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Complex Regional Pain Syndrome Related Dystonia

Exploratory Metabolomics and Therapeutic Studies

Anton Adriaan van der Plas

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Complex regional pain syndrome related dystonia: exploratory metabolomics and therapeutic studies

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Exploratory Metabolomics and Therapeutic Studies

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Anton Adriaan van der Plas
geboren te Leiderdorp
in 1974

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This PhD project was performed within TREND (Trauma RElated Neuronal Dysfunction), a Dutch consortium that integrated research on epidemiology, assessment technology, pharmacotherapeutics, biomarkers and genetics on Complex Regional Pain Syndrome. The consortium aimed to develop concepts on disease mechanisms that occur in response to tissue injury, its assessment and treatment.

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Η περιέργεια είναι η αρχή της σοφίας
(Σωκράτης)

Nieuwsgierigheid is het begin van wijsheid
(Socrates)

voor mijn ouders

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Chapter 1

General Introduction

1.1 FROM PAST TO PRESENT

Causalgia

It is 1864. The American Civil War takes its toll. At the end of the war more than 1,000,000 casualties and losses are counted. Silas Weir Mitchell, a US Army physician at the Turners Alne Hospital for Nervous Diseases in Philadelphia, is a close witness of the many casualties on the Union side. In his book "Gunshot Wounds and Other Injuries of Nerves", Mitchell describes the symptoms and signs of peripheral nerve injuries as he observed them in Unionist soldiers.¹ In a small number of men he perceives "pain which they described as 'burning' or as 'mustard red-hot' or as a 'red-hot file rasping the skin' ... The part itself is not alone subject to a deep burning sensation, but becomes exquisitely hyperesthetic, so that a touch or tap of the finger causes pain". Mitchell uses the term causalgia (from the Greek terms "kausis" or burning and "algos" or pain) to summarize the characteristics of this condition. Next to pain and other sensory abnormalities, he also describes features suggestive of:

- (1) vasomotor changes: "The burning comes first, the visible skin-change afterwards",
- (2) trophic changes: "When the acute stage has departed, the tissues become hard...",
- (3) edema: "... a painful swelling of the joints...may attack any or all articulations of a member".

The term causalgia was maintained for many years to come, although only for patients who developed symptoms after major nerve injury.

Reflex sympathetic dystrophy

The discovery of X-rays at the end of the 19th century opened up new avenues in the understanding of underlying mechanisms of diseases. In 1900 Paul Hermann Martin Sudeck, a surgeon from Hamburg, applied this new diagnostic tool to examine patients suffering from causalgia and documented a "patchy osteoporosis of the small bones of the hand or feet and the distal metaphysis of forearm or tibial bones" in these patients.² The term "Sudeck's atrophy" was introduced, which was changed to "limb dystrophy" in 1936 by Reider, a student of Sudeck.

In the first half of the 20th century, different hypotheses were proposed about the underlying mechanism of the disorder. Sudeck suggested an “exaggerated inflammatory response after injury or operation of an extremity” as a cause of the disease.³ Rene Leriche, a military surgeon during WW1, related the symptoms and signs with sympathetic disorders and documented “... I asked myself whether, by acting upon the perivascular sympathetic, I might be able to succeed in modifying the causalgia.”⁴ In one patient he exposed the brachial artery of the affected limb and removed its adventitia over a distance of 12 cm. The next day, the patient reported clear improvement of pain which supported his theory of a sympathetic disturbance underlying the disorder. Based on this experience he advocated “novocain infiltrations of the paravertebral sympathetic chain” as an effective treatment.⁵ The chronicity of the symptoms was thought to result from “a reflex travelling through the nervous system”. To capture these various pathophysiological hypotheses the nomenclature “reflex sympathetic dystrophy or RSD” was formulated.⁶ The term RSD was maintained for decades to come and exclusively used to describe symptoms of causalgia, but without evident causative nerve damage.

Complex regional pain syndrome

Based on doubts about the pathophysiological role of the sympathetic nervous system and the inherent inadequacy of the terms RSD and causalgia, a special consensus workshop of the International Association for the Study of Pain (IASP) was held in 1993 in Orlando to provide the new nomenclature “complex regional pain syndrome or CRPS”.⁷ Two types of CRPS were recognized: type I without nerve injury (replacing the term “RSD”) and type II associated with major nerve injury (replacing the term “causalgia”). This committee formulated diagnostic criteria for CRPS type I (table 1.1).⁸ Of note, the presence of an initiating noxious event, or a cause of immobilization was not considered mandatory to establish the diagnosis. The diagnostic Orlando criteria for CRPS were modified following an international consensus meeting in 2003 in Budapest (table 1.2).⁹ Compared to the Orlando criteria, the Budapest-criteria have higher specificity and also include disease-related trophic changes and motor dysfunction. The

Table 1.1. The diagnostic Orlando criteria for CRPS type 1

1	The presence of an initiating noxious event, or a cause of immobilization
2	Continuing pain, allodynia, of hyperalgesia with which the pain is disproportionate to any inciting event
3	Evidence at some time of edema, changes in skin blood flow, or abnormal sudomotor activity in the region of the pain
4	Absence of a condition that would otherwise account for the degree of pain and dysfunction

Criteria 2-4 are obligatory for a diagnosis of CRPS

Budapest criteria have recently been adopted by the IASP as the new diagnostic standard following an international cohort study, which confirmed the validity of the criteria.⁹

1.2. EPIDEMIOLOGY OF CRPS

CRPS is considered a rare disease. The incidence of CRPS in population-based studies varies from 5.5/100,000 persons per year in the USA¹⁰ to 26/100,000 persons per year in the Netherlands.¹¹ However, it should be noted that these incidence rates are based on the Orlando criteria and that the IASP has meanwhile formally accepted the Budapest criteria as the diagnostic standard. The latter are stricter, which indicates that if current diagnostic standards would have been applied, the incidence rate would have been considerably lower. CRPS can manifest at any age, but most patients are aged between 40 and 70 years with women being more frequently affected than men (ratio of 3-4 to 1).^{10,11} Patients with CRPS-related motor dysfunction are on average about 10 years younger.¹² The female-to-male ratio in this subgroup of CRPS patients is approximately 8-9 to 1.^{13,14} In about 60% of cases the upper extremity is affected and a fracture is the most common trigger (about 45%), followed by sprain (about 18%) and elective surgery (about 12%).¹¹ Spontaneous onset of the disease has been described in 3-11% of cases.^{11,15-21}

Table 1.2. The diagnostic Budapest criteria for CRPS

1	Persistent pain, which is disproportionate to any known inciting event
2	The patient must report at least one symptom in three of the following categories to fulfill the clinical diagnostic criteria and at least one symptom in four of the following categories to fulfill the research diagnostic criteria (anamnestic hints): - Sensory: reports of hyperesthesia and/or allodynia - Vasomotor: reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry - Sudomotor/edema: reports of edema and/or sweating changes and/or sweating asymmetry - Motor/trophic: reports of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nails, skin)
3	The patient must display at least one sign in two or more of the following categories during the current physical examination: - Sensory: evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch and/or deep somatic pressure and/or joint movement) - Vasomotor: evidence of temperature asymmetry and/or skin color changes and/or skin color asymmetry - Sudomotor/edema: evidence of edema and/or sweating changes and/or sweating asymmetry - Motor/trophic: evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nails, skin)
4	There is no other diagnosis that would otherwise account for the signs and symptoms and the degree of pain and dysfunction

1.3 PATHOPHYSIOLOGY OF CRPS

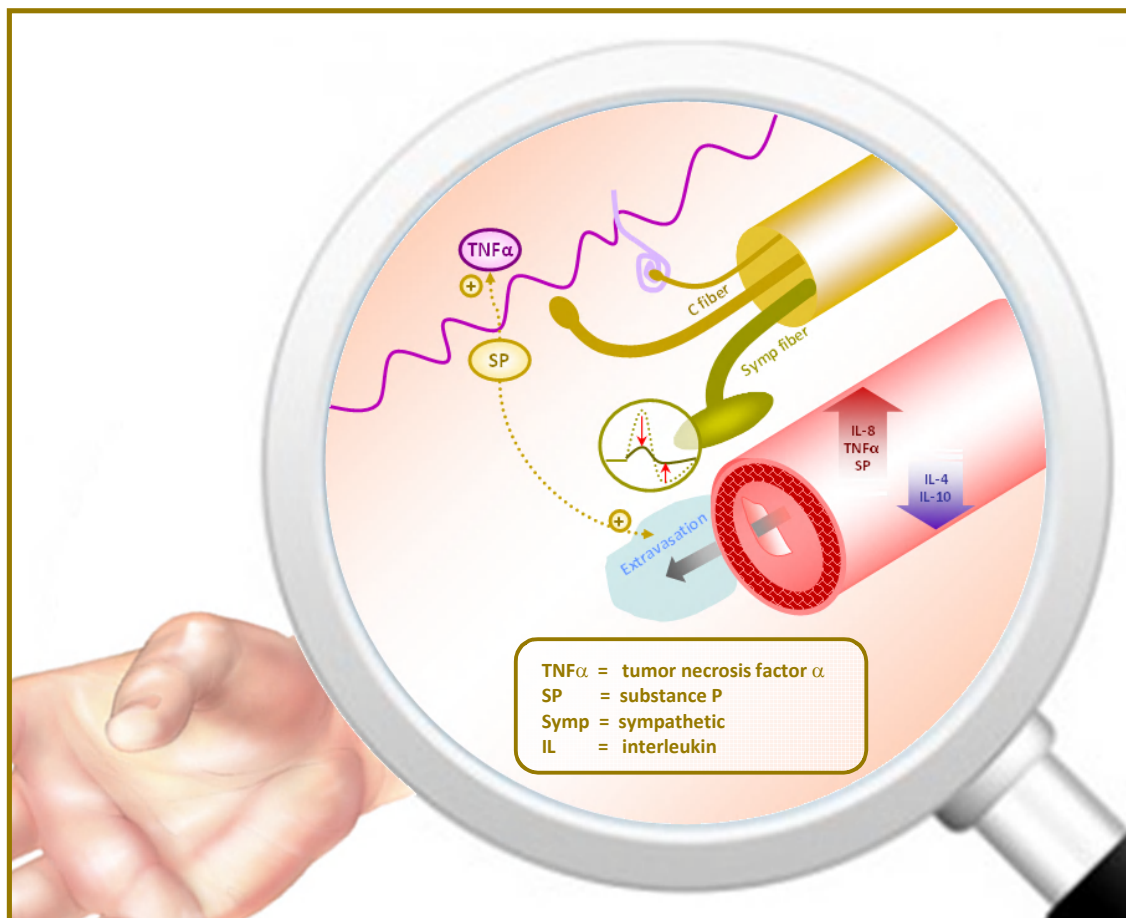
The clinical spectrum of CRPS is heterogeneous and includes sensory, autonomic, trophic, and motor changes of the affected limb (table 1.2). The different features reflect the involvement of multiple biological pathways, which most likely include exaggerated inflammation, vasomotor dysfunction, and maladaptive neuroplasticity.²²

Aberrant inflammation

In the early stage of the disease the affected limb shows classical signs of local inflammation (calor, rubor, tumor, dolor, functio laesa) in the painful area.²² These inflammatory changes in CRPS patients are likely mediated by the neuropeptide

substance P (SP; figure 1.1).^{23,24} In one studies the serum level of calcitonin-gene-related peptide (CGRP), another neuropeptide, was also found to be elevated in the acute stage of the disease, i.e. within 6 months after the onset of CRPS symptoms.²³ However, in a recent meta-analysis the pooled effect estimate of CGRP was not significantly different in patients with acute CRPS compared to controls.²⁵ Neuropeptides are released from peripheral terminals of sensory nerve fibers in skin, muscle and joints²⁶ and promote the inflammatory processes of vasodilatation and protein extravasation in tissue.²⁷ In addition, SP stimulates the production of other pro-inflammatory cytokines in keratinocytes (figure 1.1).²⁸ The concentration of the pro-inflammatory cytokine $\text{TNF}\alpha$ is elevated in skin tissue of the affected limb,²⁹ which coincides with the identification of

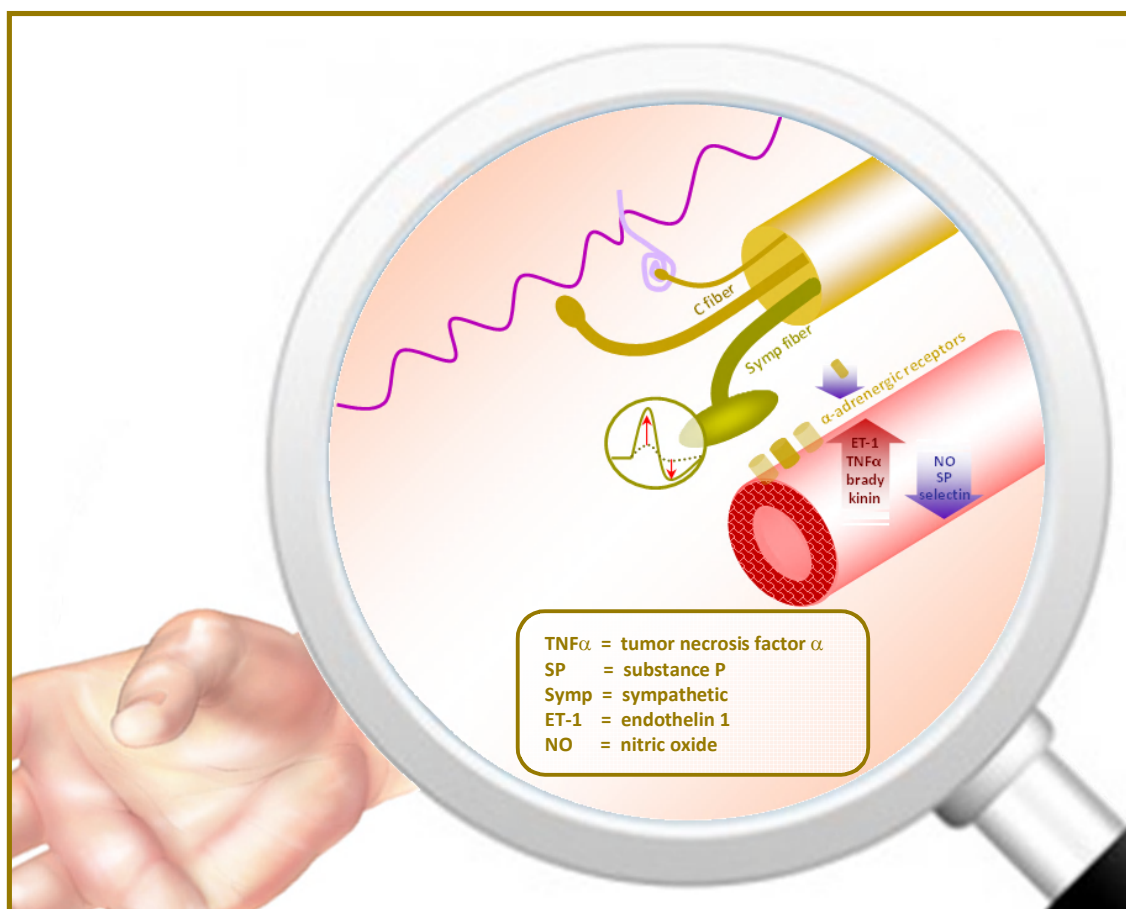
Figure 1.1. Biochemical and neurophysiological changes in skin tissue and blood in the affected limb of CRPS patients in the acute phase of disease



polymorphisms in the $\text{TNF}\alpha$ -encoding gene in genetic studies in CRPS patients (figure 1.1).³⁰ In the acute phase of disease pro-inflammatory changes can also be found in serum including increased concentrations of soluble TNF receptors and interleukin-8²⁵ and decreased concentrations of the anti-inflammatory cytokines interleukin-4 and interleukin-10 (figure 1.1).³¹ These findings indicate an increased Th1-to-Th2 ratio in the early stage of CRPS.

The serum profile of cytokines and other inflammatory peptides changes as CRPS symptoms continue for more than 6 months. Concentrations of $\text{TNF}\alpha$ and bradykinin are particularly increased, whereas serum levels of SP, sE-selectin, sL-selectin and sP-selectin are clearly decreased (figure 1.2).²⁵ During the first 6 years after onset of

Figure 1.2. Biochemical and neurophysiological changes in skin tissue and blood in the affected limb of CRPS patients in the chronic phase of disease



symptoms, concentrations of $\text{TNF}\alpha$ and interleukin-6, which are not correlated to duration or severity of disease, only gradually decrease.³² In the later stage of disease pro-inflammatory cellular changes with a raised percentage of the CD14+CD16+ monocyte/macrophage subgroup become manifest in blood.³³ However, the exaggerated inflammatory response in CRPS patients is probably not caused by a cellular mediated immune response. In a subgroup of CRPS patients serum auto-antibodies are found, which bind to an inducible autonomic neuronal surface autoantigen.³⁴ The relevance of this autoimmune process for the pathophysiology of CRPS remains to be elucidated. It is unclear if changes in pro- and anti-inflammatory cytokines in tissue and serum are already present prior to the onset of CRPS symptoms.

Vasomotor dysfunction

In the early stage of the disease the skin temperature of the affected extremity usually rises as a result of excessive vasodilatation.³⁵ A subgroup of CRPS patients with an elevated skin temperature displays unilateral inhibition of sympathetic vasoconstrictor neurons, probably due to functional changes in the CNS (figure 1.1).^{35,36} As a result, the concentration of norepinephrine in venous blood declines in the affected limb proximal to the painful area.³⁵ In the majority of CRPS patients the affected limb turns cold in the course of the following months.³⁶ The excessive lowering in skin temperature is a reflection of disturbed thermoregulatory skin perfusion (figure 1.2). In approximately 20% of patients the conversion from a warm to a cold skin temperature already occurs in the acute stage of disease.³⁷

Remarkably, in some CRPS patients who display a cold skin temperature in the affected limb, the concentration of norepinephrine is decreased in the venous blood flowing proximal to the painful area,³⁶ which suggests an increased density or affinity of α -adrenergic receptors in the skin of these patients.^{38,39}

Lowering of the skin temperature in CRPS patients may also be caused by a decreased endothelial release of nitric oxide and increased production of endothelin 1, resulting in persistent vasoconstriction in the affected limb (figure 1.2).^{40,41}

Maladaptive neuroplasticity

Disproportionate persistent pain and sensory changes in the affected limb are key features of CRPS and reflect both structural and functional changes in the CNS, a process also known as central sensitisation.⁴² Loss of inhibitory activity of normal cutaneous sensations on central sensory processing causes increased excitation of thalamocortical nociceptive networks.^{43,44} The activation and upregulation of glutamate receptors within the nociceptive circuit between spinal cord and cerebral cortex probably also play a major role in the process of central sensitization.⁴⁵ On the other hand there are indications of downregulation of the inhibitory influence of corticospinal descending pathways to spinal nociceptive neurons.^{46,47} As a result, spinal nociceptive neurons turn more responsive to peripheral input and may even depolarize spontaneously. Inflammatory cytokines in CSF, especially interleukin-1 β and interleukin-6, may also in part be responsible for the sensitization of spinal nociceptive neurons, although two studies produced contradictory results.^{13,48}

Peripheral sensitization of primary afferent nociceptors may also contribute to CRPS related pain and in turn may induce central neuroplasticity.^{49,50} In some CRPS patients sympathetic dysfunction may increase the sensitivity of nociceptors or activate primary afferent neurons causing “sympathetically maintained pain”.⁵¹

Many CRPS patients experience a distorted shape or size of the affected limb which probably originates from cortical reorganization of somatotopic maps within the primary somatosensory cortex representing the affected limb.⁵²⁻⁵⁴ Cortical reorganization is also found in the primary motor cortex and supplementary motor area, both ipsilateral and contralateral to the affected limb.^{55,56}

In conclusion, there is compelling evidence that a combination of central and peripheral mechanisms is responsible for the variety of CRPS-related symptoms. Although the role of these different mechanisms in the development and persistence of CRPS has been

clarified to a great extent, the interaction between these mechanisms remains to be elucidated.

