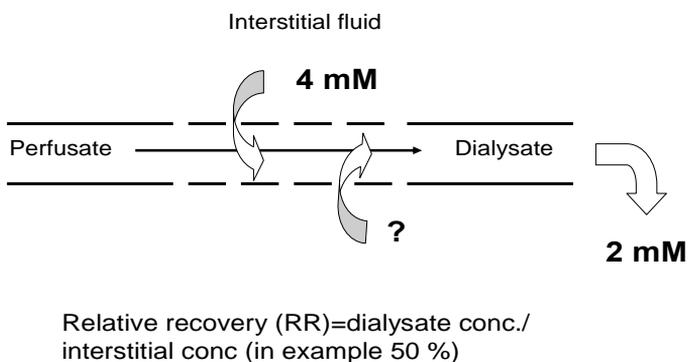


Figure VII: Relative recovery.

## Experimental protocol

Three to four weeks after clinical examination and interview, the microdialysis investigation was performed. The participants were asked not to use any medications except for paracetamol 3 days before the experimental day and were instructed not to perform any shoulder or neck-straining exercises for 48 hours before the study, except for ordinary daily work and/or leisure activities.

The participants reported to the laboratory in the morning. They finished breakfast 1-2 hours before the start of microdialysis and were only allowed to drink water during the experiment.

The participants initially completed a pain drawing to establish that the pain included the descending part of the trapezius (**Fig. VIII**). After this, the microdialysis catheters were inserted. Next, the participants rested for 120 minutes to allow the tissue to recover from

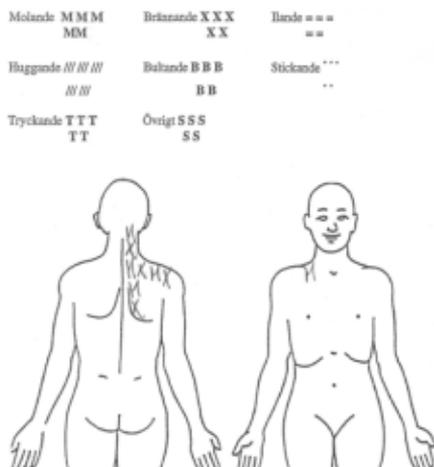


Figure VIII: Pain drawing

possible changes in the interstitial environment induced by the insertion. The following 20 minutes in rest (i.e., 120-140 minutes after catheter insertions) were used to sample sufficient dialysate for 5-HT determination – the analysis requires a sample volume of 100 µl. The next period of 20 minutes (i.e., 140-160 minutes) – still in rest - were used as baseline for the other substances and blood flow. The resting period was followed by a 20-minute repetitive, low-force exercise period. The exercise, a repetitive movement task was performed with the arm *on the same side* as the catheters. The task consisted of moving short wooden sticks (23 g) back and forth between standardized positions 30 cm apart on a pegboard at a frequency of 1 Hz indicated by an electronic metronome (Zen-on music, Tokyo, China), which has previously been used (Rosendal et al. 2005a; Rosendal et al. 2004b). The participants performed the exercise in a seated position with the pegboard placed 30 cm in front of them, measured from the elbow with the upper arm hanging vertically and the elbow in a 90° flexion. Following this exercise, the participants rested for 120 minutes (recovery).

To summarize, microdialysis samples were collected for periods of 20 minutes and the following time periods were analyzed: 120-140 minutes after catheter insertions (baseline, only 5-HT), 140-160 minutes (**baseline**), 160-180 minutes (**exercise**), 180-200 minutes (**recovery #1**) and 280-300 minutes (**recovery #2**).

#### **4.9 Visual analogue scale (Paper I-IV)**

Pain intensity was recorded using a 100 mm VAS with endpoints 0 mm “no pain” and 100 mm “worst possible pain”. During intramuscular saline infusion and cuff pressure algometry an electronic 100 mm VAS-device (controlled by the subject) with a red light display was used, connected to a computer (section 4.6 and 4.7). The use of VAS followed clinical praxis and earlier studies (Sorensen et al. 1997).

#### **4.10 Reaction time (Paper II)**

Reaction time (RT) was recorded using a 1000 Hz tone, randomly generated by a computer with intervals between 3 to 8 seconds. A timer started at the same time the tone was generated. The patient was instructed to press a button as soon as the tone was perceived. RT was defined as the time from tone generation until pressing the button. The mean of 5 consecutive tones was calculated each time and the measurement was done twice every time.

#### **4.11 Statistical methods**

Since we use VAS and other psychophysical outcome measures we have generally used non-parametric statistics. Descriptive statistics are generally expressed as mean  $\pm$  standard error of mean or as mean  $\pm$  one standard deviation.

Friedman's test was used for analysis of variance for repeated measures and the Mann-Whitney test for inter-group comparisons. Wilcoxon signed ranks test was used for intra-group comparisons. The t-test was used for some parametrical data (e.g., age and concentrations). Multivariate statistics were used in paper II and partially in paper III. Spearman's rho was used for bivariate correlations and the Pearson Chi-square or Fisher's exact tests were used for categorized variables. **Table III** gives an overview to the different methods.

Table III: Different statistical methods used in paper I-IV.

<b>Method/ Paper</b>	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
Friedman's test	X	X		
Wilcoxon signed ranks test	X	X		X
Mann-Whitney U test	X	X		X
Student's t-test			X	
Spearman's rho	X	X	X	X
Pearson Chi-Square		X		X
Fisher's exact test				X
Principal component analysis (PCA)		X		
Partial least squares regression (PLS)		X	X	
Variance component models with backwards selection			X	

## 5 Results

### 5.1 Paper I

#### 5.1.1 VAS ratings five days before and five days after testing

No significant differences in mean of scores of pain intensity (VAS) between the five days before and after testing existed for placebo ( $48\pm 18$  mm vs.  $52\pm 21$  mm;  $p=0.061$ ), ketamine ( $48\pm 18$  mm vs.  $45\pm 21$  mm;  $p=0.408$ ), lidocaine ( $48\pm 18$  mm vs.  $49\pm 23$  mm;  $p=0.764$ ) or morphine ( $51\pm 18$  mm vs.  $51\pm 20$  mm;  $p=0.697$ ).

#### 5.1.2 Effect of drug – longitudinal analysis

Generally, the three active drugs but not the placebo, was associated with significant decreases in pain intensities and unpleasantness after start of infusion. Only for unpleasantness was a significant decrease for the placebo challenge found, but it was less prominent than the decreases found for the three active drugs and the post hoc tests (after Bonferroni correction) were not significant. No order effect with respect to the different drugs was found.

#### 5.1.3 Effect of drug – comparisons between drugs

The Friedman tests revealed that significant differences existed with respect to areas under curve, mean VAS decreases and efficacy between the active drugs and placebo (**Table IV**). For the areas under curve of pain intensity (VAS) of the neck, all three active drugs showed significantly smaller areas than the placebo ( $p$ -values: 0.001-0.041) (**Table IV**). For areas under the curve of general pain and unpleasantness, only the effect of morphine differed significantly from the placebo.

The three mean VAS decrease variables as well as the three efficacy variables were significantly changed after the three active drugs compared to the placebo ( $p$ -values: 0.001-0.044) (**Table IV**).

Table IV: The effects of placebo, ketamine, lidocaine, and morphine infusion in patients with WAD with respect to the areas under curve, the mean VAS decreases throughout the test (i.e., the mean of the values obtained during drug administration and after drug administration decrease versus the baseline) and efficacy (i.e., the maximum difference between the value obtained at baseline and a single time point) of neck pain, general pain, and unpleasantness. The p-values of the statistical evaluation are in the right column (Friedman's test). Post hoc tests: \* denotes significantly different from placebo.

<i>Variables</i>	<i>Drug</i>	<i>Placebo</i>	<i>Ketamine</i>	<i>Lidocaine</i>	<i>Morphine</i>	<i>Statistics</i>
Area under curve –neck (mm s)		436099± 236573	383787± 252542*	328505±220930*	263405±250800*	<0.001
Area under curve –general (mm s)		392589± 245135	390063± 240663	356225±236977	272615±250084*	0.006
Area under curve-unpleasantness (mm s)		356932± 234016	324110±264878	302509±211483	246256±280346*	0.010
Mean VAS decrease-neck (mm)		5±12	14±13*	14±18*	18±22*	0.001
Mean VAS decrease-general (mm)		3±13	16±19*	13±17*	20±20*	<0.001
Mean VAS decrease-unpleasantness (mm)		6±12	17±18*	13±15*	18±17*	0.002
Efficacy-neck (mm)		14±15	29±20*	27±21*	26±24*	0.002
Efficacy-general (mm)		12±12	31±25*	26±19*	27±24*	0.001
Efficacy –unpleasantness (mm)		15±15	29±24*	27±20*	26±20*	0.001

#### **5.1.4 Pain duration vs. pharmacological outcome**

No significant correlations existed between the duration of WAD and any of the outcome variables listed in the left column of **Table IV** (i.e., area under curve, mean VAS decrease and efficacy). Moreover no significant differences were found when duration of pain more or less than 24 months was compared.