1.4 DYSTONIA IN CRPS

In his extensive documentation of wounded soldiers suffering from causalgia, Silas Weir Mitchell also noted “When the acute stage has departed, the tissues become hard and partial ankylosis results”.¹ It is unclear if he described an abnormal posturing of the affected limb which we now define as a fixed or tonic dystonia, or a contracture resulting from trophic changes in soft tissues. In 1984 Marsden et al comprehensively described 4 CRPS patients with muscle spasms and rhythmical jerking of the affected limb.⁵⁷ The authors stated: “In the absence of pathological information the mechanism of the abnormal movements in our patients is open to speculation. Hopefully, however, this description will allow others to recognize the syndrome of muscle spasms associated with Sudeck's atrophy after trauma as a relevant disabling entity”. The authors' wish was fulfilled in the following decades as CRPS related motor symptoms gained increasing interest both in the field of pain and movement disorders.⁵⁸ During a consensus meeting in Budapest in 2003 it was decided to include decreased range of motion and motor dysfunction as an additional criterion into a new set of diagnostic criteria for CRPS.⁹

Although the term ‘dystonia’ - i.e., not related to CRPS - was already in use for many years, a proper classification and description of dystonia was only established in the 1980's.⁵⁹ To date, dystonia is defined as an involuntary movement disorder with muscle contractions causing twisting and repetitive movements or abnormal postures.⁵⁹ Dystonia can be classified according to the distribution of symptoms, age of onset and etiology (hereditary or acquired).⁶⁰ Although different networks may be involved in the development of various types of dystonia, they likely share the involvement of sensorimotor circuits that are associated with motor learning and memory, which manifest as dystonic movements and postures.⁶¹ The term dystonia for abnormal posturing of a limb in CRPS patients, which typically present as a fixed flexion deformity

of the hand, fingers and toes and a fixed inversion deformity of the foot, therefore suggests a central underlying mechanism for this type of dystonia. This assumption is supported by (1) the spontaneous spread of dystonia to other limbs in a subgroup of CRPS patients,¹² (2) neurophysiological research indicating central disinhibition in patients with CRPS-related dystonia⁶⁴ and (3) improvement of dystonia in a large proportion of CRPS patients receiving intrathecal baclofen (ITB).^{62,63}

CRPS-related dystonia may very rapidly lead to a fixed abnormal posture^{14,65} and in approximately half of the patients the lower limb is the site of dystonia onset.¹² These features are atypical for other types of adult-onset dystonia, which are not symptomatic.⁶⁰ Hence some experts in the field of movement disorders consider CRPS-related dystonia a psychogenic movement disorder.⁶⁶ However, most other characteristics of psychogenic dystonia are not applicable to dystonia in CRPS.⁶⁷ Additionally, if psychological factors are expected to play an important role in the onset of the condition, one would assume that certain psychological traits or a particular unique psychological profile would be present either before or around the period of disease onset, but such associations with CRPS-related dystonia have not been found thus far.^{68,69} As Marsden *et al* stated in their case-report on CRPS-related dystonia: “In the absence of pathological information the mechanism of the abnormal movements in our patients is open to speculation.”⁵⁷

1.5 MANAGEMENT OF CRPS

The growing understanding of the role of different mechanisms underlying CRPS, especially in the past 10 years, stimulated the development of target-specific interventions. Indeed, different treatment strategies are now available to counteract processes of aberrant inflammation, disturbed vasomotor regulation and maladaptive neuroplasticity.⁷⁰ Particularly in the field of pain management important progress has been made. In two placebo-controlled, double-blind trials intravenous S(+)-ketamine, an N-methyl-D-aspartate receptor antagonist, was administered for 4 and 10 days in chronic CRPS patients.^{71,72} Both studies revealed significant pain relief, not only at the

time of administration but also during the following months. These findings support the hypothesis that central sensitization is the origin of CRPS-related pain.

Only a few studies were set up to evaluate the efficacy of an intervention in dystonia related to CRPS.^{62,63} In one study intrathecal administration of boluses of baclofen, a pre- and postsynaptic gamma-aminobutyric acid B receptor agonist, clearly improved dystonia in some chronic CRPS patients.⁶³ The efficacy of ITB was also observed in the screening phase of a larger study, which supports the hypothesis of central maladaptive plasticity underlying CRPS-related dystonia.⁶² In the same study, patients who reported significant improvement during ITB administration in the screening phase received an implantable pump for intrathecal delivery of baclofen. Remarkably, after one year of ITB administration, 30% of these patients failed to reach the same level of improvement as noted during the screening procedure. Systematic reviews have revealed a lack of evidence of other centrally acting drugs in CRPS-related dystonia.⁷⁰ Apart from central mechanisms involved in the development of dystonia and spasticity, pathological changes in muscle tissue, which contribute to stiffness around a joint, may occur in these conditions.^{73,74}

1.6 AIMS OF THIS THESIS

The first aim was to study the efficacy and safety of ITB (*Part I*) and intrathecal glycine (*Part II*) in patients with CRPS-related dystonia and evaluate the role of central GABA-B receptor modulation in the pathobiology of pain and dystonia related to CRPS. *Chapter 2* describes the efficacy of ITB on 10 different pain qualities related to CRPS. In *Chapter 3* the influence of different infusion rates of ITB in CRPS-related dystonia is discussed. *Chapter 4* reports on the development of chorea after baclofen in 4 CRPS patients and addresses the potential role of supraspinal GABA-B receptor modulation in the development of CRPS-related dystonia.

Chapter 5 describes the efficacy and safety of intrathecal glycine in patients with CRPS. The second aim of this thesis was to unravel pathobiological processes involved in the

development and maintenance of CRPS (*Part III*) using mass spectrometry. *Chapter 6* describes the explorative analysis of urine by capillary electrophoresis-mass spectrometry in CRPS patients. The results of ^1H -NMR based metabolic profiling of CSF in patients with CRPS-related dystonia are described and discussed in *chapter 7*.

The third aim (*Part IV*) was to find evidence of efficacy for a treatment modality with a good safety profile. In *chapter 8* the results of an explanatory study evaluating the muscle relaxant effects of intramuscular magnesium sulphate for dystonia in CRPS are discussed.

In *chapter 9* the results of all studies are summarized and future perspectives are discussed.

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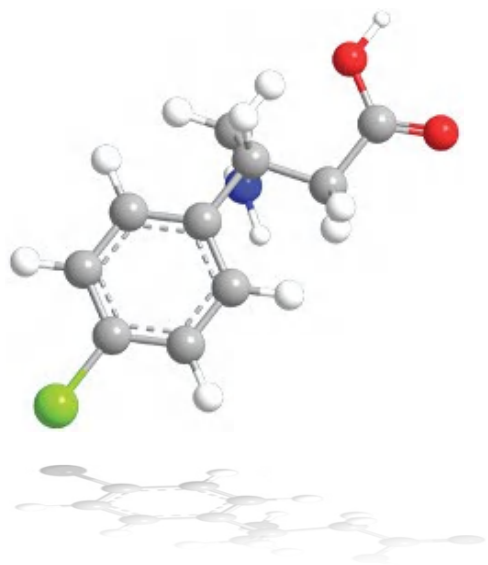
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PART I.

Intrathecal Baclofen Treatment in CRPS



Chapter 2

The Efficacy of Intrathecal Baclofen on Different Pain Qualities in Complex Regional Pain Syndrome

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ABSTRACT

Background. Complex regional pain syndrome (CRPS) is characterized by severe debilitating chronic pain. Patients with CRPS may experience various pain sensations, which likely embody different pathophysiological mechanisms. In this study, we evaluated the differential effects of central γ -aminobutyric acid (B) receptor stimulation on the different pain qualities in CRPS patients with dystonia.

Methods. The 10 pain qualities of the neuropathic pain scale, dystonia severity, and changes in use of antinociceptive drugs were evaluated every 3 months for a period of 1 year in 42 CRPS patients with dystonia receiving titrated doses of intrathecal baclofen (ITB) treatment in an open design.

Results. Using a linear mixed model analysis and controlling for global dystonia severity and the use of supplemental analgesics, we found a significant improvement in global intense pain, sharp pain, dull pain and deep pain during the first 6 months. After this period, the scores leveled off despite further improvement of dystonia and continued ITB dose escalation.

Conclusions. γ -Aminobutyric acid (B) receptor stimulation by ITB exerts differential antinociceptive effects on specific pain qualities in CRPS patients with dystonia.

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is a multifactorial disorder that is associated with an aberrant host response to tissue injury of a limb.¹ The clinical spectrum of CRPS is heterogeneous and includes sensory, autonomic, trophic, and motor changes of the affected limb, which reflects the involvement of biological pathways that underlie exaggerated inflammation, vasomotor dysfunction, and maladaptive neuroplasticity.¹ The key symptom of CRPS is spontaneous continuous pain, which is disproportionate to the inciting event.² However, patients with CRPS exhibit various qualities of pain³ depending on the presence of allodynia, hyperalgesia, vascular involvement and fixed dystonia.⁴⁻⁸ Analgesics are often used in the management of pain in CRPS, but evidence supporting these treatments is limited.⁹ Different qualities of pain may have different underlying mechanisms. Studying the modifying effects of drugs with a known action on the different pain qualities in homogeneous groups of patients may contribute to the development of future pain management strategies.⁴ γ -Aminobutyric acid (GABA)ergic circuits play a major role in the processing of different types of pain, especially at the spinal level.¹⁰⁻¹² In a previous study, we found that continuous delivery of intrathecal baclofen (ITB), a presynaptic and postsynaptic GABA(B) agonist, significantly improved the global severity of pain in patients with CRPS and dystonia.¹³ It is interesting to note that this improvement of pain was only partly explained by a decrease of dystonia, suggesting a direct antinociceptive effect of baclofen. This study therefore explored whether GABA(B) receptor stimulation by ITB exerted a differential effect on pain qualities in CRPS patients with dystonia.

METHODS

Patients

Patients with a diagnosis of CRPS-I and dystonia in at least one extremity who were participating in a trial of ITB administration were included in this study.¹³ Written informed consent was obtained from patients in accordance with the Declaration of Helsinki and the local ethics committee approved the study. The trial was registered in

the Dutch Trial Register (NTR403) on September 12, 2005 with van Hilten serving as the principal investigator. Patients were assessed for eligibility between January 2002 and January 2007. Inclusion criteria were as follows: CRPS-I according to the diagnostic criteria of the International Association for the Study of Pain (IASP)² and symptoms for at least 1 year. The IASP criteria include a combination of the following: (1) the presence of an initiating noxious event or a cause of immobilization; (2) continuing pain, allodynia, or hyperalgesia disproportionate to the inciting event; (3) evidence at some time of edema, changes in skin blood flow, or abnormal sudomotor activity in the area of pain; and (4) the absence of a condition that would otherwise account for the degree of pain and dysfunction. Criteria 2 to 4 are required for the diagnosis of CRPS. In contrast to the previously reported study, this study also included patients who developed CRPS spontaneously because recent evidence emerged showing that the clinical presentation of these patients does not differ from those in whom the syndrome developed after a trauma.¹⁴ All patients were subjected to the same study design, including a single-blind placebo run-in, dose escalation screening procedure, followed by open-label continuous ITB administration for 12 months for patients who met the responder criteria.¹³ The procedure for implantation of a pump (SynchroMed Infusion system; Medtronic Inc., Minneapolis, MN) and the verification of pump-catheter system integrity as well as the ITB dose escalation scheme were identical to those used in the previous study.¹³ The administration of ITB was started at a rate of 150 µg/day and increased in 10 to 20% steps until patients experienced a satisfactory improvement of dystonia (the patient's experience of dystonia relief) or dose-limiting side effects occurred.^{15,16} Changes of supplemental analgesics were allowed and monitored throughout the study.

Measures

In this open label study, we performed a post hoc analysis of data from a study that was designed (1) to investigate the effectiveness of ITB in a dose escalation study in a large group of patients with CRPS-related dystonia and (2) to evaluate whether ITB is effective and safe in this population over a 12-month period.¹³

This study was performed in an outpatient setting. Patients were evaluated at the start of continuous ITB administration and every 3 months for the following year.

To study the effect of ITB on the different pain qualities we used the neuropathic pain scale (NPS), which includes 2 global ratings (intensity and unpleasantness) and 8 specific ratings (sharp, hot, dull, cold, sensitive, itchy, deep and surface).⁴ This scale uses a numeric rating scale (NRS) ranging from 0 (absent) to 10 (most severe) to rate each pain quality item. Preceding each 3-month visit, patients rated their global dystonia severity (GDS) at hourly intervals for 5 consecutive days using the same NRS.¹³ A mean NRS score was calculated from all ratings in these 5 consecutive days.¹³ A medication quantification scale (MQS) was used to quantify the use of analgesics based on the number of medications used, the pharmacological class of the drug, and the dosage level.¹⁷ The MQS score was calculated by multiplying the dosage level and a detriment weight, which was assigned for 8 classes of medication. The MQS scores of all drugs of each patient were summed to obtain a total MQS score per patient.

Statistical analysis

We used a longitudinal linear mixed effects model with a heterogeneous first-order autoregressive covariance structure to test the hypothesis of change in NPS over time for each of the NPS qualities including time as a categorical variable. We analyzed the effect at each time point (3, 6, 9 and 12 months) compared with baseline and were not interested in a linear or quadratic change.

In this model, patients are treated as random effects, so the analysis can be adjusted to the pain level of each single subject. Furthermore, it adjusts for correlation between repeated measures from the same patient (assuming stronger correlations between scores that are closer in time), and allows missing data and analysis of repeated data with a variable number of measurements per subject. Because we used a linear mixed model, we examined the distribution of the residuals for all components of the NPS. An inspection of the normal probability plots showed that the distribution of the residuals was normal for all pain qualities. The analysis was controlled for the potential

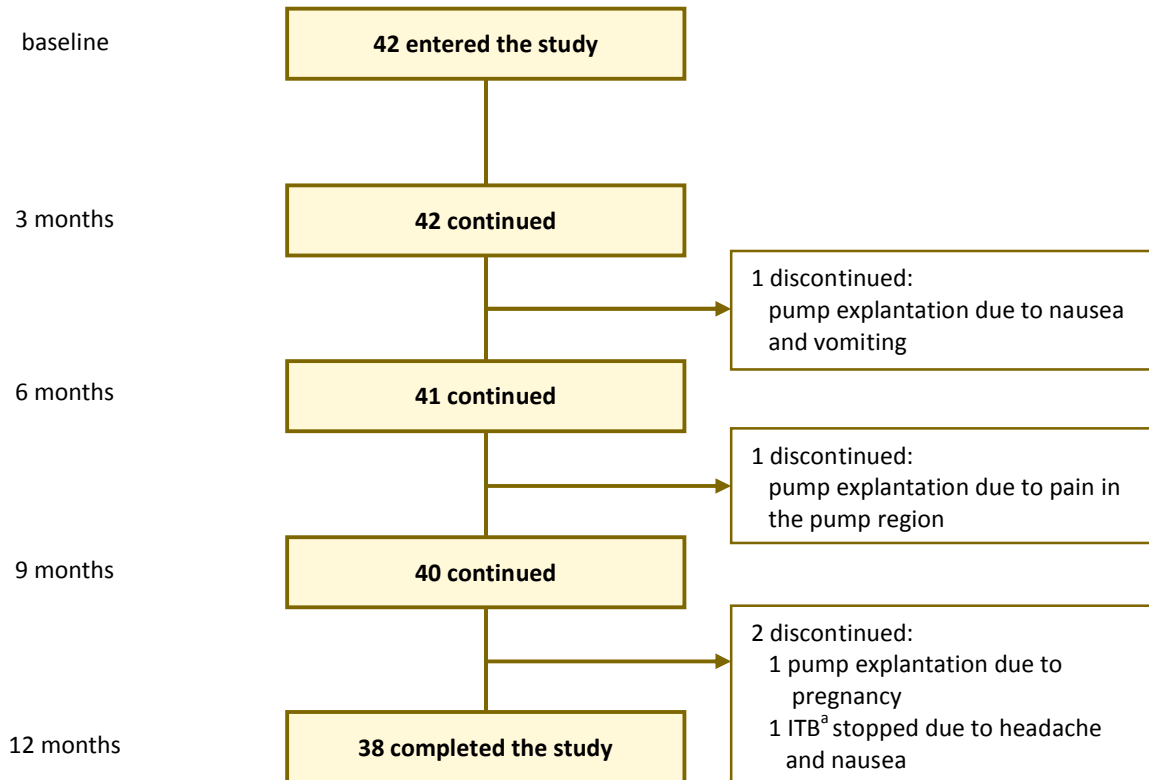
confounding effect of other analgesic medication (MQS), GDS, and the absence or presence of an inciting event triggering CRPS. All statistical tests were 2-sided, with $\alpha = 0.05$. The Cronbach α for internal consistency in our study was 0.93, and interitem correlations ranged from 0.27 to 0.90 with an average of 0.57. Because items are clearly not uncorrelated, we believe that correcting for multiple comparisons may not be necessary. The results are expressed as mean (standard deviation [SD]). Given that the present study concerns a post hoc analysis of a study that was primarily aimed at reducing dystonia,¹³ no sample size calculations with respect to pain relief were performed. Analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL).

RESULTS

Forty-two patients with a mean (SD) age of 37.3 (12.4) years and a mean (SD) disease duration of 8.8 (6.4) years were found eligible and participated in this study (figure 2.1). Forty patients (95%) were female and in 31 patients (74%) the onset of CRPS was triggered by a noxious stimulus. The number of affected extremities was 2 (24%) in 10 patients, 3 (17%) in 7 patients, and 4 (60%) in 25 patients. Four patients failed to complete the 1-year follow-up period (figure 2.1) but were included in the linear mixed model analysis. A complete list of adverse events is illustrated in table 2.1.

After correction for the use of supplemental analgesics and GDS, a significant time effect was found for global intense pain ($p=0.009$), sharp pain ($p<0.0001$), dull pain ($p=0.003$), and deep pain ($p<0.0001$) (table 2.2). This effect was characterized by a decline of scores over the first 6 months, after which the scores leveled off. We compared the course of the scores between each pair of these 4 pain qualities for the first 6 months within persons and considered the course to be similar if both pain qualities improved, worsened, or remained the same, and dissimilar if otherwise. Following this definition, a similar course of pain was found in 71% of cases for the combination of global intense and sharp pain, in 67% of cases for the combination of global intense and dull pain, in 74% of cases for the combination of global intense and deep pain, in 68% of cases for

Figure 2.1. Enrollment of patients



a. ITB = intrathecal baclofen

the combination of sharp and dull pain, in 66% of cases for the combination of sharp and deep pain and in 63% of cases for the combination of deep and dull pain. The time effect for GDS was also significant ($p=0.003$) with a continuous decline all through the 1-year follow-up (table 2.2). The mean daily dose of ITB increased every 3 months during the year (table 2.2). The absence or presence of an inciting event triggering CRPS did not significantly influence the time effect for any pain quality, GDS, MQS, or daily dose of ITB.

DISCUSSION

CRPS is associated with severe debilitating pain, which may involve different pain qualities across patients.^{4,6} These various pain qualities may result from different pain

Table 2.1. Adverse events

Adverse events	Type of event	N
ITB-related (n = 26)	Urine retention	4
	Somnolence	3
	Psychiatric ^a	3
	Nausea, vomiting	5
	Headache	2
	Fatigue	2
	Dysesthesia	3
	Hypotension, bradycardia	1
	Other ^b	3
Device-related, catheter (n = 49)	Post-dural puncture headache	37
	Dislodgement	5
	Subcutaneous fluid collection/CSF leak	3
	Occlusion/kink	2
	Compression spinal cord or root	2
Device-related, pump (n = 10)	Pump pocket infection	4
	Pain at pump site	3
	Migration of pump	2
	Ulcerations at pump site during pregnancy	1
Other (n = 22)	Worsening = CRPS symptoms	6
	Psychiatric ^c	4
	Excessive weight loss	3
	Gastrointestinal problems (unrelated to ITB)	3
	Infections (unrelated to device)	3
	Internal complications ^d	3

a. Psychosis: n = 2, depression and anxiety disorders: n = 1.

b. Diplopia, dizziness, anorgasmia.

c. Confusional state: n = 2, reactive depression: n = 1, reactive psychosis: n = 1.

d. Anemia, elevated liver enzymes, electrolyte changes.

mechanisms.⁴ This study focused on patients with CRPS characterized by dystonia. Homogeneous groups enhance the clinical, pathophysiological, and genetic coherence, which in turn may benefit research aiming to study or influence specific biological pathways. Patients in this study received baclofen, a presynaptic and postsynaptic GABA(B) agonist, which possesses analgesic properties.¹⁸⁻²⁰ The intrathecal delivery of baclofen is used to overcome the obstacle of the blood-brain barrier and thus results in greater therapeutic effectiveness concentrated at the spinal site of action.^{13,21}

Table 2.2. Scores of the Neuropathic Pain Scale for different pain qualities, global dystonia severity, use of supplemental analgesics and daily dose of intrathecal baclofen over a 1-year follow-up

	Baseline	3 months	6 months	9 months	12 months	F value	P value
NPS-item							
Global	7.6 (1.5)	6.3 (2.2)	5.7 (3.1)	5.9 (2.9)	5.6 (2.9)	(4, 64.08) = 3.70	0.009
Sharp	7.0 (2.3)	6.0 (2.5)	5.0 (3.6)	5.3 (3.4)	4.6 (3.4)	(4, 56.28) = 7.18	<0.0001
Hot	5.0 (3.0)	4.3 (3.3)	4.5 (3.5)	4.5 (3.5)	4.0 (3.3)	(4, 59.83) = 0.33	0.858
Dull	6.6 (2.4)	6.5 (2.7)	5.3 (2.8)	5.5 (3.0)	5.2 (3.1)	(4, 55.98) = 4.67	0.003
Cold	5.9 (3.4)	4.3 (3.3)	4.1 (3.6)	4.9 (3.5)	4.5 (3.8)	(4, 40.68) = 1.50	0.221
Sensitive	6.6 (3.2)	5.2 (3.7)	5.3 (3.7)	5.2 (3.5)	5.3 (3.5)	(4, 55.78) = 2.40	0.061
Itchy	2.6 (3.0)	1.5 (2.4)	1.4 (2.4)	2.2 (2.7)	1.8 (2.7)	(4, 39.65) = 0.20	0.940
Unpleasant	7.6 (1.5)	6.4 (2.2)	6.2 (2.9)	6.1 (2.9)	5.9 (2.7)	(4, 71.61) = 1.65	0.170
Deep	8.1 (1.4)	7.0 (2.2)	6.0 (3.1)	6.3 (3.0)	5.9 (3.0)	(4, 55.85) = 8.96	<0.0001
Surface	6.1 (3.2)	4.7 (3.4)	4.8 (3.5)	5.0 (3.2)	4.7 (3.3)	(4, 66.89) = 0.24	0.917
GDS	7.1 (1.7)	5.2 (2.5)	4.9 (2.8)	4.5 (2.6)	4.2 (2.4)	(4, 57.07) = 4.64	0.003
MQS	19.4 (11.6)	14.0 (12.1)	13.6 (13.4)	13.8 (12.0)	13.7 (11.8)	(4, 163.06) = 7.53	<0.0001
ITB (µg/day)	0 (0)	489 (248)	588 (295)	664 (332)	725 (344)	(4, 77.46) = 153.20	<0.0001

NPS = neuropathic pain scale; GDS = global dystonia severity; MQS = medication quantification scale; ITB = intrathecal baclofen; values are presented as mean (standard deviation)

In this study, we found a significant time effect with a reduction of pain scores during the first 6 months of follow-up for global intense, sharp, dull and deep pain. These reductions can be interpreted as clinically significant (reduction in NRS scores ≥ 2) for global intense, sharp and deep pain and to a lesser extent for dull pain.²² Notably, the time effect was independent of the course of GDS and the use of supplemental analgesics, supporting the notion of baclofen's antinociceptive effect on these pain qualities in these patients. This assumption is further supported by the significant decrease of supplemental analgesic consumption during the first 3 months. Oral administration of baclofen proved to be effective in the management of neuropathic pain in both peripheral neuropathy and central pain syndromes.¹⁸⁻²⁰ Notably, the add-on of ITB to spinal cord stimulation improved neuropathic pain over a prolonged period of time in patients who experienced insufficient benefit from spinal cord stimulation alone.²³ However, this improvement was based on a reduction of the global pain ratings. Findings of our study show that a targeted stimulation of the central GABA(B) receptor may have differential effects on various pain qualities, which to our knowledge has not been described previously. The scores for the different pain qualities leveled off after 6 months whereas GDS continued a declining course, and the dose of ITB was further escalated. The differential effects of these variables over time further support an independent role of GABAergic mechanisms on global intense, sharp, dull and deep pain qualities. Furthermore, patients were informed that the purpose of ITB was to improve dystonia severity. The continued decrease of GDS with further ITB dose escalations when improvement of different pain qualities had stabilized additionally highlights the differential roles of GABA(B) receptors in mechanisms underlying pain and dystonia in CRPS.

The onset of CRPS is generally thought to result from a noxious stimulus, although this is not an obligatory criterion for the diagnosis following the IASP criteria.² A previous study showed no significant difference in clinical characteristics between patients who developed CRPS spontaneously or after an inciting event.¹⁴ Also in this study, we found

no differences in outcomes between CRPS patients with and without an initiating trauma.

Although most studies on CRPS report a female-to-male ratio of 3-4, the high percentage of female patients in this study (95%) is in line with other studies on CRPS patients with dystonia.^{7, 24, 25}

One limitation of our study is the open-label design, which hampers the identification of a potential placebo effect, due to the absence of a control group. Ideally, a placebo-controlled, randomized design would have been performed. Because this would involve the follow-up of patients with an implanted pump without medication for 1 year, this was considered unethical. However, we believe that the somewhat differential effect of ITB on particular pain qualities over time as compared with dystonia time makes a substantial role of a placebo effect on the outcomes of this study less likely. We cannot, however, exclude a placebo effect or natural progression of the disease affecting the pattern of the different pain qualities over time.

Another limitation of the study could be the lack of homogeneity in dose escalation of ITB. However, this was inevitable because the occurrence of side effects limited dose escalation in some patients and the daily dose was maintained once a satisfactory result was achieved. Nonetheless, we noticed a trend of continuous increase of the daily dose of ITB throughout the whole 1-year follow-up period.

In conclusion ITB proved beneficial in reducing global intense, sharp, dull and deep pain and highlights the differential antinociceptive effects of central GABA(B) receptor stimulation on specific pain qualities in CRPS.

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Chapter 3

The Lack of Efficacy of Different Infusion Rates of Intrathecal Baclofen in Complex Regional Pain Syndrome

A Randomized, Double-Blind, Crossover Study

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ABSTRACT

Objective. Intrathecal baclofen (ITB) is effective in the treatment of dystonia related to complex regional pain syndrome (CRPS). In a previous study, we noted that the responsiveness to ITB declined in 30% of patients once drug delivery was switched from an external to an implanted device associated with a reduction of the infusion rate (IR).

Design. In a double-blind study, we investigated the effect of varying the IR at a fixed daily dose on the efficacy and safety of ITB in patients with CRPS-related dystonia. Patients were randomized to either slower infusion rate delivery (SIRD) or four-times faster infusion rate delivery (FIRD) for 2 weeks and were crossed over after a 1-week washout period.

Patients. Patients were eligible if they experienced no beneficial response to ITB on dystonia despite a minimum dose of 600 µg/day or because side effects limited dose escalation.

Outcome Measures. Primary outcome measures were changes in global dystonia and pain severity.

Results. There were no significant differences between the FIRD and the SIRD groups for the median change of numeric rating scale dystonia (-0.3 [interquartile range {IQR} -1.1 – 0.5]), pain (0.1 [IQR -0.8 – 1.3]), and secondary outcomes, except for the frequency of adverse events which was significantly higher during FIRD (12 vs 2). FIRD was preferred only by patients who were included because side effects to ITB prevented dose escalation.

Conclusions. Increasing the IR at a fixed daily dose is not associated with improvement of dystonia or pain but warrants further investigation in patients in whom side effects prevent further dose escalation.

INTRODUCTION

Complex regional pain syndrome type I (CRPS I) is commonly preceded by injury, usually to a limb, and is characterized by pain, disturbed blood flow, temperature regulation, and motor control of the affected area.¹⁻³ Approximately 20% of patients with CRPS develop dystonia, which is characterized by fixed flexion postures.^{1,4-7} Because dystonia in CRPS tends to spread to other limbs, the syndrome may evolve into a disabling disorder with a marked impact on quality of life.^{4,6,8} Impaired inhibitory control of sensorimotor circuits is a key pathophysiological finding in dystonia of CRPS.⁹ Baclofen stimulates pre- and postsynaptic γ -aminobutyric acid B receptors, which enhances central inhibitory activity.^{10,11} However, dystonia in CRPS is rarely controlled by oral baclofen, probably because of the drug's poor ability to pass the blood-brain barrier;^{12,13} although in some patients, beneficial effects are reported with dosages as high as 90-120 mg per day.⁴ Epidural administration of baclofen was shown to be beneficial in intractable spasticity likely because the drug is lipid soluble and may cross the dura to act on the spinal canal.¹⁴ Intrathecal delivery of baclofen overcomes the obstacle of the blood-brain barrier and results in greater therapeutic efficacy concentrated at the spinal site of action.^{15,16} However, not all patients may respond to this mode of drug delivery, which is expensive and requires an invasive implantation procedure. Hence, a screening procedure with an external pump is frequently used to evaluate the responsiveness to intrathecal baclofen (ITB) to help select those patients that are most suitable for continuous ITB delivery through an implanted pump. Against this background, we screened patients using a 2-day placebo run-in dose escalation (200-800 μ g ITB) design.¹⁶ The responder criterion for pump implantation was set at a $\geq 25\%$ improvement of global dystonia severity on two consecutive baclofen days as compared to placebo. However, after pump implantation, 30% of patients on ITB surprisingly failed to meet the responder criterion of the screening despite the use of a minimum daily dose of 600 μ g or because side effects limited dose escalation. In an attempt to find an explanation for this finding, we reviewed the procedures of our study.¹⁶ We found that the infusion rates (IRs) applied during the screening and postimplantation period differed because of the use of different baclofen

concentrations (0.5 mg/mL vs 3 mg/mL) (table 3.1). The IRs decreased once patients were switched to 3 mg/mL concentration in the postimplantation period. Two different concentrations were used because of the utilization of two pumps with different requirements for effective drug delivery. Could the lack of response to ITB in some patients, after the switch to the higher baclofen concentration, be explained by the use of lower IRs? In an animal model, higher IRs of baclofen and bupivacaine increased the drug's cerebrospinal fluid distribution; although experiments were performed in a nonphysiological setting¹⁷ and pain severity was reduced by increasing the IR of intrathecally delivered bupivacaine in patients with intractable pain.¹⁸ To our knowledge, the influence of the IR on the clinical efficacy of ITB, independent of the daily dose, has not been described to date. In this study, we compared the efficacy and safety of two ITB IRs at a fixed daily dose in patients with CRPS-related dystonia and hypothesized that the clinical efficacy may improve by increasing the IR without affecting safety as the daily dose was fixed.

Table 3.1. Drug delivery characteristics during screening procedure and post- pump implantation¹⁶

	Screening procedure	Postimplantation
Median (IQR) daily dose ITB (µg)	375 (275–500)	695 (393–838)
Concentration baclofen (mg/mL)	0.5	3
Median (IQR) infusion rate ITB (mL/day)	0.74 (0.54–0.98)	0.24 (0.13–0.28)

IQR = interquartile range; ITB = intrathecal baclofen

METHODS

Patients

The study was conducted in an ambulatory setting between April and December 2008. Patient consent was obtained in accordance with the declaration of Helsinki, and the study was approved by the local Ethics Committee. The trial was registered in The

Netherlands National Trial Register (NTR 1269). All patients were followed in our outpatient clinic and had CRPS-related dystonia in at least one extremity for which continuous ITB was administered. The patients fulfilled the CRPS criteria of the International Association for the Study of Pain (IASP) including continuing pain; allodynia or hyperalgesia disproportionate to any inciting event; evidence at some time of edema; changes in skin blood flow or abnormal sudomotor activity in the area of pain; and the absence of any condition that would otherwise account for the degree of pain and dysfunction.² Patients had received an internal programmable Synchromed EL or II® pump (Medtronic, Minneapolis, MN, USA) for continuous infusion of ITB after oral baclofen up to a minimal daily dose of 60 mg failed to provide beneficial response or side effects limited escalation of the daily dose.¹⁶ Patients were eligible for the IR study if they experienced no beneficial response to ITB on dystonia at a minimum daily dose of 600 µg, or dose-limiting side effects prohibited dose escalation of ITB. Furthermore, patients had to rate their global dystonia severity as at least 5 on a numeric rating scale (NRS) ranging from 0 (absent) to 10 (most severe) in order to be eligible for the study. Exclusion criteria were identical to those used in a previous study examining the efficacy of ITB in CRPS-related dystonia.¹⁶ In 11 patients, a screening procedure was performed using an external microinfusion pump before pump implantation.¹⁶ The remaining three patients participated in a trial with intrathecal glycine, after which they were switched to ITB without a screening procedure.¹⁹ These three patients were included in this study because they failed to improve $\geq 25\%$ in dystonia severity as a result of dose-limiting side effects to ITB. Pump-catheter system integrity was verified in all patients prior to the study, and other conditions potentially influencing dystonia severity were ruled out. At baseline, the administered daily dose varied between patients, and all patients used a baclofen solution of 3 mg/mL.

Design

In a double-blind, randomized, two-period, crossover design, solutions of 3 and 0.75 mg/mL baclofen were administered in a randomized sequence. A computer method was

used to randomly allocate patients to one of the two sequences of different IRs. Prior to the start of the study, all patients received ITB at a simple continuous rate with a 3 mg/mL concentration. To obtain two IRs at a 4:1 ratio with a fixed daily dose, concentrations of 3 mg/mL (slower infusion rate delivery) [SIRD]) and 0.75 mg/mL (faster infusion rate delivery [FIRD]) were used. A physician who was not involved in the assessments of the patients carried out the switch procedure at the start of each sequence, filling the pump reservoir with baclofen concentrations of 0.75 or 3 mg/mL. Notably, reservoir fillings were always changed, regardless of the assigned new concentration to guarantee maintenance of blinding. When the baclofen concentration is changed, a bridge bolus can be used to empty the internal pump tubing and external catheter with the old baclofen concentration with the original IR. With a fixed daily administered dose, the replacement of the 3 mg/mL concentration will result in a substantial delay before the new concentration reaches the tip of the catheter. As this concentration-dependent delay may potentially contribute to deblinding, we used the side port to carry out a procedure that included aspiration of the content of the external catheter followed by multiple boluses to replace the removed volume. As a result of this approach, the new concentration would always reach the tip of the catheter within an hour regardless of the old concentration. During the first 2 weeks of the study, the first assigned IR was used. This was followed by a 1-week open administration of the 3 mg/mL concentration of baclofen to minimize potential consequences of any carryover effect. Subsequently, over the following 2 weeks, patients received the other IR.

Outcome Measures

Patients were asked to rate the global severity of pain and dystonia using an NRS ranging from 0 (absent) to 10 (most severe) once every day starting 1 week before the first switch procedure until the end of the second treatment period. At the start and end of each treatment period prior to changing the IR, a blinded clinical assessor rated the dystonia severity using the Burke-Fahn-Marsden (BFM) scale, which is the sum of the scores of the individual body regions.²⁰ The same rater assessed the change of CRPS

signs and symptoms from baseline on a global impression scale (GIS) at the end of each treatment, ranging from -3 (much worse) to +3 (much better) with 0 for "no change". At the end of the study, patients were asked to compare both treatment periods using a patient preference questionnaire (PPQ). The PPQ consists of a 10-cm horizontal line ranging from -5 to +5. A score of 0 reflects no preference, whereas -5 or +5 expresses maximal preference for the first or second treatment period, respectively. In addition, patients were asked to indicate the reasons for their preference. At the end of each study the clinical assessor was asked to guess treatment assignment to evaluate the integrity of blinding. The primary outcome measure was the difference in change in global severity of pain and dystonia between baseline and end of each IR period. The secondary outcome measures were defined as the difference in change in BFM score between baseline and end of each IR period, the difference in GIS between both treatment periods, the PPQ score, and the frequency and severity of adverse events assessed at the end of each IR period.

Statistical Analysis

The results are expressed as median (interquartile range [IQR]). For all variables, a Wilcoxon signed rank test or Mann-Whitney *U* test was used to examine differences within and between patients, respectively. A chi-square test was conducted to compare the amount of adverse events between the FIRD and the SIRD groups. Significance was assumed at the 0.05 level. Assuming an alpha of 0.05, beta of 0.2, a standard deviation of the NRS of 2, and a correlation between measures of 0.6, we calculated that 14 patients would be required to detect a statistically significant difference of $\geq 25\%$ in the reduction of NRS scores between both IRs.

RESULTS

Demographic and Baseline Characteristics

Fourteen CRPS patients (13 women) aged 45.5 years (IQR 37.3–56.0) with a median disease duration of 12.5 years (IQR 8.0–16.3) were found eligible and participated in the

study (table 3.2). The median daily dose of ITB was 695 µg (IQR 393–838), with the median thoracic level of the catheter tip at T7 (range T2–T11). Seven patients (six women) were allocated to the FIRD-SIRD sequence; seven patients (all women) were allocated to the SIRD-FIRD sequence. All patients completed the study. A Wilcoxon signed rank test indicated there were no significant differences between baseline measures (NRS for dystonia and pain and BFM) of the first and second treatment period for each sequence, indicating a successful washout (table 3.3). Additionally, a Mann-Whitney *U* test indicated that there were no significant differences between these baseline measures of patients assigned to each treatment sequence (FIRD-SIRD or SIRD-FIRD).

Table 3.2. Demographic and clinical characteristics

Characteristics	Value
Median (IQR) age (years)	45.5 (37.3–56.0)
Sex	
Male	1
Female	13
Median (IQR) dystonia NRS	7.2 (5.9–8.0)
Median (IQR) pain NRS	6.4 (5.5–7.8)
Median (IQR) duration of CRPS (years)	12.5 (8.0–16.3)
Median (IQR) duration of dystonia (years)	12.5 (7.8–16.3)
Median (IQR) duration of ITB (years)	3.9 (2.6–5.1)
Number of affected extremities (%)	
1	0 (0)
2	1 (7)
3	2 (14)
4	11 (79)
Number of dystonic extremities (%)	
1	0 (0)
2	1 (7)
3	3 (21)
4	10 (72)
Median (IQR) Burke-Fahn-Marsden score	47 (33–62)

IQR = interquartile range; NRS = numeric rating scale; CRPS = complex regional pain syndrome; ITB = intrathecal baclofen.

Table 3.3. Baseline scores for primary and secondary outcome measures

Measure	FIRD, median (IQR)	SIRD, median (IQR)	P value
<i>Primary outcome measures</i>			
NRS for dystonia	7.4 (5.9–8.3)	7.4 (6.4–8.1)	NS
NRS for pain	6.7 (5.5–7.5)	7.0 (5.9–7.8)	NS
<i>Secondary outcome measure</i>			
Burke-Fahn-Marsden scale	50 (33–60)	48 (32–65)	NS

NS = not significant; FIRD = faster infusion rate delivery; SIRD = slower infusion rate delivery; NRS = numeric rating scale.