#### **5.1.5 Responders ( $\geq 50$ % reduction of pain intensity in the neck)**

Three out of 33 subjects had incomplete data and were not included in the following analysis. Two placebo responders were identified. Ten subjects were global non-responders (i.e., did not respond to any of the three drugs). Three subjects were responders only to one active drug (two subjects were only morphine responders and one subject was only lidocaine responder). Eight subjects were responders to two drugs (five responders to both morphine and ketamine, two responders to both ketamine and lidocaine and one responder to morphine and lidocaine) and seven subjects were responders on all three active drugs (global responders). Fourteen ketamine responders, 11 lidocaine responders and 15 morphine responders were identified. Morphine appeared to be associated with effects of longer duration than ketamine (**Fig. IXa-c**).

#### **5.1.6 Pain duration vs. pharmacological response**

The distribution of patients with duration since trauma less than two years and longer than two years was similar in the group of ketamine responders (8 patients with pain < 2 year versus 6 patients with pain > 2 years), lidocaine responders (5 vs. 6), and morphine (7 vs. 8) responders.

Figures IXa-c:

Box plots of pain intensity of the neck (VAS; mm) for responders and non-responders for the actual drug (placebo responders and subjects with incomplete data excluded) for the challenges of ketamine (*Figure IX a*; non-responders: N=14, responders: N=14), lidocaine (*Figure IX b*; non-responders: N=17, responders: N=11) and morphine (*Figure IX c*; non-responders: N=13, responders: N=15). The box plots represent pain intensity according to VAS at the following locations: after needle, di10', di20', di30', ai10', ai20', ai30', ai45', ai60' and ai120', where di10'-30' correspond to the ratings during infusion and ai10'-ai120' correspond to ratings after the infusion period.

Figure IXa

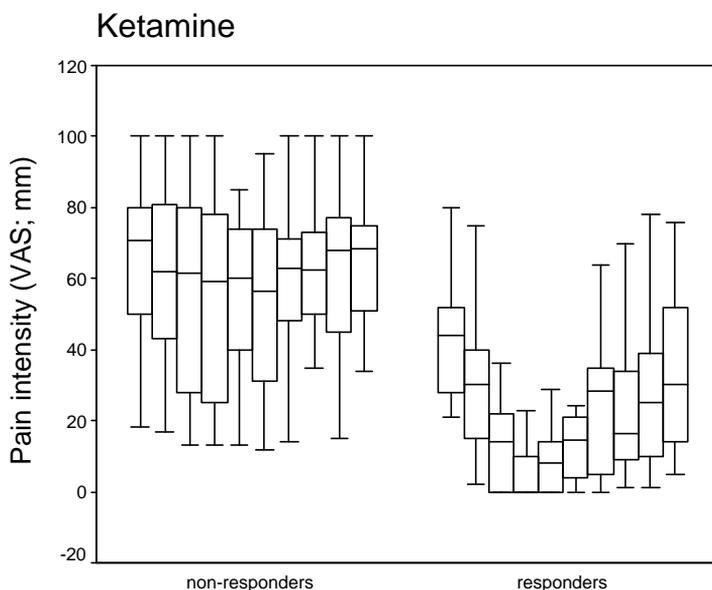


Figure IXb

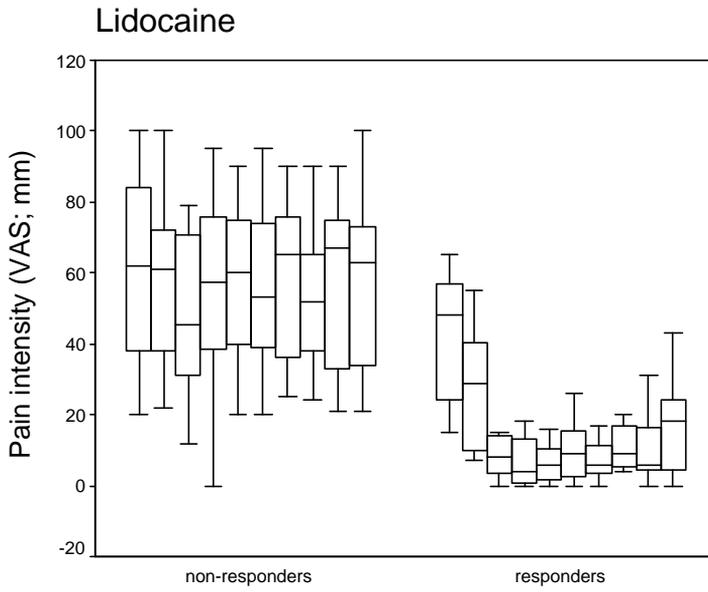
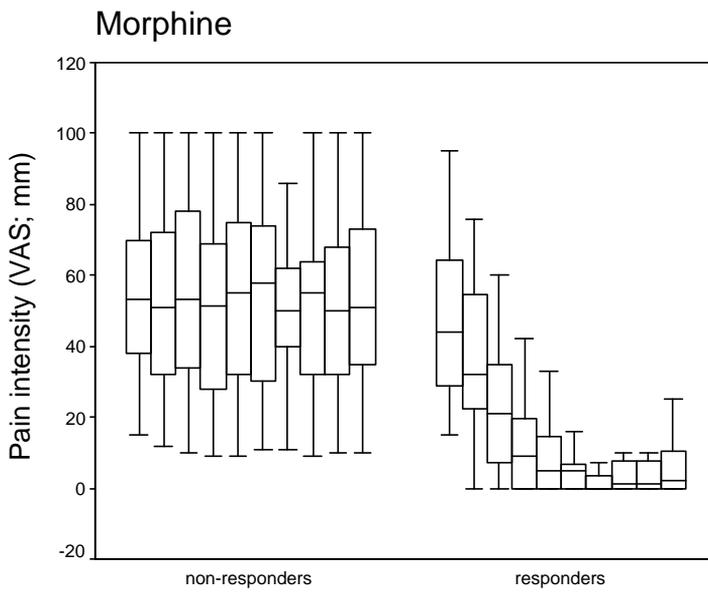


Figure IXc



### 5.1.7 Non-responders

The global non-responders had significantly higher mean pain intensities before and after the tests than the other two groups (global responders and other responders). Moreover, the non-responder group showed a significant increase in pain intensity after the test (global non-responders: VAS before:  $62\pm 11$  mm versus VAS after:  $73\pm 14$  mm,  $p=0.009$ ; global responders: VAS before:  $45\pm 21$  mm versus VAS after:  $41\pm 20$  mm,  $p=0.831$ ; others: VAS before:  $44\pm 17$  mm versus VAS after:  $45\pm 21$  mm,  $p=0.334$ ).

### 5.1.8 Experimental pain assessments

A subsample ( $n=17$ ) volunteered for the second part of the study. Three patients were global non-responders, six were global responders, and eight were responders of two drugs. Thirteen out of 17 were ketamine responders; corresponding figures for lidocaine and morphine were 9 and 12 respectively. Electrical PT was significantly lower at repeated stimulation than at single stimulation both in muscle and skin ( $p<0.001$ ). Widespread referred pain areas with proximal spread after infusion of hypertonic saline into the right TA muscle were found in some ( $n=4$ ) of the subjects. Moreover, several subjects ( $n=4$ ) indicated pain on the posterior aspect of the lower limb, which contrast to results of healthy subjects.

The intramuscular pain thresholds (PTel) at single and repeated stimulation correlated *negatively* with maximum VAS at intramuscular saline induced pain ( $r = -0.814$ ,  $p < 0.001$  and  $r = -0.692$ ,  $p = 0.003$ ). The cutaneous PTel at single and repeated stimulation correlated *positively* with VAS peak time at intramuscular saline induced pain ( $r = 0.494$ - $0.511$ ,  $p = 0.04$ - $0.05$ ).

### 5.1.9 Pain duration and pharmacological effects vs. experimental pain

No consistent pattern between duration of WAD and any of the experimental pain variables in **Table V** could be found. Nor could we find any significant differences in the experimental pain variables with respect to the responses to the pharmacological challenges alone or in different combinations. No correlations existed between the experimental pain variables and baseline pain (mean values of five days before) or the variables found in **Table IV**.

Table V: Mean values ( $\pm$  1SD) from registrations of pressure Pain Thresholds, intramuscular and cutaneous stimulation, pain intensity variables during intramuscular saline infusion, and prevalences (in absolute numbers) of local pain, and referred pain (proximal pain, distal pain, and pain on the posterior part of the lower limb) in the subgroup of 17 patients with WAD, in paper I.

<i>Variables</i>	<i>Mean</i>	<i>SD</i>
<i>Pressure algometry</i>		
Pressure pain threshold (kPa)	609.3	312.5
<i>Intramuscular and cutaneous stimulation</i>		
Intramuscular PT single stimulation (mA)	5.1	4.0
Intramuscular PT repeated stimulation (mA)	3.0	2.6
Relative decrease in intramuscular PT (%)	38.1	19.8
Cutaneous PT single stimulation (mA)	5.6	4.0
Cutaneous PT repeated stimulation (mA)	4.3	3.1
Relative decrease in cutaneous PT (%)	22.7	12.7
<i>Saline induced pain</i>		
VAS area (cms)	1860.6	624.6
VAS duration (s)	400.3	91.4
VAS peak (cm)	8.5	1.9
VAS peak time (s)	113.4	53.3
Pain area (arbitrary units)	7.0	6.3
Local pain (n)	17	
Distal pain (n)	15	
Proximal pain (n)	4	
Posterior pain (n)	4	

## **5.2 Paper II**

### **5.2.1 Background data**

There was no correlation between pain duration and habitual pain intensity (i.e., baseline). However positive correlations existed at baseline between pain duration and pain thresholds (i.e., PPT, PTeISS and PTeIRS) (Spearman's rho: 0,31-0,43 all  $p < 0,01$ ).