Results on Outcome Measures

There were no significant differences between the FIRD and the SIRD group in median change of NRS score for dystonia (-0.3 [IQR -1.1–0.5]) and pain (0.1 [IQR -0.8–1.3]) and BFM score (2 [IQR -4–13]) (table 3.4). Six patients preferred FIRD with a median PPQ score of 3.0 (IQR 1.6–4.3), and seven patients favored the SIRD with a median PPQ score of 3.0 (IQR 1.0–3.0). One patient had no preference for any of the IRs. The difference between both groups in PPQ score was not significant. After FIRD, six patients showed an improvement on the GIS (+1 in four and +2 in two patients), three patients deteriorated (-1 in two and -2 in one patient), and five patients showed no change. After

Table 3.4. Difference in change for primary and secondary outcome measures

Measure	FIRD, median (IQR)	SIRD, median (IQR)	Δ FIRD-SIRD, median (IQR)	P value
<i>Primary outcome measures</i>				
NRS for dystonia	-0.6 (-1.3–0.2)	0.0 (-1.3–0.8)	-0.3 (-1.1–0.5)	NS
NRS for pain	-0.4 (-0.8–0.4)	0.0 (-1.8–0.2)	0.1 (-0.8–1.3)	NS
<i>Secondary outcome measure</i>				
Burke-Fahn-Marsden scale	0 (0–7)	-2 (-6–0)	2 (-4–13)	NS

Negative values represent improvement in outcome measures. P values were derived from a Wilcoxon signed rank test. NS = not significant; FIRD = faster infusion rate delivery; SIRD = slower infusion rate delivery; IQR = interquartile range; NRS = numeric rating scale.

SIRD, four patients showed an improvement on the GIS (+1 in three and +3 in one patient), three patients deteriorated (-1 in one and -2 in two patients) and seven patients showed no change. There was no significant difference in change found on the GIS score between both IRs ($z = -1.66$). There was no significant effect of IR sequence on any of the outcome measures. Twelve adverse events (five moderate, seven mild) were reported in eight patients on FIRD (Table 3.5). Headache was the most frequently reported adverse event ($N = 5$), which in three patients was typical of intracranial hypotension headache and likely resulted from aspiration of catheter content during the switch procedure. Two patients reported one adverse event (both mild) while on SIRD including one patient with intracranial hypotension headache. There were significantly more adverse events on FIRD compared with SIRD ($\chi^2 = 6.78$, $df = 1$, $P = 0.01$), even after the removal of events due to intracranial hypotension ($\chi^2 = 6.97$, $df = 1$, $P = 0.01$).

Table 3.5. Adverse events reported after each infusion rate treatment

Adverse event	SIRD	FIRD
Headache	1 (7)	5 (36)
Chorea	0	1 (7)
Nausea	1 (7)	1 (7)
Hallucinations	0	1 (7)
Short-term amnesia	0	2 (14)
Light-headedness	0	1 (7)
Drowsiness	0	1 (7)

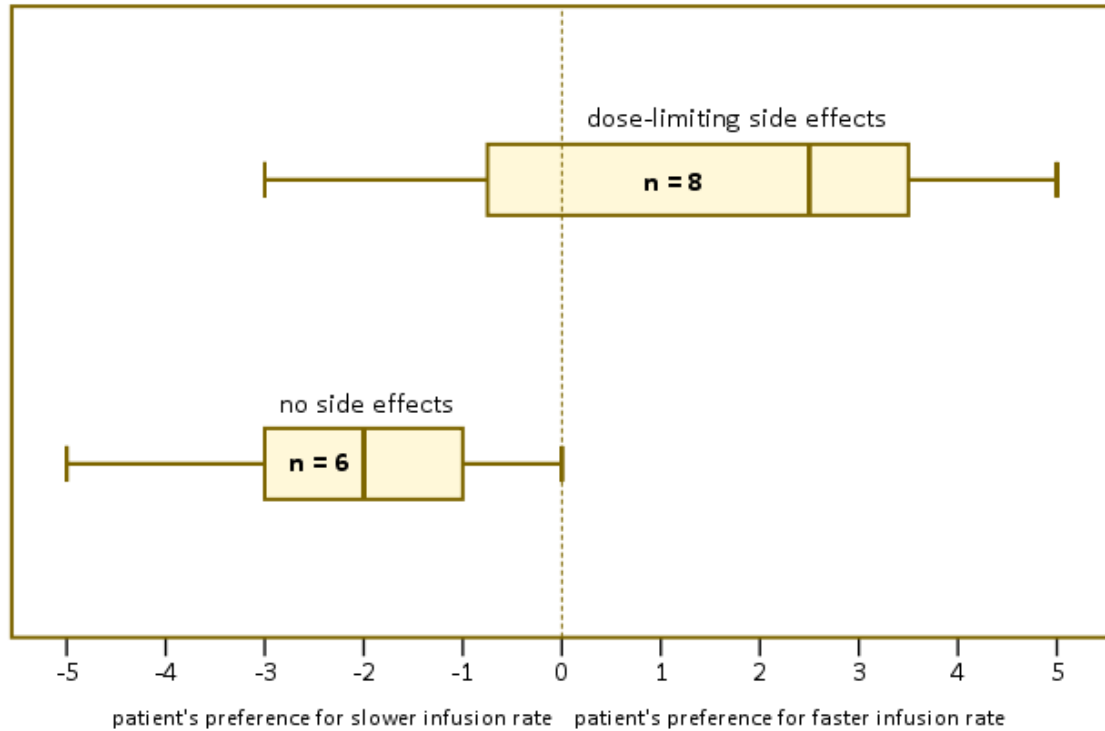
Numbers represent N (%).

SIRD = slower infusion rate delivery; FIRD = faster infusion rate delivery.

Post Hoc Subgroup Analysis

None of the demographics or clinical characteristics predicted any outcome measures except for the reason of inclusion in the study, which was related to the preference of a particular IR (Figure 3.1).

Figure 3.1. Box plot illustrating patient’s preference for patients with a poor response with and without dose-limiting side effects.



Six out of eight patients, who entered the study because side effects prohibited dose escalation of ITB, reported preference for FIRD because dystonia and pain improved (median PPQ score 2.5 [IQR -1.4–3.8]). All six patients, who entered the study because they did not respond to a minimum daily dose of 600 µg ITB without baclofen-related side effects, preferred SIRD, except one patient who reported no preference for any of the IRs (median PPQ score 2.0 [IQR 0.8–3.5]). Mann-Whitney *U* test revealed a significant difference in patient’s preference ($P = 0.03$) between groups, stratified according to “reason of participation”. No significant difference was found for any of the other primary and secondary outcomes as well as the rate or severity of adverse events between both groups ($P > 0.05$). The clinical assessor’s guess of which IR was administered was correct in 57%.

DISCUSSION

Intrathecal drug delivery has opened new avenues for the administration of drugs like baclofen that have difficulty passing the blood-brain barrier. However, once the drug is in the intrathecal space, many aspects may influence its profile of efficacy and safety.²¹ One of these aspects is the IR of a drug, which surprisingly has barely been explored.^{17,18} In a recent study on long-term effects of ITB in CRPS-related dystonia, we noted that a substantial percentage of patients, who responded to ITB during a screening procedure using an external pump, subsequently failed to respond to continuous ITB with an implanted pump.¹⁶ These poor responders included patients who failed to improve $\geq 25\%$ in global dystonia severity in spite of repeated dose escalations (up to 600 $\mu\text{g}/24$ hours) and patients in whom dose escalation was limited because of the occurrence of side effects. Interestingly, the intrathecal IR applied during the screening period exceeded the IR of the postimplantation period as much as seven times because a lower concentration (0.5 mg/mL) was used. Additionally, the daily dose at the time patients qualified for the responder criterion of the screening procedure was lower in the majority of patients compared with the daily dose administered during the postimplantation period (table 3.1). Triggered by these findings, we evaluated if different IRs at a fixed daily dose influence the efficacy and safety of ITB in patients with CRPS-related dystonia. In addition to dystonia, we evaluated the influence of ITB on pain because baclofen may exert an analgesic effect.²² Prior to the start of this study none of the patients experienced a beneficial response in pain to ITB. Both for the total group as well as the subgroups defined by reason of inclusion, we found no differences between FIRD and SIRD on any of the primary outcome measures. Blinding was adequately maintained, as the sequence of IRs was correctly guessed in approximately half of the cases. Our findings may indicate that IR differences up to a factor four do not account for the lack of response to ITB in implanted patients. Although we cannot exclude that the difference between the two IRs was too small to detect a clinically relevant difference, the use of a four-times higher IR seemed appropriate. The use of a concentration below 0.75 mg/mL would have implied an extra refilling of the pump

during the FIRD period in some patients, which may have caused debinding. The majority of patients displayed dystonia in all extremities reflecting the referral of more severely affected patients to our tertiary center. One could argue the severity of dystonia may account for the lack of response to ITB. However, only patients who had demonstrated responsiveness to ITB at an earlier stage were included in this study. Another limitation of this study could be the large variability in the daily administered dose of ITB across the patients, which reflects individual differences with respect to the dose of ITB at which patients perceived some albeit insufficient benefit (<25%) or the highest tolerated dose that did not cause unacceptable side effects. Consequently, it was not possible to equalize the daily administered dose of ITB. On the other hand, using a crossover design, patients served as their own controls with the daily dose fixed for each patient. Contrary to the primary outcomes, patient's preferences indicated significant differences with respect to the preferred IR. Except for one case, all patients who were included because they failed to respond to sequential dose escalations of ITB (without baclofen-related side effects) favored SIRD. Surprisingly, the majority of patients, who were included in the study because side effects restricted further dose escalation of ITB, reported a preference for FIRD. In a written statement delineating their preference, all but one patient in both subgroups revealed that their preference was based on the influence of the IR on dystonia and pain, which apparently outweighed the severity of adverse events.

Of note is that patients' preferences were paralleled neither by changes in primary and secondary outcome measures of dystonia nor by the clinical impression of change, which may question the responsiveness of the applied assessment scales to subtle changes. Possibly, increasing the IR under a fixed daily dose reduces the severity of side effects as compared with increasing the IR by raising the daily dose. In 11 patients, a screening procedure was performed prior to pump implantation. Interestingly, patients who participated in the study because ITB-related side effects limited dose escalation had a significantly larger difference between the IRs of the screening and postimplantation, compared with patients with a poor response without side effects

(table 3.6). All patients of the first category favored FIRD, except for one patient, who suffered from severe intracranial hypotension headache during FIRD and consequently preferred SIRD. However, it deserves emphasis that the aforementioned findings are the result of post hoc subgroup analyses and hence warrant further investigation in future studies. During the FIRD, patients reported significantly more adverse events of a supraspinal origin. As the daily dose of ITB was fixed for both IRs, this finding likely suggests a more extended intrathecal distribution of baclofen with higher IRs.

Table 3.6. Ratios of drug delivery characteristics for screening vs postimplantation period

	Poor response with no side effects	Poor response with dose-limiting side effects	z Value	P value
Median (IQR) daily dose ratio	0.4 (0.3–0.6)	1.0 (0.6–1.2)	-2.19	0.03
Median (IQR) infusion rate ratio	2.6 (2.0–3.4)	5.6 (3.6–7.0)	-2.19	0.03

The screening to postimplantation period ratio for infusion rate and daily dose for patients with a poor response with and without side effects to ITB [16]. *P* values were derived from a Mann-Whitney *U* test.

IQR = interquartile range.

Conclusions

In conclusion under a fixed daily dose, a four-times higher IR enhances the intrathecal distribution of baclofen as evident from the significantly higher number of adverse events. However, in CRPS a fourfold higher IR is not associated with clinically overt improvement of dystonia or pain. Patients in whom side effects restricted further dose escalations of ITB favored FIRD because of subjective improvement of dystonia and pain. In these patients, FIRD did not cause more side effects than in patients who failed to respond to ITB after subsequent dose escalations but who had not experienced side effects. Consequently, in the subgroup of patients in whom side effects prevent ITB dose escalation, the effect of increasing the IR of ITB at a fixed daily dose should be further investigated.

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Chapter 4

Baclofen-induced Chorea in Complex Regional Pain Syndrome-Related Dystonia

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ABSTRACT

Dystonia in complex regional pain syndrome (CRPS) is often treated with baclofen, a γ -aminobutyric acid B agonist. The authors report four CRPS-patients who developed baclofen-induced chorea, which coincided to a large extent with body regions that were initially affected by dystonia. Treatment with tiapride resolved the chorea, suggesting that striatal dopaminergic hyperactivity underlies baclofen-induced chorea.

INTRODUCTION

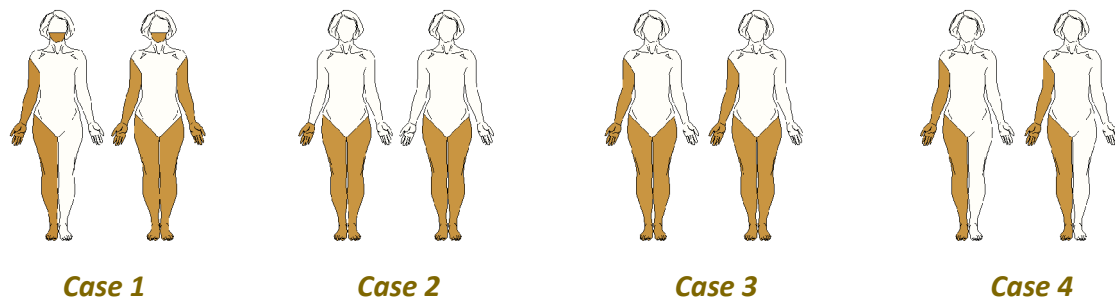
Complex regional pain syndrome (CRPS) is a chronic disorder characterized by persistent pain, vasomotor and trophic features, which usually is preceded by tissue injury.¹ Approximately 20% of patients with CRPS also develop fixed dystonia.² Baclofen, a γ -aminobutyric acid B (GABA-B) receptor agonist is commonly used in the management of spasticity of various causes, but is occasionally used in primary and secondary dystonia. To overcome baclofen's poor ability to pass the blood-brain barrier, intrathecal administration of baclofen (ITB) can be used, requiring only small doses to produce satisfactory clinical improvement. In the course of our research on ITB in CRPS patients with dystonia we encountered several cases with baclofen-induced chorea (BIC). The aim of this study is to characterize these patients and discuss potential mechanisms.

METHODS

Since 1996 approximately 300 patients have been evaluated in our clinic for CRPS-related dystonia. Patients were eligible for ITB, if they fulfilled the CRPS I diagnostic criteria of the International Association for the Study of Pain,¹ had fixed dystonia in one or more extremities, and showed an insufficient response to oral baclofen up to a minimal daily dose of 60 mg or had side effects which limited dose-escalation. Seventy-four patients who fulfilled the criteria were implanted with a programmable pump and received continuous ITB for a mean (range) duration of 48 (2 – 147) months at a median (range) daily dose of 650 (150 – 1500) μ g. The clinical characteristics of patients, in- and excluded for pump implantation, were similar.

Four patients were identified who developed chorea while on baclofen treatment (three on ITB). All patients were Caucasian and female with a mean (range) age of 52 (48 - 56) years and a mean (range) disease duration of 12 (8 - 17) years. The interval between the onset of CRPS and dystonia was less than a year in all cases. All patients showed a gradual spread of dystonia to other body parts (three multifocal dystonia and one hemidystonia; figure 4.1). None of the patients had a family history of dystonia and other causes of dystonia, including birth injury, head trauma, neuroleptic treatments

Figure 4.1. Distribution patterns of dystonia (left figure) and chorea (right figure) of four patients with CRPS. Case 1 also exhibited both dystonia and chorea in the oromandibular region. Case 1 to 3 received ITB. Case 4 received oral baclofen only.



CRPS = complex regional pain syndrome; ITB = intrathecal baclofen

were not reported or identified in the medical history or correspondence. Laboratory tests, including serum copper and ceruloplasmin as well as computed tomography or magnetic resonance imaging of the brain were normal.

The patients on ITB developed chorea at 3, 4 and 38 months after initiation of ITB at a mean (range) daily dose of 490 (315 – 650) μg with the catheter tip positioned at level L1, T7 en T10, which was comparable to patients without chorea. One patient developed chorea on oral baclofen two months after the start of this treatment at a daily dose of 70 mg. One of the patients on ITB used an oral contraceptive at the time chorea developed. However, a relation with chorea seemed unlikely since the oral contraceptive was initiated 29 years prior to the onset of chorea and its withdrawal did not improve chorea. The other three patients did not use medication with the potential to induce chorea. In all cases there were no symptoms or signs indicative of baclofen intoxication at the time chorea developed. Laboratory screening, including hematocrit and thyroid hormone tests, antinuclear factor, anti-DNA, antiphospholipid antibodies, antistreptolysin titer, acanthocytes, serum copper and ceruloplasmin and CAG repeat expansion mutation analysis for Huntington disease (performed in two patients) ruled out other causes of chorea.

In all cases chorea developed gradually, was continuous while awake, not distractible and simultaneously presented in the proximal and distal part of an extremity. None of the

patients experienced a sensory component related to the movement disorder. The distribution pattern of chorea varied between patients, but largely matched the distribution of dystonia within each patient, except for the occurrence of chorea on the contralateral non-dystonic side in patient 1 (figure 1). Patient 2 suffered from dystonia in both legs and the right hand, but developed chorea only in the lower limbs (figure 1). In all cases, dose-escalation of baclofen improved dystonia, but worsened chorea, and a reduction of baclofen dosage was associated with improvement of chorea and worsening of dystonia. In all four patients tiapride was administered, up to 300 mg in three divided doses, which successfully resolved chorea in all cases.

DISCUSSION

We present four patients who developed chorea while using baclofen (three on ITB). Dose-escalation of ITB always improved dystonia but worsened chorea and vice versa. Medical and medication history as well as laboratory tests ruled out other causes of chorea. Phenotypically, akathisia can appear as chorea. Akathisia is characterized by whole body, predominantly axial rocking movements and is associated with a sense of a need to move. In our patients the distribution pattern of movements coincided to a large extent with body regions that were initially affected by dystonia and the movements were not associated with a sensory component. A psychogenic origin was also considered unlikely since there was no acute onset or fast progression, and the movements were not distractible. Our findings thus suggest that these patients developed BIC. Hitherto, BIC has only been reported in three patients on oral treatment who suffered from various disorders.³⁻⁵ ITB is predominantly used in patients with spasticity but surprisingly no cases with BIC have been reported using this treatment. Although a referral bias to our tertiary centre likely inflated the findings, the apparent lack of reports on patients with BIC on ITB may highlight an increased susceptibility to develop BIC in patients with CRPS. The relative variability of the daily dose and long interval between the start of baclofen and the onset of chorea in our patients may question their relation. However, this is not an uncommon finding in other drug-induced

movement disorders and most likely reflects individual differences in susceptibility to drug-induced side-effects. CRPS is associated with a hyperactive state of the spinal cord (central sensitization) which is characterized by enhanced signal transmission in the pain circuitry to the cortex.⁶ In CRPS patients with dystonia, different studies have found evidence of impaired central inhibition along the neuraxis but it remains unclear if these findings genuinely reflect a pathophysiological marker of dystonia.² When baclofen is infused around the spinal cord it inhibits afferent input by stimulation of pre- and postsynaptic GABA-B receptors. Dystonia in CRPS responds to ITB which may suggest that this type of dystonia is associated with loss of GABAergic inhibition in the spinal cord.²

In CRPS, significant changes have been found in the cerebral circuitry involved in motor processing.⁷ These changes, which involve the basal ganglia and (pre)supplementary motor area have been suggested to play a role in the motor impairments encountered in patients with CRPS.⁷ Hence, in CRPS, spinal sensitization may bring about changes in supraspinal circuitry, finally leading to chorea.

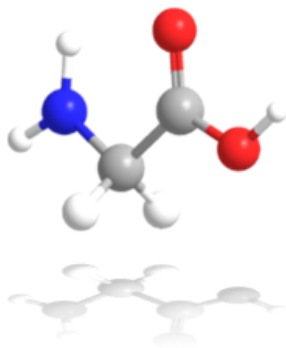
Although several mechanisms may underlie chorea, enhanced activity of striatal dopamine has been proposed as a major mechanism for the development of chorea induced by drugs including levodopa, dopamine agonists and antagonists, and lithium.⁸ Interestingly, baclofen is known to exert anti-dopaminergic activity⁹ and the drug has also been used to treat chorea in Huntington's disease and tardive dyskinesias. Tiapride is a selective D2/D3 dopamine receptor antagonist in limbic areas, the striatum, and nucleus accumbens without any affinity for other neurotransmitter receptors of the brain.¹⁰ Treatment with tiapride resolved the chorea, indicating that striatal dopaminergic hyperactivity underlies BIC in our patients. Collectively, our observations may suggest that alterations in synaptic processes mediated by GABA-B receptors underlie BIC in a subset of CRPS-patients.

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Part II.

Intrathecal Glycine Treatment in CRPS



Chapter 5

Intrathecal Glycine for Pain and Dystonia in Complex Regional Pain Syndrome

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ABSTRACT

Since glycinergic neurotransmission plays an important inhibitory role in the processing of sensory and motor information, intrathecal glycine (ITG) administration may be a potential therapy for both pain and movement disorders in patients with complex regional pain syndrome (CRPS). Aims of the current study, which is the first report on ITG in humans, were to evaluate its safety and efficacy. ITG treatment during 4 weeks was studied in CRPS patients with dystonia in the period before they received intrathecal baclofen treatment. Twenty patients were assessed and after exclusion of one patient, the remaining 19 patients were randomized in a double-blind placebo-controlled crossover study. Safety was assessed by clinical evaluation, blood examinations and electrocardiograms. Efficacy measures involved pain (numeric rating scale, McGill pain questionnaire), movement disorders (Burke–Fahn–Marsden dystonia rating scale, unified myoclonus rating scale, tremor research group rating scale), activity (Radboud skills questionnaire, walking ability questionnaire), and a clinical global impression (CGI) and patient's global impression score (PGI). Treatment-emergent adverse events were generally mild to moderate and not different from placebo treatment. During ITG treatment growth hormone levels were slightly increased. Although there was a trend to worsening on the CGI and PGI during ITG treatment, there were no significant differences between ITG and placebo treatment in any of the outcomes. ITG given over 4 weeks was ineffective for pain or dystonia in CRPS. Although no serious adverse events occurred, further studies are required to rule out potential neurotoxicity of ITG.

INTRODUCTION

Complex regional pain syndrome type I (CRPS) which is more common in women and often preceded by a trauma, is characterized by spontaneous pain, edema, changes in skin temperature and color, hyperhidrosis, and motor disturbances.^{1,2} The latter mainly include fixed dystonia of the distal extremities.^{3,4} The pathophysiology of CRPS is still unclear but over the last decade there is increasing evidence showing that different mechanisms may contribute to its broad clinical spectrum.^{1,5,6}

The initial symptoms of CRPS have been attributed to a perturbed regulation of inflammation in which both C and A δ sensory nerve fibers (neurogenic inflammation) and the immune system of the skin are involved.⁷⁻¹⁰ Peripheral inflammation or injury may in turn lead to profound changes in the processing of sensory input at the spinal level, a process known as central sensitization.^{11,12} As a result, pain may become chronic and allodynia and hyperalgesia may develop. Additionally, these central changes may corrupt the normal control of motor circuits.^{13,14}

Compelling evidence from neurophysiological studies that focused at the spinal or cortical level in patients show that disinhibition is a key characteristic in the involvement of the central nervous system in CRPS patients with and without dystonia.¹⁵⁻¹⁸

Cutaneous C and A δ afferents are linked to spinal interneuronal circuits that mediate nociceptive withdrawal reflexes (NWRs).¹⁹ Interestingly, both sensitized NWRs in animal models and pain and dystonia in CRPS patients respond to the intrathecal administration of the GABA_B agonist baclofen (ITB), which enhances spinal GABAergic inhibition.²⁰⁻²²

In addition to GABAergic circuits, glycinergic circuits may also be involved in central sensitization. In rats, peripheral inflammation-induced central sensitization has been associated with loss of glycinergic inhibition.²³ Following peripheral inflammation or spinal PGE₂ injection, mice with a glycine receptor deficiency show a reduced pain sensitization.^{24,25} In animal models of neuropathic pain, intrathecal glycine (ITG) reduced^{26,27} or prevented²⁸ hyperalgesia. Besides involvement in afferent processing, glycine may play a prominent role in the control of motor functions. Strychnine is a glycine receptor antagonist, and poisoning with this drug results in overwhelming

muscle spasms, rigidity and tremor.²⁹ Glycine receptor mutations in both humans and animals result in spasms, tremor and myoclonia,^{30,31} motor features that bear a remarkable similarity to those reported in CRPS.

In view of the important role of disinhibition in chronic CRPS and the potential role of glycinergic mechanisms in pain and motor processing, drugs such as glycine that enhance glycinergic inhibition may provide a new mode of treatment in CRPS. Because glycine is abundantly present in food, it would seem plausible that this potentially dangerous inhibitory neurotransmitter has limited access to the CNS. Indeed, two animal studies have reported a poor blood-brain barrier (BBB) passage of glycine.^{32,33} As a consequence, glycine requires intrathecal administration (ITG) to explore its role in the management of pain and dystonia in CRPS.

Aims of the current study were to evaluate the safety and efficacy of ITG in patients with CRPS. We here report the results of a double-blind randomized placebo-controlled crossover trial evaluating 4 weeks of ITG treatment in 20 chronic CRPS patients.

METHODS

Subjects were male or female out-patients, at least 18 years of age, with a clinical diagnosis of CRPS with dystonia who were referred to the movement disorders outpatient clinic of the Department of Neurology and were candidates for ITB treatment. Patients who qualified for ITB treatment were requested to participate in the current study, which was performed in the period before ITB treatment started. Patients were referred by physicians throughout the Netherlands. Inclusion criteria were CRPS-I according to the diagnostic criteria of the International Association for the Study of Pain,³⁴ clinically significant fixed dystonia in one or more extremities, and symptoms for at least 1 year. Exclusion criteria were satisfactory relief of symptoms with conventional treatments including oral baclofen, pregnancy, breastfeeding, childbearing potential without using effective contraception, clinically significant psychiatric illness, suspicion of poor compliance, or involvement in legal proceedings concerning compensation for CRPS. All patients were evaluated by a psychiatrist to exclude psychiatric comorbidity. In all subjects

a programmable SynchroMed pump (Medtronic, Minneapolis, MN) for continuous IT administration was implanted. The catheter was introduced in the subarachnoid space (L2-L3) under X-ray guidance with the distal tip of the catheter placed in the midthoracic region. The catheter was then tunneled subcutaneously and connected to the pump. Pump-catheter system integrity was verified post-operatively. We aimed to recruit 20 patients, which was considered a reasonable sample size for a first safety study. Patient consent was obtained according to the Declaration of Helsinki and the study was approved by the Ethics Committee of the Leiden University Medical Center.

N = 1 experience

To date no published studies on ITG in humans are available. The dose schedule of ITG in the current study was based on our experiences in one CRPS patient who progressed to generalized dystonia and did not respond to ITB. After consent was obtained from both the local Ethics Committee and the patient, a last resort therapy with ITG was started. We noted a sustained and prominent decrease in pain with moderate effects on dystonia at a dose of 30 mg/24 h. ITG was administered for 1 year and no side-effects occurred.

Study design

We used a double-blind randomized placebo-controlled crossover design. Randomization was done by a computer-generated list and took place at the Department of Pharmacy. Treatment allocation remained concealed from patients and investigators (including those who performed the assessments) throughout the study. Every subject received two intrathecal treatments: 21 mg/mL glycine solution during 4 weeks, and sodium chloride 0.9% (w/v) during 4 weeks (placebo), with a tapering and wash-out period in-between both treatments: tapering in 1 week (3 equal dose reductions with an interval of 48 h), followed by a wash-out period of 1 week. The carry-over between treatments was considered minimal because the plasma half-life of IV glycine ranges from 30 to 60 min³⁵ (the half-life of ITG is unknown). Placebo and ITG have the same watery

appearance, which made unblinding by inspection of the administered substances impossible. Treatment was started at 8 mg/24 h and was increased weekly with 8 mg/24 h. Unless side-effects occurred, glycine administration reached a daily dose of 32 mg/24 h in the last week of the glycine period. Higher dose administrations were not studied because of the associated short filling interval (< 1 month) of the pump, which was considered not feasible in clinical practice.

An independent data safety board monitored the study. The committee monitored the safety of the patients by evaluating the treatment-emergent adverse events (AEs). The study is registered with the Netherlands Trial Register, number NTR499.

Outcome measures

Safety assessments included history taking, physical examination and routine blood assessments (every other week) and electrocardiograms (every other week). Pain was evaluated with a numeric rating scale (NRS) for pain, and the McGill pain questionnaire.³⁶ A TSA-II thermal sensory analyzer (Medoc Ltd., Ramat Yishai, Israel), using a thermode placed on both hands (thenar eminences), was used to assess detection thresholds of temperature change (method of limits).³⁷ Efficacy on movement disorders was studied with the Burke-Fahn-Marsden (BFM) Dystonia Rating Scale³⁸, unified myoclonus rating scale (Sections 2-4)³⁹ and tremor research group rating scale (Items 1-8).⁴⁰ Assessment of activity level included the Radboud skills questionnaire⁴¹ (if arms were involved) and the walking ability questionnaire⁴² (if legs were involved). Change of CRPS signs and symptoms was rated on a global impression scale: both the investigator (clinical global impression, CGI) and the patient (patient's global impression, PGI) assessed the change during treatment on a scale ranging from -3 (very poorly) to +3 (very well). Success of the blinding was investigated by asking both the patient and investigator to guess which treatment was administered.

Plasma glycine and growth hormone analysis

Blood samples for glycine measurement were taken at the last day of treatment (before tapering). Blood was collected in EDTA (ethylene diamine tetraacetic acid) tubes and directly cooled with melting ice. Plasma was isolated after centrifugation at 20°C for 7 min at 1500g and stored at -20°C until analysis. Glycine was determined on a Biochrom 30 automated amino acid analyzer (Biochrom, Cambridge, UK) as previously described;⁴³ however 250 µM L-methionine sulfone (Sigma, St Louis, MO) was used as the internal standard and a short buffer program of 50 min was used.

Because in a previous study in children, IV glycine increased growth hormone (GH) levels,⁴⁴ blood levels of GH were monitored at baseline and at the last day of each of the interventions.

Statistical analysis

To study differences between 4 weeks of ITG treatment and 4 weeks of placebo treatment, the paired *t* test (if data were normally distributed) or Wilcoxon test (if not) were used. Significance was assumed at the 0.05 level. For all tests, the SPSS software package version 14.0 (SPSS Inc., Chicago, IL) was used.

RESULTS

No patients refused to participate in the study in the period before their ITB treatment started. Patients were aged 26-58 years (19 women, 1 man). One patient withdrew her consent after implantation. The remaining 19 patients entered the study (table 5.1). In one patient, the study was ended prematurely because participation was experienced as too burdensome (she received ITG during 21 days, and no placebo).

Safety

Treatment-emergent AEs were found in 15 patients. The most frequently reported AEs were drowsiness, headache, dysesthesia and nausea and vomiting (table 5.2).

Table 5.1. Baseline characteristics - before pump implantation ($n = 19$)

Characteristic	Value
Median age, years (IQR)	41 (34-51)
Sex	
Male	1
Female	18
Median duration of CRPS, years (IQR)	9 (5-17)
Number of dystonic extremities (%)	
1	0
2	6 (31)
3	3 (16)
4	10 (53)
Modified Rankin scale (%) ⁴⁵	
No significant disability	0
Slight disability	7 (37)
Moderate disability	5 (26)
Moderately severe disability	7 (37)
Severe disability	0

CRPS = complex regional pain syndrome type I; IQR = interquartile range.

The proportion of patients with one or more AEs was similar during ITG (12/19 = 63%; 18 AEs) and placebo treatment (9/18 = 50%; 13 AEs; $\chi^2 = 0.23$, $df = 1$, $p = 0.63$).

Serious AEs did not occur. Other AEs during ITG treatment were categorized as mild to moderate. In one patient, a mild but persistent exacerbation of pain and dystonia occurred, which began at the first day of ITG treatment.

Plasma glycine and GH measurements

Mean serum glycine concentrations were not different between ITG treatment (242 μM , range 106-461) and placebo treatment (241 μM , range 105-499) at 28 days of treatment ($p = 0.75$, paired t test).

At baseline, four patients had GH values (8.8, 9.8, 25.9 and 70.6 mU/l) above the normal range (0.0-5.0 mU/l). GH was significantly increased at day 29 of ITG treatment (median 2.1, interquartile range [IQR] 0.7-5.3, maximum 30.7 mU/l) compared to day 29 of placebo (median 0.9, IQR 0.5-4.2, maximum 13.4 mU/l; $p = 0.031$, Wilcoxon test). There

Table 5.2. Treatment-emergent adverse events

Adverse Event	ITG (n = 19)	Placebo (n = 18)
Drowsiness	3	2
Headache	2	2
Dysesthesia	1	3
Dysgeusia	1	-
Photopsia	-	1
Restless legs	1	-
Nervousness	1	-
Emotional lability	2	-
Nausea or vomiting	1	3
Palpitations	-	1
Polyuria	1	-
Unpleasant feelings	1	2
Myalgia	2	-
Fever (1 day)	1	-
CRPS exacerbation	2	-
Total	19	14

CRPS = complex regional pain syndrome type I; ITG = intrathecal glycine.

were no significant abnormalities in other clinical laboratory tests or electrocardiograms.

Efficacy

There were no significant differences in any of the outcome measures, between ITG and placebo treatment (table 5.3). During ITG treatment, one patient reported improvement (PGI +2), and nine reported worsening (PGI -3 in one patient, -2 in four, and -1 in four). The CGI score showed improvement in one patient during ITG treatment (+2), and worsening in five (-3 in one, and -1 in four). During placebo treatment, two patients reported improvement (PGI +2), and four reported worsening (-2 in three, and -1 in

Table 5.3. Summary of secondary outcome events (medians (IQR) are presented)

Outcome event	Range scale ^a	Intrathecal glycine		Placebo		P value ^b
		Baseline	4 weeks	Baseline	4 weeks	
Pain (numeric rating scale)	<u>0</u> - 10	6 (4 - 8)	6 (5 - 8)	7 (5 - 8)	7 (5 - 7)	1.0
<i>McGill pain questionnaire</i>						
Number of words chosen	<u>0</u> - 20	12 (7 - 13)	13 (8 - 14)	12 (8 - 14)	12 (8 - 14)	0.20
Pain rating index	<u>0</u> - 63	21 (11 - 28)	20 (13 - 32)	22 (14 - 28)	19 (13 - 31)	0.49
<i>Thermal sensory analyser (°C)</i>						
Cold detection threshold (ΔT)	0.1 - 17.0	1.3 (0.3 - 4.7)	1.0 (0.3 - 3.8)	1.0 (0.4 - 5.6)	1.0 (0.4 - 4.2)	0.70
Heat detection threshold (ΔT)	0.1 - 18.5	1.4 (0.5 - 5.1)	1.8 (0.9 - 4.3)	2.3 (1.2 - 6.1)	2.0 (0.8 - 4.0)	0.23
Burke-Fahn-Marsden dystonia rating scale	<u>0</u> - 120	32 (14 - 44)	35 (12 - 48)	27 (12 - 41)	31 (16 - 48)	0.60
<i>Unified myoclonus rating scale</i>						
Myoclonus at rest	<u>0</u> - 128	0 (0 - 8)	0 (0 - 5)	0 (0 - 1)	0 (0 - 1)	0.35
Stimulus sensitivity	<u>0</u> - 17	0 (0 - 1)	0 (0 - 0)	0 (0 - 1)	0 (0 - 1)	0.13
Myoclonus with action	<u>0</u> - 160	10 (0 - 20)	0 (0 - 16)	3 (0 - 20)	4 (0 - 17)	0.96
Tremor research group rating scale (item 1-8)	<u>0</u> - 76	2 (0 - 6)	2 (0 - 6)	1 (0 - 6)	2 (0 - 6)	0.96
Radbound skills questionnaire (total)	1 - <u>5</u>	4 (3 - 5)	4 (3 - 4)	4 (3 - 4)	4 (3 - 4)	0.18
<i>Walking stairs questionnaire</i>						
Walking in the house	0 - <u>10</u>	9 (8 - 10)	9 (6 - 10)	9 (6 - 10)	9 (7 - 10)	0.77
Walking outside	0 - <u>10</u>	10 (7 - 10)	10 (7 - 10)	10 (9 - 10)	10 (9 - 10)	0.23
Patient's global impression	-3 to + <u>3</u>		0 (-1 to 0)		0 (0 - 0)	0.32
Clinical global impression	-3 to + <u>3</u>		0 (-2 to 0)		0 (0 - 0)	0.74

a. Best score is underlined

b. ITG at 4 weeks versus placebo at 4 weeks (Wilcoxon signed rank test)

one), the CGI showed improvement in none, and worsening in one (-1). The effect of placebo was 0% for the pain NRS and -15% for the BFM Dystonia Rating Scale.

Patient's and investigator's guesses of which treatment was administered were incorrect in 57% and 59%, respectively. There was no significant effect of sequence (ITG-placebo or placebo-ITG) on the pain NRS and BFM dystonia rating scale baseline scores.

DISCUSSION

Central disinhibition plays an important role in CRPS and enhancing the GABAergic inhibitory status through intrathecal administration of baclofen has proven beneficial in the treatment of dystonia and to a lesser extent on pain.^{21,22,46} Against this background, we were interested if similar results could be obtained by enhancing glycinergic inhibition in CRPS patients. Because no information is available on the tolerability of this mode of glycine administration in humans, this study firstly focussed on the safety of ITG and secondly evaluated its efficacy in doses up to 32 mg/24 h. No major AEs occurred in 19 CRPS patients treated with ITG over 4 weeks. The proportion of most frequently reported AEs (drowsiness, headache, dysesthesia and nausea and vomiting) were similar for ITG and placebo treatment. Compared to placebo, median GH values increased during ITG treatment (0.9 versus 2.1 mU/l). GH levels still remained in the normal range of 0-5.0 mU/l, and were smaller than the increases that are observed with intravenous GH. Notably, the absence of functional disturbances in our patients is at best only a surrogate marker for the absence of neurotoxicity of ITG.⁴⁷ Evolving deficits may not be revealed by functional indices for an extended period of time, whereas histological examination demonstrates a continuing event.⁴⁸ Consequently, our findings are insufficient to assume that ITG is not associated with neurotoxicity and further studies are required to address this issue.

Over the dose-escalation period of 4 weeks with doses up to 32 mg/24 h we did not find evidence of efficacy of ITG. Several explanations for this lack of efficacy of ITG are possible. First, it is possible that not glycinergic but GABAergic mechanisms play a key role in central disinhibition of CRPS. Second, ITG may have been administered in an

insufficient dose, although GH-increases indicate that the doses were pharmacologically active. Third, effective synaptic concentrations of glycine are regulated by glycine transporters which mediate its uptake into nerve terminals and adjacent glial cells.⁴⁹ Hence, the lack of efficacy of ITG could result from a compensatory ITG-mediated increased activity of glycine reuptake transporter mechanisms. In this case, selective inhibitors of glycine transporter could be more efficacious. Finally, however, our patient and clinician-based impression scores may hint at another explanation. Although the primary outcomes showed no difference, there was a trend on the patient and clinician-based impression scores to show deterioration during ITG. Nine patients worsened during ITG, versus four during placebo treatment according to the patient-based PGI score. In line with this, the investigator-based CGI indicated that five patients got worse during ITG, versus one patient during placebo treatment. Although, the PGI and CGI were not significantly different from zero, the trend towards a deterioration of signs and symptoms with ITG suggests that glycine may play a pathophysiological rather than a therapeutic role in CRPS. This could be caused by the dual action of glycine, which serves both as an obligatory coactivator of the spinal excitatory NMDA receptor, and as a neurotransmitter at the inhibitory strychnine-sensitive glycine receptor.^{50,51} It is possible that the excitatory effects prevailed in the applied dose range. In CRPS, chronic pain, allodynia, and hyperalgesia, are assumed to result from central sensitization, a state reflecting enhanced synaptic transmission efficiency of neurons in the dorsal horn of the spinal cord.¹¹ In central sensitisation, the NMDA receptor is upregulated, and open studies using NMDA antagonists memantine and ketamine have reported beneficial effects on pain in CRPS.⁵²⁻⁵⁴ Hence, ITGs efficacy could depend on the state of the NMDA receptor and stimulation of the excitatory glycine receptor of the upregulated NMDA receptor may potentially worsen symptoms such as pain, explaining the trend of poorer ratings of patients and physicians when patients were using ITG.⁵⁵⁻⁵⁷ Interestingly, intrathecal administration of 2-amino-5-phosphonopentanoate, an NMDA receptor antagonist, unmasked the analgesic action of glycine in rats.⁵⁸ Hence, future studies could analyze whether or not NMDA inhibition, administered prior to or

simultaneously with ITG may enhance its therapeutic potential under circumstances of central sensitisation. Because intrathecal administration of NMDA inhibitors is neurotoxic,⁵⁹ this hypothesis can only be tested with orally or intravenously administered agents.