### **5.2.2 Plasma concentrations**

The target level of ketamine was 100 ng/ml at R1-R3. The target level was approximately achieved at R1 in both drug combinations (i.e., KET/P and KET/REMI) and without any significant difference (**Table V**). However in both drug combinations the plasma concentrations of ketamine increased from R1 to R2 and R3. Moreover there were significantly higher plasma concentrations of ketamine at R2 and R3 for the KET/REMI than for the KET/P.

### **5.2.3 Habitual pain and reaction time**

The three combinations with active drugs were associated with significant decreases in habitual pain intensity over time (**Table V**). For placebo (P/P) a significant change was only noted when the fourth measurement (R3) was included. KET/REMI had significantly larger decreases in habitual pain than P/P. After 1 ng/ml remi/placebo (R1) also KET/P differed from P/P. With respect to habitual pain intensity seven patients turned out to be non-responders, two patients were placebo-responders. Six patients responded to all three pharmacological combinations. Among four patients responding to two combinations three responded to KET/P and KET/REMI, one responded to P/REMI and KET/P. One patient responded only to the combination KET/P.

It was not possible to predict group membership (i.e., responder or non responder) using PLS regression based on the relevant variables (i.e., excluding the pain intensity variables (VAS) during the different drug challenges).

No differences in RT existed between the four combinations of drugs except at the last measurement R3, the post hoc test revealed a significantly better reaction time for P/P than for the P/REMI combination (**Table VI**).

Table VI: Intensity ratings of habitual pain (VAS; cm), and reaction times (s) for the four different combinations of drugs; mean values  $\pm$  one standard deviation (SD) are given. Plasma concentrations (ng/ml) of ketamine (P-ketamine) are also reported for the two relevant drug combinations; the target levels were 100 ng/ml at R1-R3. The statistical evaluations concern the effect over the three time points (i.e., before (R0), after 1 ng/ml remi/placebo (R1) and after 2 ng/ml remi/placebo (R2)) and four time points (i.e., R0-R3) (for P-ketamine R1-R3 including post-hoc) using Friedmans test; p-values are given. In the row under each time point (in italics) are given the result of the statistical test (Friedmans test) concerning differences with respect to baseline (R0) between the four different combinations of drugs and if significant also the result of post hoc tests (\* denotes significant difference; ne= non equal = significantly different). na denotes not applicable.

Ketamine or placebo	R0		R1		R2		R3		Statistics over time (R0-R3)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
<b>VAS (cm)</b>											
Placebo	4.2	2.0	4.0	2.0	3.6	2.2	3.3	2.3	3.3	2.3	0.005*
Placebo	3.6	2.4	3.2	2.3	2.2	2.1	1.5	2.0	1.5	2.0	0.000*
Ketamine	3.9	2.2	2.6	2.4	1.9	2.2	1.8	2.1	1.8	2.1	0.000*
Ketamine	3.5	2.3	2.1	2.1	1.3	2.0	1.0	1.7	1.0	1.7	0.000*
<i>Statistics drug combinations</i>			<i>0.001*</i>		<i>0.013*</i>	<i>P/P ne</i>	<i>0.045*</i>	<i>P/P ne</i>	<i>0.045*</i>	<i>P/P ne</i>	<i>KET/REMI</i>
			<i>KET/REMI.</i>			<i>KET/REMI</i>					
			<i>P/REMI ne</i>			<i>P/REMI ne</i>					
			<i>KET/REMI</i>			<i>KET/REMI</i>					
<b>Reaction time (s)</b>											
Placebo	198.8	31.8	215.6	37.5	216.4	40.5	212.5	36.4	212.5	36.4	0.002*
Placebo	201.4	23.2	229.6	38.5	227.4	48.0	268.2	73.0	268.2	73.0	0.000*
Ketamine	205.1	35.0	224.4	31.1	224.3	32.8	228.3	32.0	228.3	32.0	0.002*
Ketamine	204.9	55.7	241.9	57.2	239.7	64.7	253.8	76.6	253.8	76.6	0.001*
<i>Statistics drug combinations</i>			<i>0.208</i>		<i>0.598</i>		<i>0.014*</i>	<i>P/P ne</i>	<i>0.014*</i>	<i>P/P ne</i>	
								<i>P/REMI</i>			
<b>P-Ketamine (ng/ml)</b>											
Ketamine	0.0	0.0	80.06	28.35	91.71	23.96	98.74	24.79	98.74	24.79	0.012*
Ketamine	0.0	0.0	96.08	31.54	117.49	30.49	135.02	26.31	135.02	26.31	(R1 ne R2, R3) <0.001*
<i>Statistics drug combinations</i>			<i>ns</i>		<i>0.011*</i>		< <i>0.001*</i>		< <i>0.001*</i>		(all different)

#### **5.2.4 Pressure pain thresholds**

The PPT of the infraspinatus muscle increased over time for the three combinations with active drugs but not for P/P. The increases in PPT differed significantly between KET/REMI and P/P after 1 ng/ml remi/placebo (R1) and between P/REMI and P/P after 2 ng/ml remi/placebo (R2).

PPT of the tibialis anterior muscle increased over time for the KET/REMI combination. A significant difference in amount of increase in PPT was only found for the KET/REMI in relation to P/P after 2ng/ml (R2).

#### **5.2.5 Intramuscular electrical stimulation**

Pain thresholds for single and repeated (temporal summation) electrical stimulation increased significantly over time both for P/P and for the active drug combinations. At R1, KET/REMI generally had significantly higher values than the other combinations. At R2, P/REMI and KET/REMI had significantly higher values than the other combinations for PTel-SS and PTel-RS.

#### **5.2.6 Intramuscular saline infusion**

Three out of four variables of pain intensity after saline infusion (VAS area, VAS mean and VAS peak) were significantly reduced by the two drug combinations containing remifentanyl (i.e., P/REMI and KET/REMI) when compared to P/P.

The number of subjects with local pain, pain distal to the malleol and pain proximal to the malleol were significantly lower when the two combinations containing remifentanyl (i.e., P/REMI and KET/REMI) were given.

The three active drug combinations had significantly less local and total pain areas of the leg after infusion of hypertonic saline compared to placebo. Moreover, KET/REMI reduced area of local pain most, while KET/REMI and P/REMI reduced total pain area equally significant.

### **5.2.7 Side-effects**

No marked differences in side effects existed between KET/REMI and P/REMI regarding expected side effects (i.e., sedation, dreams, hallucinations, pruritus and nausea).

However the combination KET/REMI had a relatively high number of subjects with other side-effects: double vision, changed body perception, tachycardia, cold sweat, dry mouth, feeling of warmth in hands and dizziness.

### **5.2.8 Multivariate analysis**

In order to understand and to take into consideration the multivariate correlations between the different outcome variables a PCA was made that included all four different tests of each person. Two significant components ( $R^2_{\text{cumulative}}=0.49$ ) were identified (data not shown). According to the first component (p1), pain after saline infusion (i.e., mean VAS and presence of local pain) correlated negatively with several of the PPT variables and the P<sub>Tel</sub>-SS and P<sub>Tel</sub>-RS variables. Thus low pain thresholds at pressure algometry and IM electrical stimulation were associated with high pain intensities during infusion of hypertonic saline. The second component (p2) mainly reflected the positive intercorrelations between the four pain intensity ratings. Hence, the variables obtained during the acute tests (PPT, P<sub>Tel</sub>-SS/RS and pain intensity during IM saline infusion) were generally uncorrelated with the pain intensity ratings.

The individual scores relating to the two principal components were finally used as two multivariate uncorrelated outcome variables (the first reflecting experimental pain sensitivity and the second habitual pain intensity). There existed significant differences in scores between the four drug combinations with respect to the two multivariate outcome variables ( $p<0.001$  for both), the three active drug combinations differed significantly from P/P for both multivariate outcome variables. The most prominent changes with respect to placebo were found for KET/REMI, followed by P/REMI and KET/P. For both multivariate outcome variables KET/REMI differed significantly from KET/P.

### **5.3 Paper III**

#### **5.3.1 Algometry (PPT)**

The average PPTs were lower in WAD than CON in right and left trapezius muscles as well as left and right tibialis anterior muscles (all  $p < 0.001$ ). There were significant positive correlations between PPTs of trapezius and tibialis anterior (Spearman's rho: 0.79-0.87; all  $p$ -values:  $< 0.001$ ).