We studied a population of severely affected CRPS patients with long disease duration. Consequently, this patient sample involved a selection of treatment refractory patients, who are not representative for all CRPS patients. However, it would be unethical to evaluate ITG in acute stage patients, in whom symptoms might resolve spontaneously or following less invasive treatment options.

There is no data on the magnitude of the placebo responses in trials evaluating chronic treatment in CRPS patients with dystonia until now. In our previous study, placebo responses to two intrathecal saline injections were 4% and 8 %, respectively.²¹ Interestingly, there was no detectable placebo response in the current study during 4 weeks of intrathecal treatment. This finding might be useful for designing future trials on this subject.

In conclusion, ITG in doses up to 32 mg/24 h during 4 weeks was not associated with serious adverse events, but further studies are required to rule out potential neurotoxicity of ITG. Although results from animal studies were promising, this study did not find efficacy on pain or dystonia in patients with chronic CRPS. Several potential explanations for this finding could be addressed in future studies.

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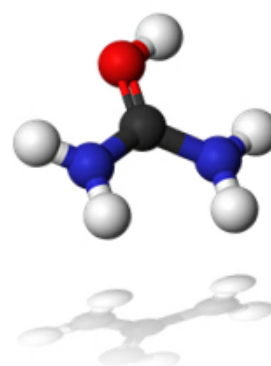
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Part III.

Exploratory Studies Using Mass Spectrometry



Chapter 6

Explorative Analysis of Urine by Capillary Electrophoresis-Mass Spectrometry in Chronic Patients with Complex Regional Pain Syndrome

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ABSTRACT

Complex Regional Pain Syndrome (CRPS) is characterized by various combinations of sensory, autonomic and motor disturbances. Pain disproportionate to the severity and duration of the inciting event is the most devastating symptom. Diagnosis of CRPS is difficult as the underlying mechanisms remain unclear. To try to derive metabolic indicators potentially characteristic for CRPS, we applied capillary electrophoresis time-of-flight mass spectrometry (CE-ToF-MS) to the explorative analysis of urine. The CE-ToF-MS method provided fast and stable metabolic profiles of urine samples. The mean intraday and interday CVs were <2% and <9% for migration times and peak areas, respectively, demonstrating robustness of the method. With the use of multivariate chemometric analysis, discrimination between urine samples from CRPS patients and controls was obtained, emphasizing differences in metabolic signatures between CRPS-diseased patients and controls. Several compounds, such as 3-methylhistidine, were responsible for discriminating the samples. The biological relevance of these compounds with regard to CRPS is discussed. Thus, CE-ToF-MS-based metabolic profiling of urine from CRPS patients and controls revealed metabolites that differentiate between diseased and control, illustrating the usefulness of this approach to get more insight into the pathology underlying CRPS.

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is a chronic pain condition characterized by a combination of sensory, vasomotor, and trophic changes. About 25% of the patients with CRPS develop movement disorders, like tremor, myoclonia and dystonia.^{1,2} The syndrome is more common in women and usually is preceded by tissue injury like a fracture or surgery. Following tissue injury, the body may respond with a series of specific reactions aiming to repair the damage, promote wound healing and recruit host defence mechanisms. Indeed, there is some evidence to suggest that multiple biological mechanisms responsible for inflammation, vasomotor dysfunction and pain may become involved in CRPS.³⁻¹⁰ Collectively, these facts indicate that in the acute phase of CRPS the body response to trauma may turn aberrant. Still, in a substantial number of patients the disease course becomes chronic. In the majority of the patients, CRPS remains restricted to one extremity; however the syndrome may spread to other extremities without a new trauma.¹¹⁻¹³ These more affected chronic phenotypes have a much younger age at onset, which might suggest a genetic predisposition.¹³ Mechanisms that play a role in this phase of the syndrome are largely unknown, and even if such mechanisms are suggested, they are seldom based on solid biochemical data. Consequently, explorative “omics” based approaches, such as metabolomics, could be a good starting point for further delineation of mechanisms underlying CRPS. At first glance, metabolic profiling is quite similar to other “omics” approaches, but unlike genomics and proteomics which often only indicate a potential for physiological (pathophysiological) effects, metabolomics data provide insight into the end points of biochemical activities of the organism.¹⁴⁻¹⁶ The output of a metabolomic experiment could either be treated as “a metabolic signature” or “phenotype” and used for building up diagnostic routine, or extrapolated to other “omics” data in order to reveal systemic mechanisms of pathology. Metabolic profiling of body fluids has a broad analytical basis: nuclear magnetic resonance spectroscopy (NMR), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS).¹⁷ This diversity of analytical methods, in a

way, reflects the diversity of chemico-physical properties of compounds composing the human metabolome. At the end, the final selection of a method depends entirely on the type of sample and the “target” group of metabolites. For example, to access the inflammatory axis of CRPS, one might consider urine as a sample of choice. The urinary metabolome consists mainly of polar and ionic compounds;¹⁸ and therefore, CE is a very attractive separation technique as it provides a high-resolution separation for highly polar and ionic compounds.^{19,20}

Recently, we have demonstrated the analytical quality and feasibility of a CE-MS method for the profiling of amino acids and related compounds in urine from patients with urinary tract infection.²¹ In the present study, we show that this CE-MS method can be used for metabolic profiling of human urine and we have applied this method for the first time to the explorative analysis of urine samples to investigate changes in metabolite patterns between patients with CRPS and control subjects.

MATERIALS AND METHODS

Chemicals

PB (hexadimethrine bromide) and a 25% (m/v) aqueous solution of poly(vinyl sulfonate) (PVS) sodium salt were from Sigma-Aldrich (Steinheim, Germany). A 10% (m/v) PB solution was prepared by diluting PB with Milli-Q water. A 5% PVS solution was made by diluting the purchased PVS solution with Milli-Q water. The polymer solutions were passed through a 0.22 µm filter and stored at 4°C. Formic acid was from Riedel de Haën (Seelze, Germany). Ammonium hydroxide 25% was from Merck (Darmstadt, Germany). All L-Amino acids with a concentration of 1 mM of each in 0.1 M hydrochloric acid were purchased from Sigma-Aldrich. HPLC-grade methanol was supplied by Biosolve BV (Valkenswaard, the Netherlands). Hippuric acid, 3-methylhistidine, xanthine, guanosine, L-carnitine, phenylalanine-glycine, N-methylnicotinamide were purchased from Sigma-Aldrich. Standard solutions were prepared with water taken from a Milli-Q water purification system or with background electrolyte (BGE) (Millipore, Bedford, MA, USA). BGE solution was prepared by dissolving formic acid in Milli-Q water (1 M, pH 1.8).

Patients and urine samples: subjects and CRPS diagnosis

Thirty-four consecutive patients (table 6.1) from the department of Neurology of the Leiden University Medical Center fulfilled the CRPS criteria of the International Association for the Study of Pain (IASP). Thirty-one patients also had developed fixed dystonia of one or more extremities. Other causes of dystonia had been excluded using appropriate blood and imaging studies (computed tomography or magnetic resonance imaging) of the brain. All patients were also evaluated concerning the interval that had

Table 6.1. Demographic, CRPS characteristics and treatment (n=36).

Demographic and injury		CRPS characteristics		Previous treatment		Current treatment	
<i>Age</i> , median (range)		Spontaneous pain	36	<i>For CRPS</i>		<i>For CRPS</i>	
41 (17 – 60) ^a		Mechanical allodynia	33	NSAIDs ^f	15	NSAID's	10
<i>Gender</i> ^b		Color changes	30	Opioids/tramadol	19	Opioids/tramadol	19
Male	6	Edema	29	Antidepressants	21	Antidepressants	14
Female	30	Hyperhidrosis	20	Anticonvulsants	16	Anticonvulsants	10
<i>Affected limb</i>		Temperature changes	31	Baclofen	22	Baclofen	14
Upper	30	Motor changes ^d	34	Benzodiazepines	17	Benzodiazepines	11
Lower	26	1 limb	13	Epidural/blocks	6	P.T/O.T	26
<i>Type of injury</i>		2 limbs	7	Topical agents	5	TENS ^h	1
Contusion	15	3 limbs	5	P.T/O.T ^g	27	SCS ⁱ	1
Fracture	8	4 limbs	9	Acupuncture	1	Scavengers	4
Surgery	7	Trophic changes	26	TENS ^h	6	<i>For other conditions</i>	
Other	6	<i>NRS pain</i> , ^e		SCS ⁱ	2		
<i>Symptoms duration</i> , median (range):		median (range)		Ketamine i.v.	3	Antihypertensives	9
72 (1 – 340) ^c		7.5 (5 – 10)		Mannitol i.v.	8	Other	29
				Scavengers	8		
				<i>For other conditions</i>			
				Antihypertensives	10		
				Other	31		

a. Median (range) age of controls = 27 (21 – 41) years

b. Gender of all controls was female

c. Duration in months

d. Dystonia was present in 31 patients (86%)

e. Numeric rating scale for pain

f. Non-steroidal anti-inflammatory drugs

g. Physiotherapy (P.T) and Occupational therapy (O.T)

h. Transcutaneous electrical nerve stimulation

i. Spinal cord stimulation

elapsed between onset of CRPS symptoms and time of sampling, medication used at the time of sampling, and previous therapy. The pain intensity was evaluated using a numeric rating scale. Patients with renal or bladder pathology were ruled out in order to limit confounding conditions. All patients were examined at our outpatient clinic for movement disorders. None of the patients had a family history of dystonia and other causes of dystonia, including birth injury, head trauma, neuroleptic treatments were not reported or identified in the medical history or correspondence. Laboratory tests, including serum copper and ceruloplasmin and computed tomography or magnetic resonance imaging of the brain were normal. Control subjects were recruited from volunteers working in our hospital and selected to match the overall age and sex distribution of the patients, and had no history of disease. Only females, who reported no significant medical history and used no medication, were included to optimize the homogeneity of the group. This comprised the number of control subjects, who were eligible for participating in the study.

Patients and urine samples: urine collection and analysis

All samples were collected early in the morning between 4.00 and 7.00 a.m. to minimize potential confounding influences of circadian rhythms of constituents excreted in the urine. Patients used standard 50 mL non-sterile containers to store urine temporarily. The same day patients visited the hospital and within 10 h after micturition (act of urinating), samples were transferred to nonsterile polypropylene micro tubes of 0.5 mL and then stored at -80°C. A total of 100 µL of each urine sample from controls and patients were pooled and used as quality control (QC) samples. Prior to CE-MS analysis, the urine samples were thawed to room temperature, mixed with BGE (1:1 v/v) and centrifuged (13200 rpm) for 5 min. The sample sequence started with five QC samples spiked with amino acids at different concentration levels for the measurement of a calibration plot, which was followed by a nonspiked urine sample. Next, urine samples from 18 control subjects and 36 patients were analyzed in an alternating way. This

sample sequence was randomized with QC samples to evaluate the performance of the CE-MS method during the analysis of the sequence.

Instrumentation and procedures

The CE experiments were carried out on a Beckman Coulter PA 800 instrument (Beckman Coulter, Fullerton, CA). Fused-silica capillaries were from Composite Metal Services (The Chase, Hallow, UK) with a total length of 130 cm and an internal diameter of 50 μm . Capillaries were noncovalently coated with a bilayer of Polybrene (PB) and poly(vinyl sulfonate) (PVS). Formic acid (1 M, pH 1.8) was used as BGE. Sample injections were performed hydrodynamically for 90 s at 90 mbar (1.3 psi). Prior to sample injection, a small plug (50 mbar, 9 s) of 12.5% ammonium hydroxide was injected for pH-mediated stacking. Electrophoretic separation was performed at +30 kV and the capillary temperature was set at 25 °C. For more experimental details we refer to ref 21. MS was performed using a micrOTOF orthogonal-accelerated time-of-flight (TOF) mass spectrometer (Bruker Daltonics, Bremen, Germany). Spectra were collected with a time resolution of 1 s. Postrun internal mass calibration was performed using sodium formate cluster ions $\text{Na}+(\text{HCOONa})_{1-9}$ ranging from 90.9766 to 430.9137 m/z . CE-MS coupling was realized by a coaxial sheath liquid interface (Agilent Technologies, Waldbronn, Germany) with methanol-water-formic acid (50:50:0.1, v/v/v) as sheath liquid. The following spray conditions were used: ESI voltage was -4.5 kV; sheath liquid flow, 4 $\mu\text{L}/\text{min}$; dry gas temperature, 180°C; nitrogen flow, 4 L/min; nebulizer pressure, 0.5 bar. Micellar electrokinetic chromatography (MEKC) combined with UV detection was used for the determination of 3-methylhistidine in urine samples. The employed experimental procedure has been described in detail elsewhere.²²

Analytical validation

The procedures for the determination of linearity of response for amino acids in BGE and in QC samples were described in a previous paper.²¹ The limit of detection (LOD) for amino acids in BGE and QC samples was calculated as the concentration yielding a S/N

of 3 via extrapolation of the S/N-ratio produced by the lowest concentration used for the construction of calibration plots of amino acids in BGE and QC samples, respectively. Intra- and interday variation of migration time and peak area were determined for 12 endogenous urinary metabolites (L-arginine, L-valine, L-glutamic acid, L-phenylalanine, L-histidine, hippuric acid, N-methylnicotinamide, creatine, xanthine, L-carnitine, guanosine and phenylalanine-glycine) with different physicochemical properties. The intraday variation for migration time and peak area was determined by analyzing ten replicates of a pooled urine QC sample. The interday variation was determined by analyzing a QC sample on 5 different days. Recovery was evaluated in a previous study.²¹

Data analysis

The raw data files were converted to netCDF format. For nonlinear alignment of the data and feature detection of the data, we used an open source XCMS software.²³ CE-MS data were processed using default settings (see documentation for XCMS, <http://metlin.scripps.edu/download/>) except for parameters: “steps” of xcmsSet method set to 3, and “bw” of group method set to 20. The normalization of the data was performed using nonparametric algorithm.²⁴ The table of ion features and normalized intensities was imported into SIMCA-P+ (version 12.0; Umetrics) software for further multivariate analysis. Principal component analysis (PCA) and partial least-squares - discriminant analysis (PLS-DA) were performed both on original data and on data after orthogonal signal correction (OSC). PLS-DA models were constructed after removal of extreme outliers and validated using permutation test with 100 permutations. To identify metabolites of interest, rational chemical formulas were generated based on internally calibrated monoisotopic masses within 10 mDa mass error, using the *SmartFormula* tool within the *DataAnalysis* (DA) software package (Bruker Daltonics). The chemically-reasonable formulas were submitted to metabolome databases: Kyoto Encyclopedia of Genes and Genomes (KEGG) ligand database,²⁵ the Human Metabolome Database (HMDB),²⁶ and the METLIN database.²⁷ The isotopic

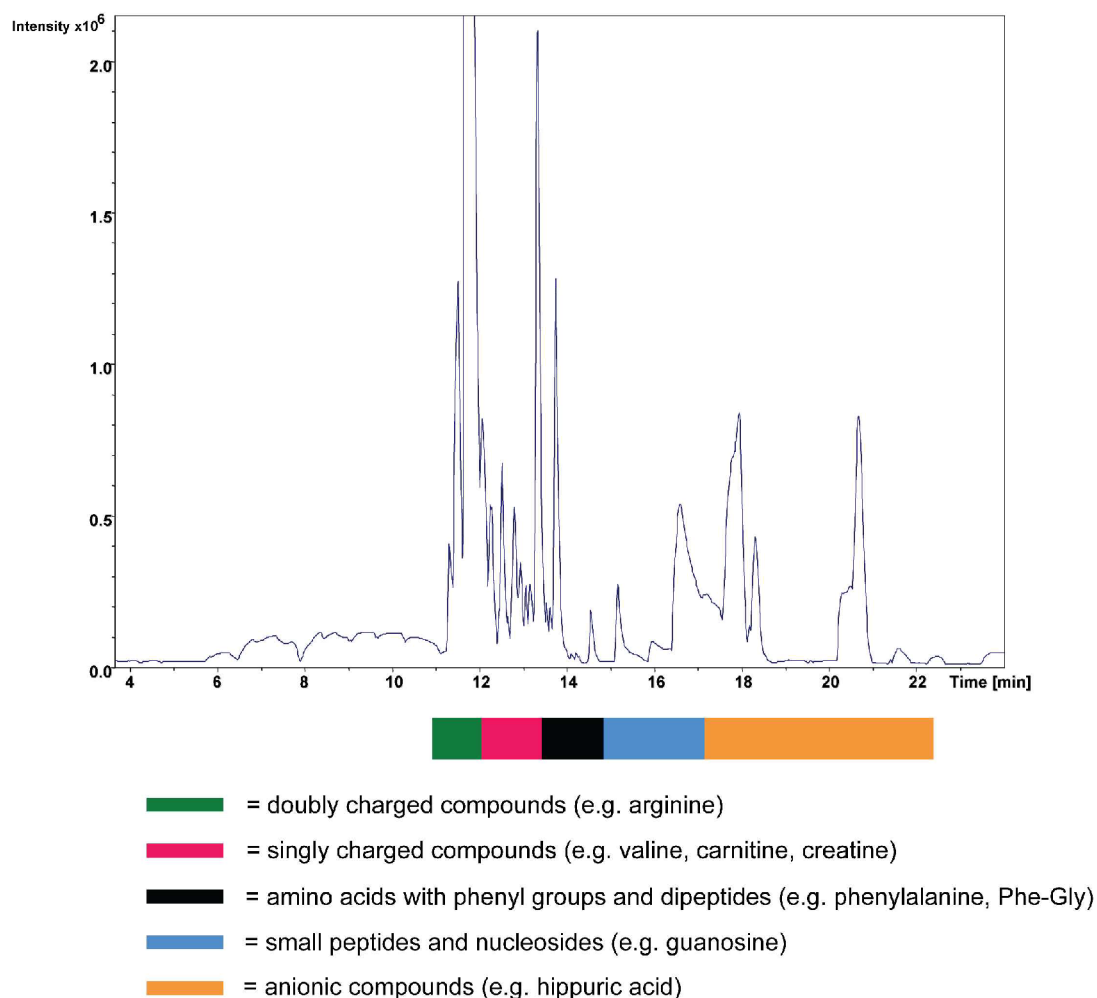
distribution patterns of the matched metabolite candidates were simulated with the *Simulate Pattern* tool of DA and compared with observed mass spectra to reduce further the number of potential elemental compositions.^{28,29}

RESULTS

Evaluation of CE-ToF-MS method for metabolic profiling

Recently, we have developed a CE-ToF-MS method for the analysis of amino acids in human urine using capillaries noncovalently coated with a bilayer of Polybrene (PB) and poly(vinyl sulfonate) (PVS).^{20,21} The PB-PVS coating proved to be very reproducible yielding stable CE-MS patterns of amino acids in human urine with favorable migration time repeatability (RSDs <2%). The CE-ToF-MS method showed linear responses between 0.5 and 250 μ M for most amino acids ($R^2 > 0.99$), indicating a good dynamic range. Here, we demonstrate that our method can be used for the profiling of ionogenic compounds with electrophoretic properties similar to amino acids (e.g. amines, small peptides and related metabolites). In CE, all cationic compounds migrate toward the cathode (in our set-up towards the MS). Therefore, in principle all cationic compounds can be analyzed using the PB-PVS CE-MS configuration. To analyze all cations simultaneously, a very low pH electrolyte, 1 M formic acid (pH 1.8), was used to confer a positive charge on all cationic metabolites, making them amenable to MS analysis. Figure 6.1 shows a typical total ion electropherogram of a urinary profile from a control subject obtained by CE-ToF-MS analysis. A metabolic profile of positively charged compounds in the m/z region from 50 to 450 was obtained in less than 18 min. Approximately 300 compounds were detected within a total separation time of 24 min. (using an injection volume of ca. 100 nL). The legend below figure 6.1 shows the type of compounds detected in the given migration time intervals (indicated by a different colour). At the low pH conditions used for separation, all compounds with a basic functionality are positively charged, allowing their migration toward the MS detector. Compounds with a higher number of amine groups in their structure, such as lysine or

Figure 6.1. Total ion electropherogram of a urine sample from a control subject obtained by CE-ToF-MS analysis. The legend below the electropherogram shows the type of compounds migrating in the specified colored time regions.



arginine, migrated first (10-11 min). Next a group of metabolites comprising amino acids (e.g., valine, leucine, threonine, proline), vitamins (e.g. carnitine) and amines (e.g., creatine and N-methylnicotinamide) migrated together with small peptides like phenylalanine-glycine (12-14 min). Subsequently, the slower migrating amino acids comprising phenyl moieties (such as phenylalanine and tyrosine) migrated and then nucleosides like guanosine (14-17 min). Acidic metabolites, such as hippuric acid, are slightly anionic at pH 1.8 and, therefore, migrate just after the EOF. Hence, the CE-ToF-

MS method is capable of separating different classes of metabolites simultaneously in human urine, as required for metabolic profiling.

The analytical quality of the data derived from the CE-ToF-MS analysis of QC samples was evaluated. The intra- and interday variation of migration time and peak area were determined for a number of endogenous urinary metabolites with different physicochemical properties (supplemental table 6.1) and are stated in table 6.2. The intraday and interday CVs were <2% and <9% for migration times and peak areas, respectively, demonstrating robustness of the method. Analytical recovery was determined in a previous study.²¹ In brief, five QC samples before and after addition of known metabolite concentrations (5, 25 and 100 μ M) were analyzed. Recoveries ranged from 75% to 99% and the CVs were 7%-11% for all metabolites. The LODs for amino acids in human urine varied from 85 to 280 nM (S/N = 3).

We also evaluated the performance of the MS detector with respect to mass accuracy. For this, the m/z value of histidine was determined in 10 urine samples of control

Table 6.2. Precision data (CV, %) for migration time and peak area of endogenous compounds in human urine analyzed by CE-ToF-MS.

Classification	Compound name	Intraday (n=10)		Interday (n=5)	
		Migration time	Peak area	Migration time	Peak area
Amino acid	L-Arginine	1.5	5.9	1.4	6.4
	L-Valine	1.1	8.9	1.2	8.5
	L-Glutamic acid	1.2	7.9	1.2	8.2
	L-Phenylalanine	0.8	7.9	1.0	7.5
	L-Histidine	1.2	6.8	1.2	7.3
Organic acid	Hippuric acid	1.1	6.5	1.3	6.9
Amine	Creatine	0.9	8.6	1.1	7.8
Pyridine base	N-Methylnicotinamide	1.1	5.5	1.0	7.0
Purine	Xanthine	1.2	7.0	1.2	7.6
Vitamin	L-Carnitine	1.2	7.7	1.2	7.8
Nucleoside	Guanosine	1.3	6.4	1.2	7.3
Small peptide	Phe-Gly	1.4	7.6	1.5	7.5

subjects. The observed mass deviation from the theoretical m/z value of histidine was less than 2 mDa (supplemental figure 6.1), indicating a stable performance of the ToF-MS instrument.

Metabolic profiling and multivariate statistics

We have used the CE-ToF-MS method for the metabolic profiling of urine samples from 36 patients with CRPS and from 18 control subjects (for the analytical workflow, see supplemental figure 6.2). Data sets were subjected to a preprocessing routine – migration time alignment and normalization – and then, to an explorative analysis using a set of chemometrical methods. The PCA scores plot revealed only weak tendency for grouping of control samples and patient samples. At the same time, there were no indications of grouping based on acquisition time, sampling time or gender of subjects (supplemental figure 6.3). To enhance class separating variance and at the same time to get an overview of metabolites contributing to it, we used a supervised data analysis method: PLS-DA. As data were acquired in two large acquisition blocks over 2 days, we split our data in two parts: data acquired on day one (18 patients and 9 controls) was used as a training set (to build a model) and data acquired on day two (18 patients and 9 controls) was used as a prediction set (to test the model). The PLS-DA score plot demonstrates an obvious visual clustering based on a two-class model (figure 6.2). The quality of the PLS-DA model was verified by a correlation coefficient R^2 (goodness to fit) and a cross-validated correlation coefficient Q^2 (goodness of prediction), respectively. A good prediction model is achieved when $Q^2 > 0.5$. The PLS-DA model showed a R^2 of 0.96 and a Q^2 of 0.855 indicating a good fitting and prediction ability. Then, we used data acquired on the second day (second acquisition block) to test the predictive power of our two-class model. Figure 6.3 shows that a majority of the samples were predicted correctly. Thus, the CE-ToF-MS method is capable of dissecting the metabolic signatures discriminating cases and controls. The strongest identified loadings from our model are listed in table 6.3. The identification of compounds was based on accurate mass measurements, analysis of isotopic profile and migration time information. The

Figure 6.2. Partial least squares-discriminant analysis (PLS-DA) model based on CE-ToF-MS data of urine from control subjects and patients with CRPS. (A), PLS-DA model based on CE-ToF-MS data of urine samples measured on day 1; R^2 of 0.96 and a Q^2 of 0.55. (B), Validation of the model using 100 permutations.

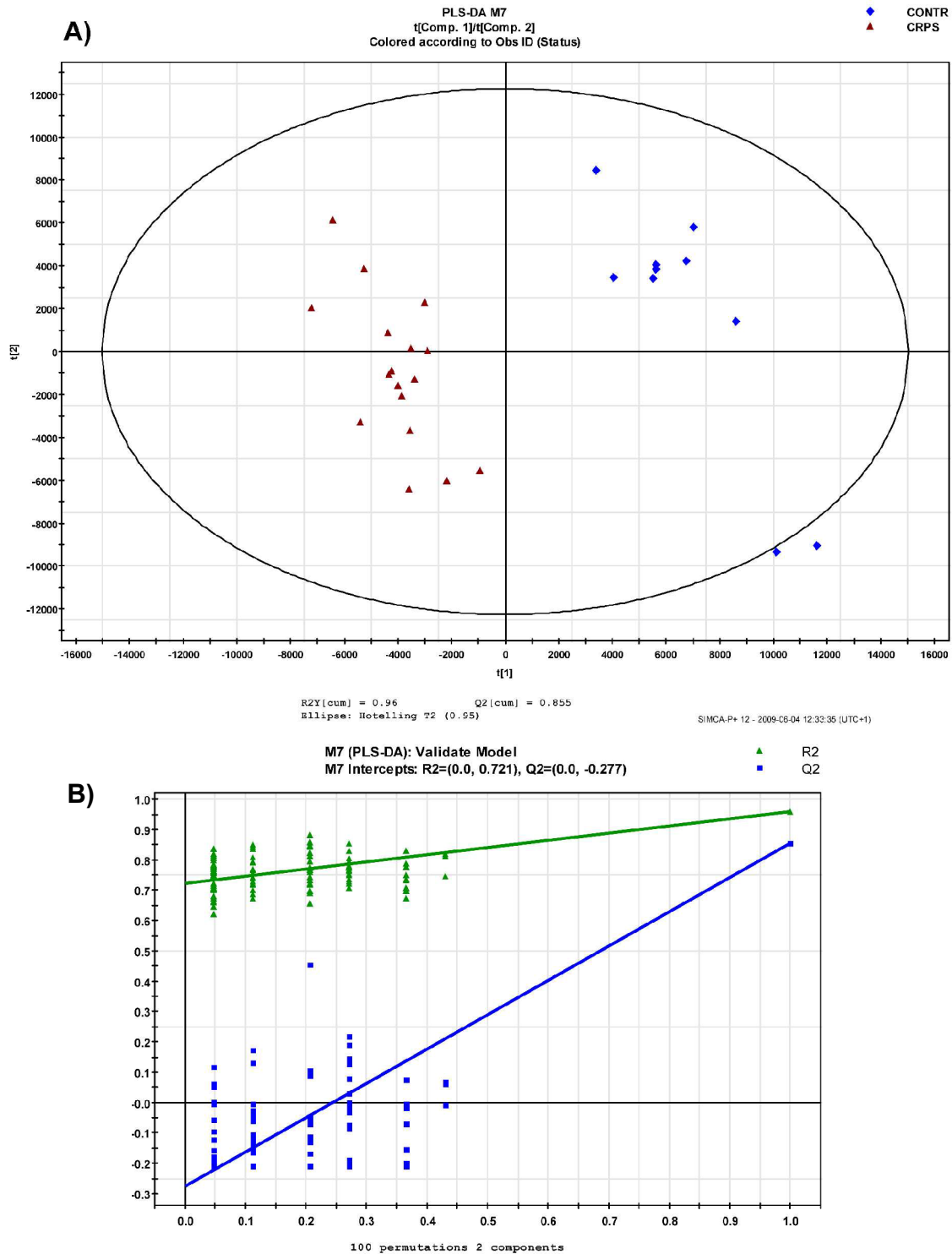
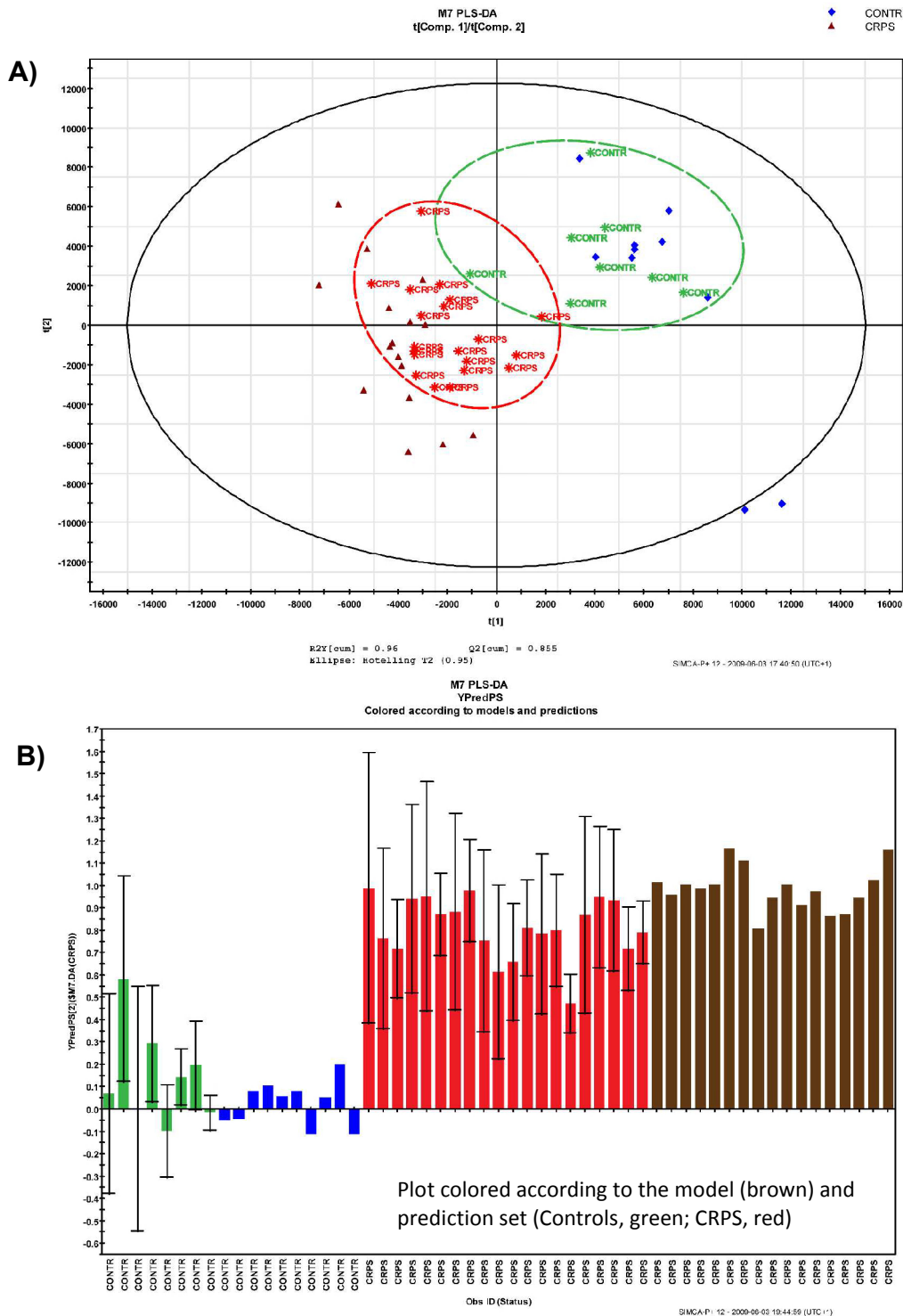


Figure 6.3. Predictive ability of PLS-DA model based on urine samples measured on day 1. (A), PLS-DA scatter plot showing the prediction of urine samples from control subjects and patients with CRPS measured on day 2; (B), Column plot displaying the predicted response value for all responses from the last component.



molecular formula obtained was subsequently introduced in, for example, The Human Metabolome Database and METLIN database to obtain the identification of the metabolites.

Table 6.3. Summary of metabolites identified that showed most significant differences between the two sample sets.

Name	Molecular formula	<i>m/z</i> observed	<i>m/z</i> calculated	Error (mDa)	Increased/decreased ^a
3-Methylhistidine	C7H11N3O2	170.09185	170.09240	0.6	↑
2-Aminobenzoic acid	C7H7NO2	138.05429	138.05495	0.7	↑
3-Amino-octanoic acid	C8H17NO2	160.13341	160.13321	-0.2	↑
Hippuric acid	C9H9NO3	180.06658	180.06552	-1.1	↓
Histidine	C6H9N3O2	156.07529	156.07675	1.5	↓
Proline betaine	C7H13NO2	144.10248	144.10191	-0.6	↓
Creatine	C4H9N3O2	132.07590	132.07675	0.9	↓

a. Concentration increased or decreased compared to controls

DISCUSSION

Hitherto, the diagnosis of CRPS is fully based on clinical symptoms and signs and potential mechanisms of disease have only partly been elucidated. This situation reflects most likely the complex origin of CRPS and the fact that, so far, hypothesis-driven strategies have provided a limited contribution to our understanding of the syndrome. Here, we present an attempt to use an alternative approach, namely, explorative analysis of the metabolome of urine, a body fluid which so far never has been analyzed systematically in CRPS. The urinary metabolome consists mainly of polar and ionic compounds and the choice of CE as a separation technique is, therefore, a logical one as in CE compounds are separated on the basis of their charge-to-size ratio. CE is especially suited for the high-resolution separation of highly polar and charged compounds. Other important advantages of CE are cost efficiency (fused-silica capillaries instead of expensive LC columns and low consumption of chemicals) and little or no need for

sample preparation. The only drawback of CE is its limited concentration sensitivity due to the nanoliter-scale sample volume loaded into the capillary. The sensitivity, however, can be improved by the use of in-capillary pre-concentration techniques, such as pH-mediated stacking. The preconcentration technique used here is based on the injection of a short plug of alkaline solution prior to a long hydrodynamic injection of an acidified urine sample. With this approach, basic and zwitter-ionic compounds such as amino acids were preconcentrated within the capillary resulting in detection limits at the low nanomolar range. This approach also exemplifies the compatibility of pH-mediated stacking for the direct analysis of complex samples while minimizing off-line sample handling.

Advantages of using a mass spectrometer as a detector, and especially the favorable characteristics of a ToF mass analyzer have been discussed in literature before.³⁰ Moreover, we have recently shown the applicability of our CE-ToF-MS method for urine analysis. In our previous publication,²¹ we have put main emphasis on the analysis of amino acids. Here, we show that our method covers a range of classes of metabolites: amino acids, small peptides, nucleosides and even organic acids (figure 6.1, supplemental table 6.1). Some organic acids, such as hippuric acid, for example, can be (partly) negatively charged at pH 1.8, and although behaving as an anion during the CE separation, they can be detected as protonated molecules in positive ion mode MS. To sum up, all small compounds carrying a positive charge at pH 1.8 can be analyzed within 17.5 min (before electro-osmotic flow) with this method. Although the CE-ToF-MS method is able to simultaneously analyze charged, low-molecular weight compounds comprising various metabolite classes, it is not effective for the separation of neutral compounds and large biomolecules such as fatty acids, sugars and steroids and larger peptides. Therefore, the analysis of urine samples of CRPS patients and controls by UPLC-ToF-MS and GC-ToF-MS methods has the potential to greatly expand the coverage of the urinary metabolome, thereby increasing the chance of finding more metabolic indicators potentially related to CRPS.

The CE-ToF-MS method has been used for metabolic profiling of urine, and so far, such an approach has not been carried out in CRPS. We have shown that metabolic profiles of urine of CRPS diseased-patients show a composition which is substantially different (table 6.3). Considering the relatively small group size and the potential heterogeneity among subjects, it is not surprising that differences between patients and controls are not reflected in the main axis of a variance represented by PCA plot. Hence, a supervised method of analysis is more obvious choice. Indeed, the PLS-DA model not only revealed class dependent variance, but predicted almost correctly an external hold-out set. Another important output of PLS-DA is the possibility to estimate and rank the influence of individual features on our model with VIP (variable influence on the projection). In theory all features above threshold ($\alpha \geq 1$) are considered to be significant for the given model, but in practice, the threshold depends on the size of the data set. A limited number of samples may result in overfitting of the model, and therefore, only molecules with $\alpha \geq 3$ and standard deviation significantly lower than the ranking factor were selected for molecule assignment and identification. Structural assignment of molecules, based on mass position, isotopic pattern, migration time and extensive use of open source metabolome databases (METLIN, KEGG, HMDB), provided a realistic chemical identity of all candidates. However, if for compounds like histidine, hippuric acid and creatine this assignment hardly could be questioned, for n-amino-octanoic acid and proline betaine it remains the best guess possible of the available data. Methylhistidine also belongs to a special case. Although the calculated elementary composition and migration time both point unequivocally to a methylated form of histidine, it is impossible to distinguish between 1- or 3-methylhistidine. As correct assignment of methylhistidine isomer was essential for later data interpretation (see below), we used a MEKC method to confirm that the isomer was indeed 3-methylhistidine (supplemental figure 6.4).

The power of metabolomics lies not in the ability to build allusive pathways starting from one or few detected metabolites, but in its multiplex characterization.²⁷ This is underscored in this study – each single metabolite by itself failed to show a significant

difference between both groups, whereas taken together, a distinct multivariate metabolic signature of CRPS emerged. Although our approach revealed a rather solid model, the interpretation of its biological significance remains a challenging task. The patient group, as presented in table 6.1, shows a great heterogeneity. Nevertheless, no abundant drug metabolites have appeared as a significant classifier in our models. All metabolites that contributed significantly to a class separation in our model belong to the category of so-called “usual suspects”, in other words reflect metabolites which are frequently reported as discriminators in clinical and toxicological studies.³¹ Thus, at the current stage, we have not made an attempt to allocate them into pathways and to build around those pathways a mechanistic hypothesis. For example, it is impossible to explain whether a positive correlation of aminobenzoic acid results from changes in the tryptophan metabolism or whether it reflects a change of gut microflora. The same is true for the interpretation of changes of hippuric acid and proline betaine concentration. However, our analysis did reveal a conspicuous increased urine clearance of 3-methylhistidine in CRPS patients. 3-Methylhistidine is formed as a result of post-translational methylation of actin and myosin. This amino acid is not reused in protein synthesis; thus, its elevated clearance in urine is a rather specific indication of catabolism of muscle tissue, a finding which has been associated with conditions like chronic physical stress, starvation, injury of muscle tissue, primary muscle diseases and malignancy.³² Since many of the latter causes of muscle catabolism have been excluded, this finding may indicate that in chronic CRPS factors like physical stress and dietary intake need to be further explored. The shift in the muscle tissue homeostasis may also be triggered by pro-inflammatory cytokines, which are assumed to play a role in the pathogenesis of CRPS.³³ However, further research is necessary to clarify the contribution of muscle protein catabolism and influence of inflammatory processes.