#### **5.3.2 Pain intensity (VAS)**

Before insertion of the microdialysis catheter, pain was  $53 \pm 4$  mm in WAD and  $0 \pm 0$  mm in CON with a highly significant difference between groups ( $p < 0.001$ ) at all time points throughout the experiment (**Fig. X**). In WAD during the low-force exercise, VAS continuously increased above baseline (160 min) and peaked at the end of exercise at  $71 \pm 24$  mm ( $p < 0.001$ ) followed by a slow decrease during recovery, but still significantly increased when compared to baseline (all  $p < 0.001$ ) (**Fig. X**).

#### **5.3.3 Blood flow**

There was no overall significant difference in trapezius muscle blood flow, measured as outflow to inflow ratio of  $^3\text{H}_2\text{O}$ , between WAD and CON. In both groups, blood flow increased (i.e., decreased value) to similar levels during exercise ( $p < 0.001$ ) and both groups had similar course throughout the rest of the experiment (i.e., no significant time\*group effect).

#### **5.3.4 Lactate and pyruvate**

In both WAD and CON, interstitial [lactate] changed throughout the experiment (time effect:  $p < 0.01$  and  $p < 0.05$ , respectively), but without a significant overall group difference. However, the development throughout the test differed significantly between the two groups (i.e., significant time\*group effect:  $p < 0.05$ ). Hence, the interstitial [lactate] was significantly increased at recovery #1 in the WAD group and thereafter it decreased. At recovery #2, interstitial lactate was significantly lower than baseline levels in CON but not in WAD (**Fig. XI**).

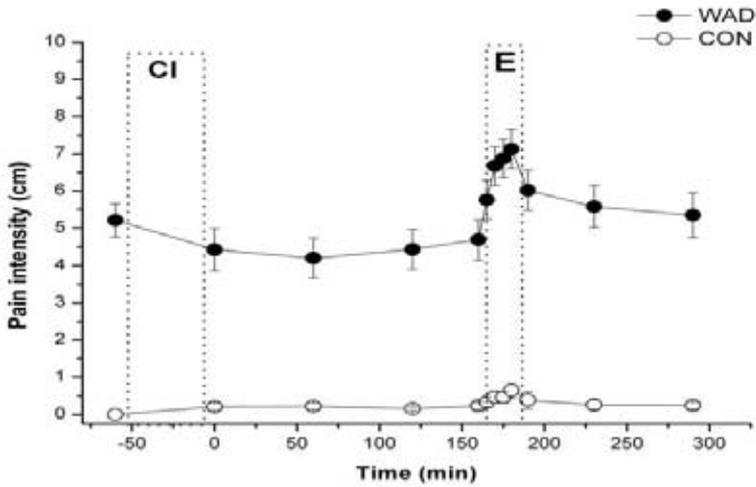


Figure X: Pain intensity throughout the experiment (CI = catheter insertion; E = Exercise)

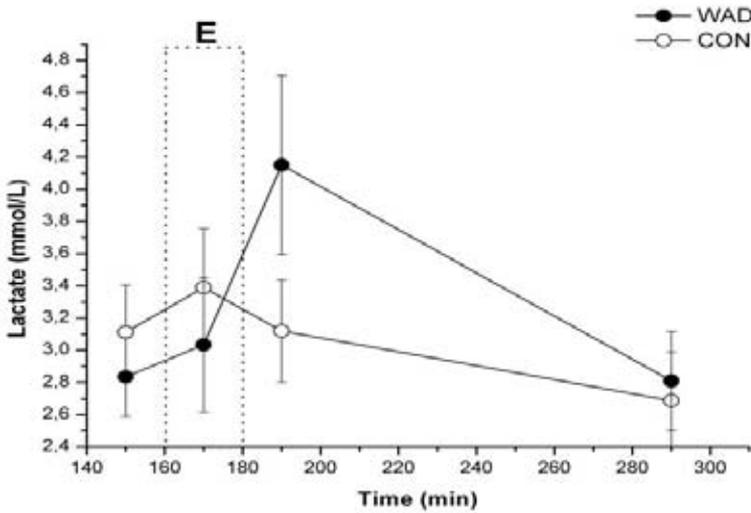


Figure XI: [Lactate] throughout the experiment (E = exercise)

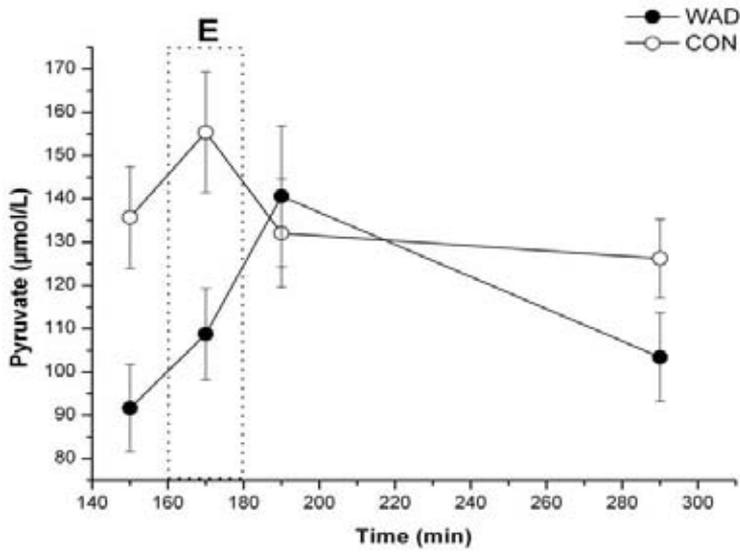


Figure XII: [Pyruvate] throughout the experiment (E = exercise)

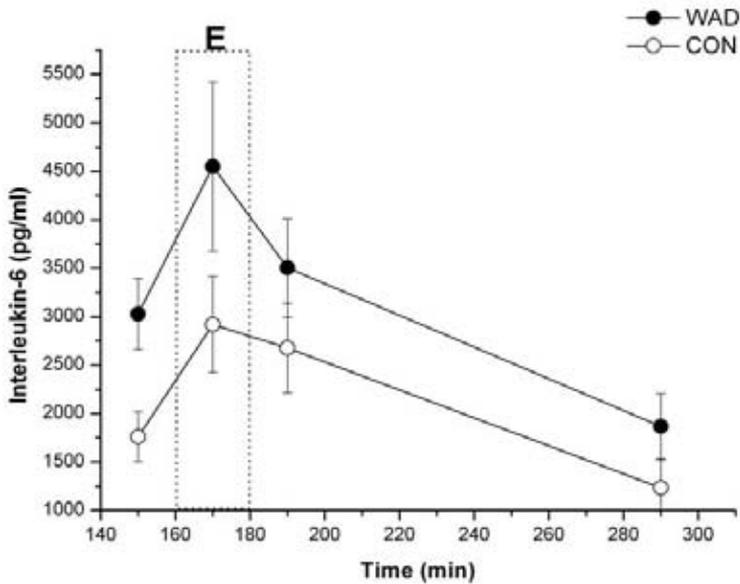


Figure XIII: [IL-6] throughout the experiment (E = exercise)

Interstitial [pyruvate] differed significantly overall between groups (i.e., group effect:  $p < 0.05$ ); CON generally had higher [pyruvate] than WAD. A significant group difference was found between CON and WAD at baseline when tested separately ( $p = 0.008$ ). The development throughout the test also differed between the two groups (i.e., significant time\*group effect;  $p < 0.05$ ). Hence, the concentrations of pyruvate at baseline and at recovery #2 were significantly higher in the CON group than in WAD group. In the CON group, a peak in [pyruvate] occurred during exercise followed by a decline. In contrast, significant changes in [pyruvate] occurred throughout the test ( $P < 0.01$ ) in WAD with a peak in [pyruvate] at recovery #1 ( $p = 0.004$ ) and with a decrease at recovery #2 (**Fig. XII**).

### **5.3.5 Potassium ( $K^+$ )**

Dialysate [ $K^+$ ] did not differ overall between groups and there was no different development throughout the test (i.e., a non-significant time\*group effect). However, the within group analysis in CON showed that the [ $K^+$ ] at recovery #2 was significantly lower than baseline value. This was not found in WAD.

### **5.3.6 Interleukin-6 (IL-6)**

The WAD group had overall significantly higher interstitial [IL-6] ( $p = 0.024$ ) than the CON group (**Fig. XIII**). A significant group difference was found between CON and WAD at baseline ( $p = 0.008$ ). The development throughout the test was similar to that of [IL-6] of the CON group. However, the peak value during exercise was not significant due to the large inter-individual variation in [IL-6] in the WAD group.

### **5.3.7 Glutamate**

No significant differences in interstitial muscle glutamate concentrations were found overall throughout the test between WAD and CON groups. In response to exercise, glutamate concentrations increased – either during (CON) or immediately post-exercise (WAD) ( $p < 0.05$  and  $p < 0.05$  respectively) where after it progressively decreased. The

concentration of glutamate was significantly lower at recovery #2 than at baseline in the WAD group.

### **5.3.8 Serotonin (5-HT)**

Interstitial muscle 5-HT concentrations measured at rest between 120-140 min differed significantly between the WAD and CON groups ( $10.5 \pm 3.4$  versus  $3.8 \pm 1.3$  nmol/l;  $p=0.05$ ).

### **5.3.9 Regression of group membership**

Group membership (CON (coded 0) or WAD (coded 1)) was regressed using the biochemical and blood flow variables as regressors (X-variables). A significant model was achieved ( $R^2=0.44$ ,  $p<0.05$ ). As expected from the univariate comparisons concentrations of pyruvate, IL-6, and  $[K^+_{\text{recovery}\#2}]$  were important for predicting group assignments. If also PPT of trapezius were included as regressors (X-variables) in the regression of group membership, the explained variation rose as expected ( $R^2=0.64$ ,  $p<0.05$ ) and the two PPT variables of trapezius were the most important regressors.

### **5.3.10 Regression of overall pain intensities in the WAD group**

The significant regression of overall pain intensity in the WAD group ( $R^2=0.54$ ;  $p<0.05$ ) identified [5-HT], potassium concentrations at recoveries #1 and #2, [pyruvate<sub>baseline</sub>], [glutamate<sub>recovery#2</sub>], [lactate<sub>recovery#1</sub>], and the three out of four blood flow measurements as the most important regressors. Positive correlations existed between pain intensity and [5-HT],  $[K^+_{\text{recovery}\#1}]$ , [lactate<sub>recovery#1</sub>], and the blood flow measurements.

### **5.3.11 Regression of Pain intensities during exercise**

The four pain intensity ratings obtained during the exercise period were simultaneously regressed using the biochemical and blood flow variables of baseline and exercise as X-variables. In conclusion, pain intensities during exercise in the WAD group correlated positively with [5-HT] and blood flow and negatively with [pyruvate<sub>baseline</sub>].

## **5.4 Paper IV**

### **5.4.1 Habitual pain**

We found no significant differences in habitual pain intensity of neck-shoulders when the ratings before and after the experiment were compared in the WAD-group.

### **5.4.2 Cuff pain threshold**

Pain tolerance thresholds (PTT) showed a significant difference both in the arm (near painful area) and leg (outside painful area) between the groups. PTT were significantly lower in the leg than in the corresponding arm in both groups. The pain tolerance intensity (VAS at PTT) did not differ at all between the groups (i.e., both patients and controls endured the same perceived pain rating (VAS) before pushing the stop button). Prominent differences were observed for the pressure-VAS Area (PVA) during cuff pressure algometry in the leg, where the area under the pressure-VAS curve (**Fig Va**) was significantly lower in the WAD-group.

### **5.4.3 Characteristics during tonic cuff stimulation**

Ten minutes constant pressure at 25 kPa showed a significant difference in the time-VAS area (**Fig Vb**) with a larger area in the WAD-group. Also the VAS-peak registration during this session differed significantly with higher ratings for the WAD group, the latter was also true for the session with calculated pressure level. The mean pressure levels calculated were 36.4 kPa in the control group and 28.5 kPa in the WAD-group. The temporal summation index was significantly higher for the WAD group with a quick and high VAS-peak. One third of the subjects (WAD) and four of the controls had a negative slope after VAS-peak, indicating adaptation to pain, however on the group level there was no difference in adaptation between the groups. Three patients in the WAD-group aborted the registration with the “escape” button (time with pain after 0 pressure was achieved varied between 13-155 seconds).

#### 5.4.4 Saline induced muscle and referred pain

The time-VAS area (AUC) registration showed significantly larger areas in the WAD group compared to controls.

Pain areas in the leg after saline infusion were expanded in the patient group, though not significant (**Fig XIV**). Number of referred pain areas, pain distal to malleol and actual referred pain area did not differ significantly between the groups. However only the patient group displayed proximal referred pain ( $n=3$ ) and had a total of 16 referred pain areas compared to 3 in the control group.

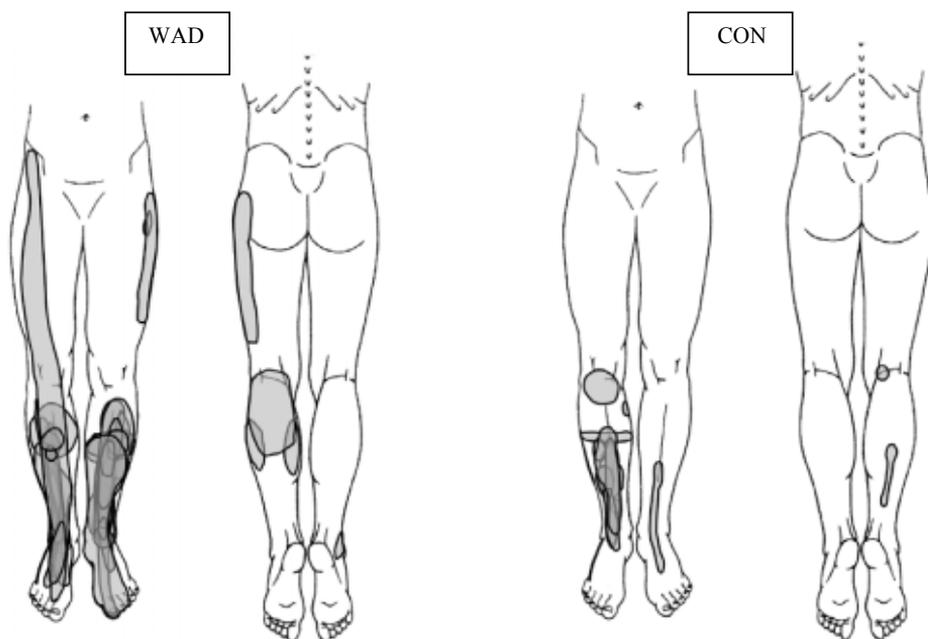


Figure XIV: Pain areas in the leg after saline infusion (pain areas from all subjects are superimposed, CON = Controls)

#### **5.4.5 Correlation between the two pain modalities (AUC vs PVA)**

AUC during saline infusion correlated negatively with the pressure-VAS area both within the WAD group ( $R=-0.44$ ,  $p=0.027$ ) and the control group ( $R=-0.66$ ,  $p=0.038$ ); all subjects taken together:  $R=-0.55$ ,  $p=0.001$ .

## **6 Discussion**

### **6.1 Pharmacological challenges**

The major findings in Paper I and II were:

- 1) The responses to pharmacological challenges were heterogeneous, but the majority of patients responded to two or more drugs with different target mechanisms (subgroups exist in chronic WAD) (Paper I and II).
- 2) Approximately one third of the subjects could be classified as non-responders to drugs (Paper I and II) (i.e., 33-35% respectively).
- 3) The pattern of responses to the pharmacological challenges did not show any significant relationships with pain duration or with the experimental pain sensitivity (Paper I).
- 4) The pharmacological combination of ketamine and remifentanyl (KET/REMI) was most effective in alleviating habitual pain intensity compared to the single drugs alone and placebo (Paper II).
- 5) The experimental pain sensitivity was significantly modulated by the drug combinations containing remifentanyl (P/REMI and KET/REMI). However the combination KET/REMI was significantly more effective in several aspects (Paper II).
- 6) The multivariate analysis revealed that changes in habitual pain and experimental pain sensitivity were uncorrelated during the pharmacological challenges (Paper II).

### 6.1.1 Central sensitization

A state of hyperexcitability in nociceptive neurons has been widely discussed during recent years, several mechanisms behind this phenomenon are suggested (Henriksson and Sorensen 2002; Lang et al. 2007; Petersen-Felix and Curatolo 2002; Suzuki et al. 2004; Woolf and Salter 2000):

- 1) Peripheral sensitization (heterosensitization)
- 2) Central sensitization of nerve cells at the spinal level (dorsal horn)
- 3) Deficient inhibition (segmental or supraspinal level)
- 4) Descending facilitation (i.e., spino-bulbo-spinal loop)
- 5) LTP (Long Term Potentiation) or “pain memory”
- 6) Changes at the thalamic, subcortical and cortical levels

The term central sensitization is sometimes used as an “umbrella” term for all central phenomena leading to enhanced pain perception, in this thesis we have mainly used the more strict definition of “enhanced responsiveness of nociceptive neurons in the CNS” (Sandkuhler 2007).

Central sensitization is characterized by temporal summation (i.e., increased firing of nociceptive nerve cells in the dorsal horn in response to *repetitive* stimuli of the same intensity from nociceptors in the periphery), increased receptive fields and allodynia/hyperalgesia (Suzuki and Dickenson 2002). Experimentally, increased areas of referred pain can be observed (Arendt-Nielsen and Graven-Nielsen 2003). Central sensitization is often discussed in relation to the NMDA-receptor complex, however considering the complexity of the nervous system it can not be ruled out that other receptors and ion-channels can be involved in this mechanism (Petersen-Felix and Curatolo 2002).

Furthermore several of the mechanisms involved interact and there is no relationship described between symptoms and clinical findings at examination and the mechanisms involved in chronic pain. Using drugs with specific target mechanisms (paper I and II)

and nociceptive stimulation of different intensity, frequency and modality (paper I-IV) a combined approach to extract differentiated information was achieved.

### **6.1.2 Target mechanisms**

In papers I and II we found significant responses to several analgesic drugs. At the individual level, the majority responded to two or more drugs, which might imply that some patients have several active pain mechanisms that have to be targeted in clinical practice. Several analgesic drugs simultaneously with effects at different levels and receptors might be necessary when designing the pharmacological treatment of the individual patient (Petersen-Felix and Curatolo 2002). A combination of drugs in low doses also increases the chance of reducing pain with fewer side effects (Baranowski 2003). In clinical practise, intravenous pharmacological challenges are made with the purpose of understanding the mechanisms that are important for the individual patient and to give clues to oral treatment (Baranowski 2003; DelleMijn et al. 1998). The use of pharmacological challenges in this thesis was based on our aim to assess different mechanisms possibly involved in the state of chronic WAD.

Fourteen out of eighteen drug responders (paper I) and 11 out of 11 (100%) drug responders (paper II) respectively were responders to ketamine, which indicates an involvement of the NMDA-receptor and a state of central hyperexcitability in a large subgroup of subjects with chronic WAD. The test-retest reliability of ketamine response is insufficiently known but repeatable response was demonstrated in one small study with fibromyalgia patients (Graven-Nielsen et al. 2000). The corresponding relationships for morphine/remifentanil were 15/18 (paper I) and 7/11 (paper II).

Nociceptive barrage - for instance, from zygapophysial joints (Barnsley et al. 1995; Lord et al. 1996; McDonald et al. 1999), or other structures - in a large subgroup of subjects could explain the opioid response. Furthermore it is likely that a state of hyperexcitability would be alleviated by strong opioids as well (i.e., decreased nociceptive input) (Suzuki and Dickenson 2002). Eleven out of eighteen drug responders responded to lidocaine (paper I), the importance of this finding is not clear since effects on both first-order neurones and higher levels of nociception can be expected with lidocaine (Rogers et al.

2006). Effects of lidocaine on the processing of the affective dimensions of pain in the anterior cingulate cortex and insular cortex has been suggested (Carroll 2007). We do not interpret lidocaine-response as a sign of neuropathic pain, however it suggests that in this subgroup of patients, sodium channels may be involved in the transmission and perception of pain (Rogers et al. 2006). Involvement of the “emotional” medial forebrain structures must be taken into account for all drugs used in this thesis since both NMDA- and opioid-receptors are abundant in the central nervous system.

### **6.1.3 Pharmacological response or non-response**

The relationship between anatomical lesions and clinical pain is not known. Muscle injury in contrast to injuries to ligaments and discs would normally heal in a few weeks and reasonably not cause chronic pain according to some authors (Barnsley et al. 1994). However, little is known about the extent and duration of trauma and nociception necessary to induce persistent plastic changes at the level of peripheral receptors, at the spinal cord, or at higher centres. In patients that have been involved in a motor vehicle accident, some special circumstances exist that is believed to influence the development and maintenance of chronic pain. Thus, some subjects develop acute and sometimes chronic psychological sequels (for instance cognitive deficits and/or post traumatic stress symptoms). Depression is prevalent as a secondary symptom to chronic WAD and is associated with pain intensity and widespread pain (Peolsson et al. 2007). In addition, factors related to compensation might influence chronic pain, although these influences on course and outcome are complex (Mayou and Bryant 2002). Some studies of WAD indicate that pain before the trauma increases the risk for chronic pain development (Sterner et al. 2003), which can be due to already sensitized pain system or patterns of cognition and behaviours. Psychological factors may modulate or even modify the response to drugs.

Clinical significant pain reduction of approximately 30% on an 11-point numerical pain rating scale has been suggested as a response (Farrar et al. 2001). However we followed earlier praxis when defining a responder by at least 50 % reduction (Sorensen et al. 1997; Verdugo and Ochoa 1994).

The percentage of global non-responders is higher than previously reported by Sorensen et al. among subjects with fibromyalgia 33-35 % vs. 17% (Sorensen et al. 1997). Among subjects with chronic low back pain, 25% were classified as non-responders (Sorensen et al. 1996). In this context it is important to state that we only have investigated the effects of three active drugs and other drugs might have effects in the present subgroup of non-responders. Other systems and receptors important for transmission of pain (not directly targeted in this study) could be involved, nitric oxide synthase (NOS) antagonists and NSAID have been shown to reduce hyperalgesia (Dickenson 1995). The NK<sub>1</sub>-receptor (i.e., receptor for substance P) seems to play a central part in the long-term potentiation of pain (Suzuki et al. 2004), furthermore substances in the spinal cord can modulate the efficacy of opioids (e.g., CCK)(Dickenson 1994). Insufficient opioid or NMDA-antagonist response might represent an individual with less efficient *endogenous pain control*. This is an important pathway thru which psychosocial factors could modulate pain response (i.e., enhanced facilitation) (Suzuki et al. 2004). One interesting aspect is if this subgroup (non-responders) actually represents individuals with long-lasting (less reversible) alterations in the nociceptive nervous system, earlier discussed as “modification”. Non-responders showed significant higher VAS-scores five days before and after the pharmacological challenge than responders. The clinical importance of this finding is unclear at the present, but raise several lines for further investigations such as pain mechanisms involved, aetiology, differences in coping strategies and psychosocial status.

The results of the pharmacological challenges indicate that different combinations of mechanisms are activated and hyperexcitability is present in a subgroup of subjects with chronic WAD. Such heterogeneity can be due to factors such as aetiology, pain processing, genetics and psychosocial factors.

#### **6.1.4 Pharmacology and experimental pain (Paper II)**

We used a target controlled infusion system for Paper II. The chosen infusion algorithm aims at a preset target concentration and a computer regulates the infusion rate according to the algorithm, this regime minimizes side effects. There is also an advantage with

respect to safety reasons to use an extremely short acting  $\mu$ -agonist as remifentanyl ( $T_{1/2}$ = 3-10 minutes). In a recent study the  $\mu$ -receptor agonist *alfentanil* showed prominent modulating effects on experimental muscle pain in healthy subjects (Schulte et al. 2003). In the present study no correlation was found between the clinical habitual pain and the experimental pain sensitivity during the pharmacological challenges. Several explanations for this are plausible: 1) experimental acute pain and chronic WAD-related pain recruit different sets of pain processing mechanisms and maybe even pain modalities (i.e., different nociceptive transducers activated) 2) different levels of facilitation and inhibition in the descending control could be activated and 3) differences in the sensitivity of the outcome measures.

The chosen experimental pain outcome parameters were modulated by both drug combinations containing remifentanyl, the same is true for temporal summation (TS) (i.e. repeated electrical stimulation). However, PPT of TA and local pain area in leg after saline infusion were most efficiently modulated by the ketamine/remifentanyl combination. Ketamine enhanced the remifentanyl effect on IM electrical pain when the lowest target concentration of remifentanyl was used. The same modality specific effect was demonstrated in a recent study on healthy volunteers (Luginbuhl et al. 2003). When assessing nociceptive flexion reflex after electrical stimulation of the sural nerve a significant reduction of the stimulus-response curve was demonstrated when combining ketamine with morphine (Bossard et al. 2002). However, the authors were unable to demonstrate effect on wind-up. Remifentanyl in higher concentration or in combination with ketamine reduced temporal summation. A higher ability to inhibit temporal summation was expected for ketamine alone (Arendt-Nielsen et al. 1995), although earlier studies have mainly evaluated fibromyalgia patients or healthy controls (Graven-Nielsen et al. 2000; Luginbuhl et al. 2003; Sorensen et al. 1998). In this study electrical pain assessments were made outside painful area, this may reflect a less pronounced state of central hyperexcitability compared to fibromyalgia.

Experimental muscle pain (electrical, chemical and mechanical) can be regarded as stimulation of mainly group III and IV afferent nerve fibres (Graven-Nielsen and Arendt-

Nielsen 2002). This could at least partly explain why remifentanyl alone is more powerful when experimental pain is compared to habitual chronic pain. In contrast to acute experimental pain where the nociceptive input is obvious, the situation is probably far more complex in chronic WAD. Since we did not use a control group in paper II we used the experimental pain assessments mainly for the purpose of: 1) allowing mechanism-based comparisons (e.g. ketamine/remifentanyl effects on temporal summation) 2) providing a model for acute muscle pain as reference.

### **6.1.5 The impact of duration on pharmacological response (Paper I)**

In fibromyalgia patients, ketamine response was linked to duration of widespread pain (Aspegren-Kendall et al. 2003), which contradict the results in this study. If changes in the periphery and peripheral and central nervous system occur early in the chronic stage, there is a risk that a relationship between the duration of WAD and the pattern of responses to the pharmacological challenges was not detectable with the subjects in this study who had a pain-duration from 5 to 96 months.

## **6.2 Experimental pain assessments**

*(In part also discussed under section 6.1.4)*

### **6.2.1 The impact of duration on experimental pain (Paper I and II)**

In paper I we found no correlation between duration of pain and experimental pain outcome. However in paper II positive correlation existed at baseline between pain duration and pain thresholds for pressure and electrical stimulation (both single and repeated). The findings in paper II could indicate a state of adaptation either in the periphery or in central pathways (i.e., enhanced inhibition). Further studies are needed to confirm the results in paper II.

### **6.2.2 Manual pressure algometry (Paper III)**

WAD patients had significantly lower pressure pain thresholds than controls both in trapezius and tibialis anterior muscles (paper III) despite that the WAD group clinically

had a pain condition without widespread pain. Our results agree with Scott et al. who also found generalized hypersensitivity in WAD (Scott et al. 2005). The biochemical findings in paper III may indicate the contribution of a peripheral component (i.e., the trapezius muscle) in this hypersensitivity even if there was no significant regression of PPTs (for details see paper III). However both group membership (WAD/CON) and pain intensity were possible to regress.

### **6.2.3 Computerized cuff pressure algometry (Paper IV)**

The main finding was the presence of generalized hypersensitivity to mechanical stimulation (cuff pressure algometry) outside painful area in subjects with chronic WAD. Moreover facilitated temporal summation of mechanical stimulation was found.

Pressure-pain tolerance thresholds were significantly lower in the legs and arms (well outside spontaneous painful area) in patients with chronic WAD. In agreement with this we found that the pressure-VAS areas in the legs were significantly smaller in the WAD group. The slope of the pressure-VAS curve can be interpreted as a psychophysical indicator of pain sensitivity to pressure (i.e., stimulus-response function). Since pressure increases with constant rate a steeper slope is equivalent to a smaller area under the curve and a shorter time from pain detection until tolerance threshold is reached (**Fig Va**).

We did not find any significant differences in PDT between the two groups in the arm or leg. A recent study on fibromyalgia patients, in contrast to the present study, reported significantly lower pressure pain thresholds (i.e., PDT) compared to controls during cuff pressure algometry (Jespersen et al. 2007). One reason for this difference could be the fact that fibromyalgia patients by definition both have widespread pain and generalized hyperalgesia, which was not the case in this group of chronic WAD-patients.

Fibromyalgia may be associated with more prominent alterations in the nociceptive nervous system or even in the periphery. The results during cuff pressure algometry are in agreement with the findings discussed above (6.2.2).

Significantly lower PTT recorded in the leg compared to the arm (in both groups), was unexpected for two reasons: 1) the individual muscle volume in the arm is smaller and an

increased sensitivity could be expected (Graven-Nielsen and Arendt-Nielsen 2002), 2) In paper II we found the pressure pain thresholds in the infraspinatus muscle to be lower compared to the leg (i.e., TA).

Since saline infusion in the tibialis anterior muscle was the last item of assessment, this relative hypersensitivity in the legs can not be explained by experimentally induced hypersensitivity (Graven-Nielsen and Arendt-Nielsen 2002). The arm assessment was however performed as the last cuff pressure algometry item and an effect of generalized descending inhibitory control can not be ruled out.

During the temporal profiles (**Fig Vb**) different stimulus-response patterns were found between WAD-patients and controls, the patient group immediately showed higher VAS ratings (including VAS-peak ratings) and facilitated temporal summation. Enhanced effect of temporal summation, expanded referred pain areas and generalized hyperalgesia to pressure are all findings strongly associated with central sensitization (Arendt-Nielsen and Graven-Nielsen 2003; Arendt-Nielsen et al. 2003; Mense 1993).

#### **6.2.4 Saline-induced muscle and referred pain (Paper I and IV)**

The intramuscular hypertonic saline infusion in the leg produced significantly larger time-VAS areas (AUC) in the WAD-group than in controls. Based on pain drawings after saline infusion the pain areas in the leg were expanded in the patient group (**Fig XIV**). Significant differences in AUC after hypertonic saline infusion in the tibialis anterior between healthy controls and WAD patients were also found in the study of Koelbaek Johansen and co-workers (Koelbaek Johansen et al. 1999). However in their study, in contrast to the present study of local or regional pain associated with WAD, almost half of the subjects reported widespread pain. Proximal spread of referred pain after intramuscular hypertonic saline only existed in the patient group in paper IV, in paper I this phenomenon was demonstrated among 4 subjects. Proximal spread of referred pain has been reported in a study of patients with WAD (Koelbaek Johansen et al. 1999) and another study of FM (Sorensen et al. 1998). Several studies have shown expanded referred pain areas associated with different clinical conditions such as: fibromyalgia, temporomandibular pain disorders and chronic osteoarthritic knee pain (Bajaj et al. 2001;

Sorensen et al. 1998; Svensson et al. 2001). Expanded referred pain areas with proximal spread of projection is an indicator of central sensitization and corresponds to basic neurophysiologic experiments in rats with proximal segmental spread of hyperexcitability (Arendt-Nielsen and Graven-Nielsen 2003; Hoheisel et al. 1993).

## **6.3 Microdialysis**

### **6.3.1 Serotonin (5-HT)**

WAD had higher [5-HT] than controls. Though pyruvate, [IL-6], and [K<sup>+</sup>] were more important than 5-HT for the discrimination between WAD and CON, [5-HT] had the strongest correlation with pain intensity in WAD.

5-HT is a potent stimulus for activation of muscle nociceptive free nerve endings (Mense 1993) and it mediates pain and hyperalgesia (Babenko et al. 1999; Graven-Nielsen and Mense 2001; Mork et al. 2003). The higher ratio of intramuscular to blood [5-HT] in chronic masseter myalgia (Ernberg et al. 1999) together with our results of WAD and with the six-fold higher [5-HT] in work-related trapezius myalgia (TM) (Rosendal et al. 2004b), indicate that 5-HT is involved in peripheral nociceptive processes in chronic myalgia. The differences in [5-HT] may be related to different mechanisms sensitizing the muscle (i.e., primary muscle injury or secondary/referred pain area). Serotonin (5-HT) by itself will not cause significant pain or hyperalgesia when injected into a muscle but rather sensitize for algogenic substances (i.e., bradykinin)(Babenko et al. 1999). The effects of 5-HT depends on which receptor is activated, in central pathways 5-HT may both inhibit or facilitate pain transmission, a regulatory role in the periphery can not be ruled out.

### **6.3.2 IL-6**

WAD had higher [IL-6] than CON (**Fig XIII**) and also higher than TM (Rosendal et al. 2005a). IL-6 is a pro-inflammatory cytokine involved in nociception, hyperalgesia and in sickness response (Hoheisel et al. 2005; McMahon et al. 2005; Page 2005; Sommer and Kress 2004; Watkins and Maier 2005; Verri et al. 2006). TNF- $\alpha$  triggers the release of

IL-6 (Cunha et al. 2005; Sommer and Kress 2004). Even though there is some evidence suggesting effects of cytokines on primary afferent neurones the major effects are indirect, releasing other cytokines and final inflammatory substances (Cunha et al. 2005; Sommer and Kress 2004; Verri et al. 2006). Our finding that the pain intensities in WAD did not correlate well with [IL-6], might be in agreement with the observations that the major effects of cytokines are indirect. Another reason for this lack of correlation might be due to that muscles release IL-6 during exercise (Langberg et al. 2002; Ostrowski et al. 1999). Patients with chronic WAD have a relative inability to relax the trapezius muscle in short pauses (Elert et al. 2001; Fredin et al. 1997) and thus having a more sustained muscle activity pattern which per se might increase the [IL-6], but on the other hand these levels of contraction according to surface electromyogram in short pauses are relatively low (Elert et al. 2001; Fredin et al. 1997). It was not possible due to pain to obtain maximal reference contractions in a valid way and determine the relative level of exercise in the present design. Hence, there is a risk that WAD patients worked at a somewhat higher relative level than the CON during the short exercise, but that cannot explain the significantly higher [IL-6] at baseline.

It was recently suggested that IL-6 is a co-regulator of local metabolism during exercise and increased [IL-6] may be the underlying cause of increased [lactate] and [pyruvate] in the myalgic muscle of TM (Rosendal et al. 2004a; Rosendal et al. 2005b). However, the high [IL-6] of WAD was not associated with *simultaneous* increases in [lactate] (**Fig XI**), but it cannot be ruled out that there was a delayed effect since [lactate] peaked during the recovery.

### **6.3.3 Pyruvate, lactate and blood flow**

WAD had a significantly lower [pyruvate] than CON. In TM – with a pain condition also clinically affecting the neck-shoulder region – were found significantly higher concentration than in CON (Rosendal et al. 2004b). In both WAD and CON, [pyruvate] increased somewhat during exercise, but thereafter different patterns were found. In CON, decreases followed at the two recovery time-points, whereas in WAD [pyruvate] peaked at recovery #1 followed by a relatively steep decrease (**Fig XII**). Lactate is now

considered as an active metabolite, capable of moving between cells, tissues and organs where it may be oxidized as a fuel or reconverted to form pyruvate or glucose (Gladden 2004; Philp et al. 2005; Robergs et al. 2004). Both WAD and CON showed tendencies to increase in [lactate] during exercise (**Fig XI**). In CON, [lactate] thereafter decreased and was significantly lower than baseline at recovery #2; such a pattern was also found in TM (Rosendal et al. 2004b) even though the overall level was significantly higher. Philp et al. suggested that lactate assists in the detection of exercise stress before tissue damage occurs (Philp et al. 2005). In WAD [lactate] peaked at recovery #1 with a significant higher level than baseline, an occurrence that does not support this suggestion. The importance of lactate *per se* as pain mediator can be questioned since it only activates muscle nociceptors at supraphysiological concentrations (Mense 1993). However, a combined effect together with other substances – e.g., 5-HT,  $K^+$ , and pyruvate – cannot be excluded.

The changed lactate and pyruvate dynamics in response to exercise and recovery with a lower level of pyruvate in WAD may indicate changes in the lactate-pyruvate metabolism via lactate dehydrogenase isoforms (Philp et al. 2005). Another explanation might be related to blood flow alterations since studies of chronic pain in trapezius indicate disturbances in blood flow regulation (Elvin et al. 2006; Larsson et al. 1998; Larsson et al. 1999; Larsson et al. 1994; Sandberg et al. 2005). However, the present groups had identical values in blood flow. Invasive methods might *per se* change blood flow (Sandberg et al. 2005), but in the present study both groups were exposed to the same trauma (i.e., catheter insertion). In WAD, pain intensities showed positive correlations with blood flow, which contradicts an earlier report of WAD (Larsson et al. 1994). Exercise hyperemia is the result of an integrated response of several vasodilator mechanisms and one vasoactive compound may take over when the formation of another is compromised (Clifford and Hellsten 2004), one of these may be  $K^+$  that was important in the regressions of pain intensities.

## **6.4 Methodological considerations**

### **6.4.1 Paper I**

In this paper the time span between pharmacological challenge and experimental pain assessment resulted in a drop out of patients. The patient group was also somewhat more heterogeneous since not all cases were the victims of a traffic accident. Patients with widespread pain were not excluded. However the lack of association between duration of pain and pharmacological and experimental responses more likely depended on the lack of subjects with really short pain duration.

### **6.4.2 Paper II**

The intention was to select patients referred from primary care for evaluation at the pain clinic. This group may not be representative for the whole population of patients with chronic WAD, however the relatively small fraction that develops long term disability after neck trauma is more likely to be targeted in this Paper. The problem of reproducibility of pharmacological response has been discussed both in relation to paper I and II (i.e., section 6.1.2). A recent study on FM was able to predict response to oral dextromethorphan (a NMDA-antagonist) with an intravenous ketamine challenge (0,1 mg/kg) (Cohen et al. 2006). Unfortunately there is no substantial support in the literature and in future studies a repeated challenge could be of value. Regarding the experimental pain assessments, most values were based on mean calculations of three measurements.

### **6.4.3 Paper III**

It might be argued that the overall differences between WAD and CON may be due to differences in relative force level during work and/or contraction duration of trapezius as discussed above. However the major results concerning IL-6 and pyruvate (and 5-HT only measured at baseline) are found already at baseline before the exercise. One possibility is to exclude the brief exercise in future studies in order to avoid the pain intensity increase in patients with pain, but such a design makes it impossible to detect changed dynamics in response to exercise and recovery. Such changes were found for lactate and pyruvate and might be early signs of a pathophysiological process. The [IL-6]

in WAD, CON and TM (all women) (Rosendal et al. 2005a) were several times higher than those obtained from healthy men (Rosendal et al. 2005b). The reasons for these differences are unclear and deserve further investigation.

#### **6.4.4 Paper IV**

The same group of patients was used in paper III and IV, however since the experimental procedures in paper IV were performed as the first item this should not bias the outcome. The methods used in paper IV are not expected to affect muscle metabolism or biochemistry in the shoulder, nor pressure pain sensitivity. Moreover at least 2-3 weeks separated study IV and III.

The optimum sample size would have been 50 persons (25 WAD-patients and 25 controls), due to mainly financial reasons this design was not possible. The weaknesses of this study are mainly the underpowered design (small control group) and the slightly higher mean age in the control group.

In the future a multivariate data analysis could be considered. The cuff pressure algometry technique does not depend on the practical skill of the examiner (in contrast to ordinary pressure algometry), however instruction to the subject regarding the various thresholds and the use of the “stop button” is crucial. This instruction bias was minimized by using the same 2 examiners for all sessions.

## 7 Summary and Conclusion

- Opioid sensitive neurons and NMDA-receptors seem to be active in a majority of cases with chronic WAD in our studies. From a mechanism-based point of view this can be consistent with long term excitation and sensitization of nociceptors in peripheral tissues and/or central sensitization.
- A significant share of WAD patients does not respond to NMDA-antagonist or opioid, this may indicate involvement of other pain mechanisms such as activation of other receptors or channels, plastic changes in the nervous system or impaired endogenous pain control.
- A humble attitude to the phenomenon of “non-response” is justified, since new drugs, better knowledge of mechanisms and better diagnostic capabilities may alter this picture.
- Experimental pain assessments indicate a state of central hyperexcitability in WAD, with widespread hypersensitivity even in regions not affected by pain. Central sensitization and deficient inhibition of pain from higher levels in the nervous system may explain this finding.
- Painful trapezius muscle associated with WAD show biochemical aberrations compared to healthy controls. The changes indicate a peripheral pain component from muscle tissue and this may sustain the pain condition.
- Mechanisms both in the periphery and the central nervous system (including psychological) seem to interact. Chronic WAD is a heterogeneous condition and several subgroups can be identified.

## 8 Future prospects

- The heterogeneity of this chronic pain condition implies a more diversified approach regarding research, diagnostic efforts and treatment/rehabilitation.
- Since modulation of pain and even long-lasting alterations of the nervous system seem to be an integral part of WAD, the emphasis should be on early interventions in further studies.
- Larger clinical trials with early assessment of the nociceptive nervous system, more effective pain management and early intervention (i.e., multidisciplinary rehabilitation) should be made.
- In future studies easy administrable quantitative sensory techniques like cuff pressure algometry and hypertonic saline could be used as outcome parameters together with assessments of functioning, depression and quality of life.

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## Sammanfattning på svenska

Kronisk smärta efter whiplashtrauma (pisksnärtvåld), orsakar betydande lidande och nedsatt funktionsförmåga. Cirka 1-4 personer per 1000 invånare drabbas årligen av akut smärta efter whiplashtrauma mot halsryggen, ungefär 10 % av dessa löper risk att få betydande kvarstående besvär.

Syftet med avhandlingen har varit att med hjälp av experimentella tekniker öka kunskapen om de komplexa mekanismer som ligger bakom smärta vid kroniska whiplashrelaterade tillstånd (eng. Whiplash Associated Disorders = WAD).

Flera olika metoder för att framkalla akut djup vävnadssmärta (elektrisk, kemisk samt tryck) har använts.

Smärtstillande läkemedel (morfin, remifentanil, lidocain samt ketamin) tillfördes intravenöst och effekter på både whiplashrelaterad smärta (studie I och II) och experimentell tillfogad smärta (studie II) har jämförts. I studie III har även smärtande skuldermuskel analyserats (mikrodialys) hos WAD-patienter och friska kontroller med avseende på smärtmedierande ämnen, samt omsättning av metaboliter före, under och efter arbete. I studie IV jämfördes känsligheten för stimulering med två olika smärtmodaliteter, mekanisk (cuffalgometer) och kemisk (intramuskulär koksalt), med friska kontroller.

Huvuddelen av patienterna upplevde smärtlindring av morfinpreparat men även av NMDA-antagonisten ketamin. Ketamin blockerar NMDA-receptorer i nervsystemet, aktivering av NMDA receptorn tros ligga bakom fenomenet central sensitisering (ökad retbarhet hos nervceller i ryggmärgen). Att kombinera remifentanil (morfinliknande preparat) med ketamin visade tilläggs effekt jämfört med enbart remifentanil. Ungefär en tredjedel av patienterna uppvisade inte någon betydande smärtlindring vid något av läkemedelstesterna. Andra receptorer och jonkanaler än de som påverkas av de testade läkemedlen kan vara involverade, smärtfacilitering (förstärkning av smärtimpulser) eller bristande smärtinhibition (hämmning) från högre centra i hjärnstam och hjärna kan vara en annan förklaring. Experimentell smärtstimulering med tryck (utanför smärtande område) visade i WAD-gruppen en ökad smärtekänslighet både vid kortvarig och längre stimulering samt förändrade smärtareor i benet vid koksaltstimulering jämfört med friska kontroller. Studie III visade högre nivåer av 5-HT (serotonin) och IL-6 i muskulatur samt avvikande metabolism jämfört med kontroller. 5-HT är associerat med muskelsmärta och IL-6 med inflammation, muskelmetabolism och smärtekänslighet.

Perifert aktiverade smärtreceptorer och/eller central sensitisering tycks föreligga hos merparten av den undersökta gruppen, förändrad smärtmodulering från högre centra i hjärnstam och hjärna kan vara en bidragande mekanism. Förändringarna uppträder sannolikt tidigt i förloppet, huruvida reaktionsmönstret kan finnas hos vissa individer redan före ett trauma går inte att dra några säkra slutsatser om. Mekanismerna bakom smärta vid kronisk WAD är multipla och samspelet komplext. I kommande kliniska studier kan det vara av intresse att studera effekterna av tidig intervention (rehabilitering och/eller smärtlindring) samt värdera experimentella smärtmetoder som prognostiskt instrument över tid.

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