Conclusion

In this work, it is shown that CE-ToF-MS is a very attractive method for metabolic profiling of human urine, especially applicable in the case of high risk explorative

studies, which have to be performed with minimal costs and consumption of patient material. With this method, we identified a urinary metabolomic signature of chronic CRPS patients. Although our findings have to be validated in a further study with larger numbers of patients and age- and sex-matched controls, the results of this study unexpectedly highlight a conspicuous increased muscle catabolism. Since this condition may further compromise the physical health state of patients, this explorative study identified a potentially important issue for future research in CRPS.

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SUPPLEMENTAL TABLE AND FIGURES

Supplemental table 6.1. Names and physico-chemical properties of the test mixture metabolites.

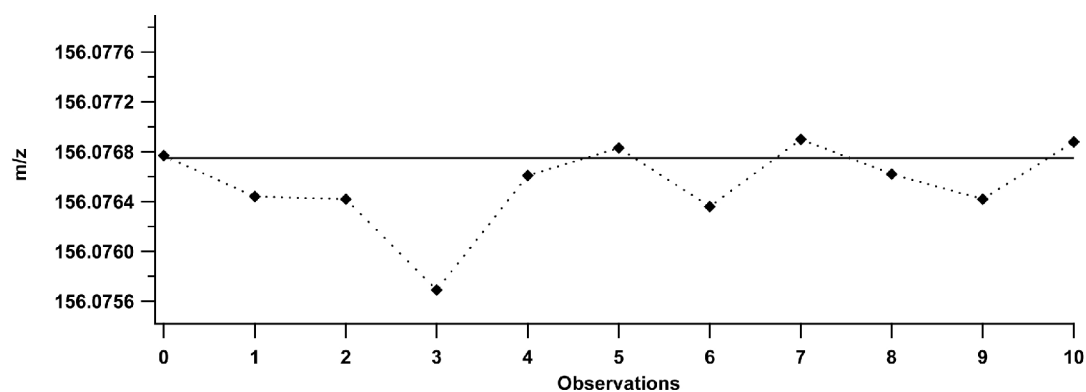
Classification	Compound name	MW ¹	pKa ² acid	pKa ² base	Log P ³
Amino acid	L-Arginine	174.11168	2.01	9.04	-3.86
	L-Valine	117.07898	2.29	9.74	-2.28
	L-Glutamic acid	147.05316	2.10	9.47	-3.54
	L-Phenylalanine	165.07898	2.58	9.24	-1.35
	L-Histidine	155.0695	2.30	9.60	-2.66
Organic acid	Hippuric acid	179.05824	3.60	-	0.24
Amine	Creatine	131.06947	2.67	11.02	-1.58
	N-Methylnicotinamide	136.06366	3.50	-	-0.21
Purine	Xanthine	150.01778	0.80	7.44	-1.04
Vitamin	L-Carnitine	161.10519	3.80	-	-3.90
Nucleoside	Guanosine	283.09167	1.60	9.21	-2.06
Small peptide	Phe-Gly	222.1005	2.34	9.09	0.03

1. Monoisotopic mass

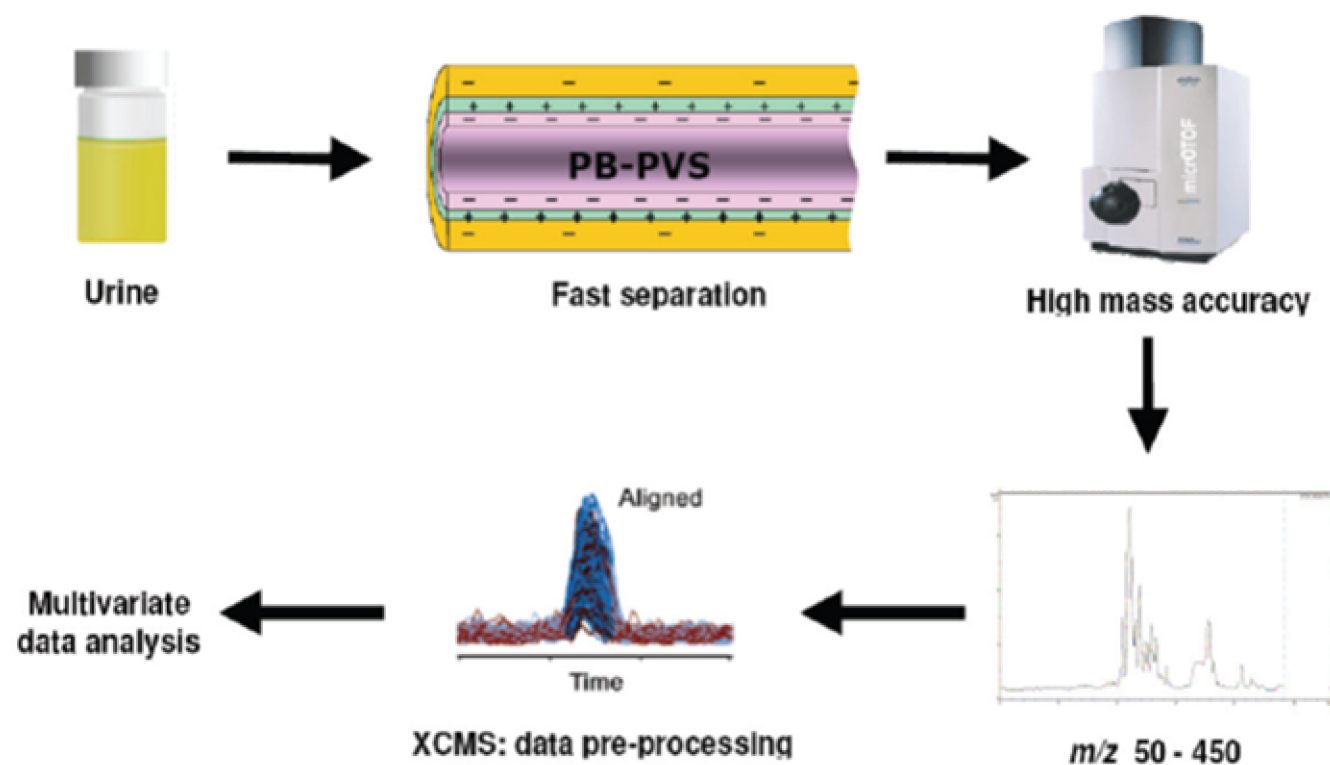
2. Data taken from CRC Handbook of Chemistry & Physics 89th Edition

3. Predicted by ALOGPS software

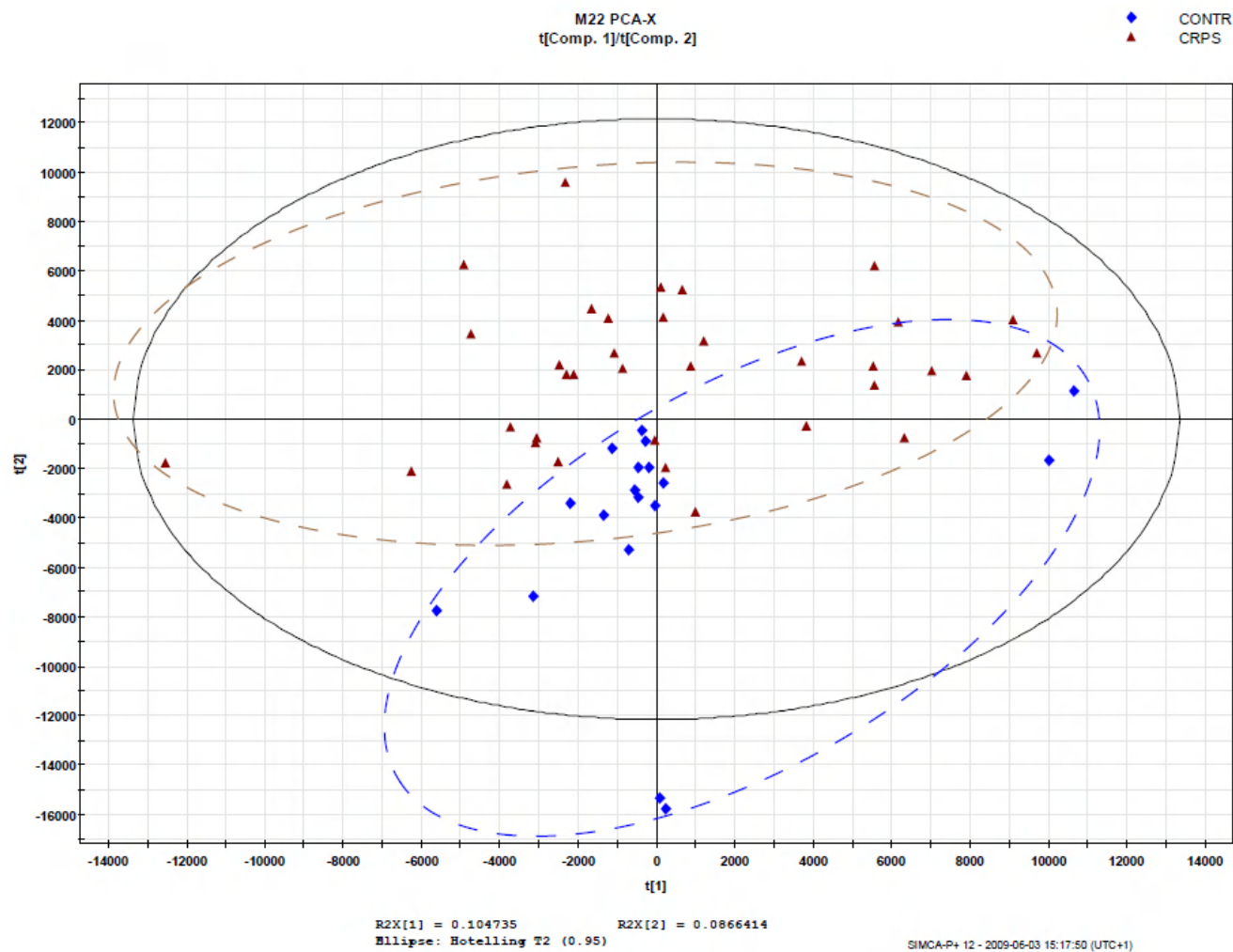
Supplemental figure 6.1. Instrument performance with respect to mass accuracy. The observed mass deviation from the theoretical m/z value of histidine less than 2 mDa over 10 consecutive runs of urine samples.



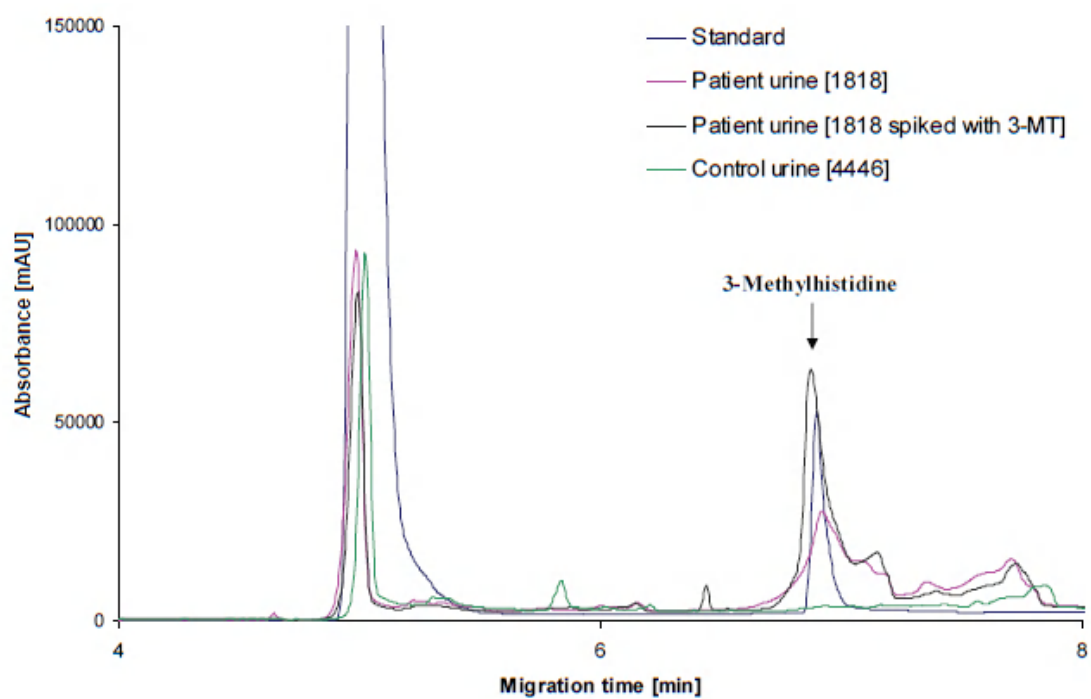
Supplemental figure 6.2. Analytical work flow used for metabolic profiling of urine samples.



Supplemental figure 6.3. PCA score plot colored according to observation ID; Unsupervised model shows no strong clustering of patients and controls for the first two principal components. Total variance covered by the PC1 and PC2 is about 10% and 9% respectively.



Supplemental figure 6.4. MEKC-UV electropherograms of 3-methylhistidine (3-MT). Four overlaid electropherograms are shown: (1) 3-MT dissolved in water (standard), (2) patient urine, (3) patient urine spiked with 3-MT at a concentration of 100 μ M, and (4) control urine.



Chapter 7

¹H-NMR Metabolic Profiling of Cerebrospinal Fluid in Patients with Complex Regional Pain Syndrome

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Pain

(accepted for publication)

ABSTRACT

Background. In Complex Regional Pain Syndrome (CRPS) related dystonia, compelling evidence points at involvement of the central nervous system, but the underpinning pathobiology is still unclear. Thus, to enable a hypothesis free, unbiased view of the problem and to get new insight into the pathobiology of dystonia in CRPS we have applied an exploratory metabolomics analysis of cerebrospinal fluid (CSF) of patients with CRPS related dystonia.

Methods and Findings. ¹H-NMR spectroscopy in combination with multivariate modeling were employed to investigate metabolic profiles of a total of 105 CSF samples collected from patients with CRPS related dystonia and controls. We found a significantly different metabolic profile of CSF in CRPS patients as compared to controls. The differences are reflected already in the first two principle components of the PCA model which is an indication that the variance associated with CRPS is stronger than variance caused by such classical confounders as gender, age or individual differences. A supervised analysis generated a strong model pinpointing the most important metabolites that contributed to the metabolic signature of patients with CRPS related dystonia. From the set of identified discriminators, the most relevant metabolites were 2-keto-isovalerate, glucose, glutamine, lactate, which all showed increased concentrations and urea which showed a decreased concentration in CRPS subjects.

Conclusion. Our findings point at a catabolic state in chronic CRPS patients with dystonia which likely is associated with inflammation.

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is a multifactorial disorder which is associated with an aberrant host response to tissue injury of a limb and predominantly affects women.²⁸ The clinical spectrum is heterogeneous and includes chronic pain, autonomic and trophic changes of the affected limb which reflect the perturbed regulation of biological pathways that underlie aberrant inflammation, vasomotor dysfunction, and maladaptive neuroplasticity.²⁸ Approximately 25% of the patients with CRPS develop dystonia which is characterized by sustained contractions of distal flexor muscles resulting in an abnormal posture.⁴⁶ Dystonia in CRPS may spread to other limbs and has a poor prognosis.^{17,50} Although compelling evidence suggests that abnormal motor function in CRPS is associated with changes within the central nervous system,^{27,31,49} the pathobiology underlying dystonia in CRPS is still unclear. Given its location in the body, cerebral spinal fluid (CSF) is by definition the body fluid that should best reflect the functional status of the central nervous system (CNS) and as such may provide an excellent medium for the search of the markers related to the pathobiology of dystonia in CRPS. Three previous hypothesis driven studies in CRPS patients with dystonia explored the role of CSF cytokines but found inconsistent results.^{3,4,34} Consequently, exploratory, hypothesis generating approaches, such as metabolomics, could be a good alternative for further delineation of biological pathways involved in dystonia in patients with CRPS. Metabolomics refers to a study of the metabolome: the “complete” set of the metabolites (the end products and intermediates of the metabolism) to be found within a biological sample.^{36,37,39} As the metabolome provides the most direct reflection of a physiological status of an organism, metabolomics is increasingly integrated in clinical studies as an essential functional readout. ¹H-NMR spectroscopy is the commonly accepted “golden standard” of the modern metabolomics.²⁶ Being one of the most standardized screening technologies in modern biomedical sciences, ¹H-NMR spectroscopy is the method of choice for screening of a large number of clinical samples. Additionally, the nondestructive processing of samples by ¹H-NMR spectroscopy leaves the option open for consecutive sample analysis on an additional analytical platform.

In this study we used ^1H -NMR spectroscopy on CSF in search for metabolites reflecting common pathobiology underlying dystonia in CRPS.

METHODS

Patients and controls

CSF samples were obtained from patients with a diagnosis of CRPS-1 and dystonia in at least one extremity who qualified for participation in one of three trials on intrathecal administration of baclofen, glycine or methylprednisolone.^{32,33,51} Patients were referred by physicians throughout the Netherlands. Inclusion criteria were: CRPS-1 according to the diagnostic criteria of the International Association for the Study of Pain (IASP)¹ and symptoms of CRPS for at least 1 year. Other causes of dystonia were excluded using appropriate blood tests, nerve conduction studies and imaging (computed tomography, magnetic resonance imaging) of the spinal cord and brain. Exclusion criteria for participating in the above mentioned trials were: satisfactory relief of symptoms with conventional treatments including oral baclofen, pregnancy, breastfeeding, childbearing potential without using effective contraception, clinically significant psychiatric illness, suspicion of a poor compliance, or involvement in legal proceedings concerning compensation for CRPS.

Controls were selected from subjects who underwent a lumbar puncture for spinal anesthesia because of a surgical intervention, including urologic (e.g. transurethral resection of a urinary bladder tumour), orthopaedic (e.g. total knee prosthesis), vascular (femoropopliteal bypass), gynaecologic (vulvectomy) and general (e.g. lipoma excision) surgery. Otherwise, controls did not suffer from neuropathic pain or neurological diseases. Patient consent was obtained according to the Declaration of Helsinki and the study was approved by the Ethics Committee of the Leiden University Medical Center.

CSF acquisition and sample processing

CSF samples (5 mL) of 57 patients were collected prior to the administration of intrathecal trial medication. In 41 of these patients CSF was obtained from a lumbar

puncture with a 15-gauge Tuohy introducer needle preceding the positioning of a percutaneous catheter that was introduced into the subarachnoid space (L3-4) and advanced to the lower thoracic region to administer trial medication in case of participation in the baclofen or glycine trial. CSF was collected in 16 patients who participated in the methylprednisolone trial, by means of a lumbar puncture with a 20-gauge spinal needle prior to the administration of trial medication.

In 61 controls CSF (5 mL) was obtained from a lumbar puncture with a 20-gauge spinal needle prior to spinal anesthesia for a surgical intervention. Neither patients nor controls had any ongoing or recent infection at the time of the sample collection.

After CSF was obtained, a small amount was used for leucocyte and erythrocyte count. Subsequently, CSF was centrifuged at 1790g for 5 minutes and supernatant was collected. Thereafter, supernatants were frozen and stored at -80° C. In all subjects, the complete procedure was performed within 2 hours and the concentration of erythrocytes in CSF was less than 1000 per μL . All subjects gave written informed consent prior to inclusion, and the study was approved by the hospital ethics committee.

Preparation of CSF samples for ^1H NMR spectroscopy

After thawing, 500 μL of individual CSF samples were mixed with 300 μL of 0.9 % saline solution each (vortex). Samples were then placed on cooled sample racks (6°C) after transfer into 96 deep-well plates. For sample preparation 60 μL of 50 mM phosphate buffer (pH 7.0) in D_2O containing 1.2 mM sodium 3-trimethylsilyltetraduterio-propionate (TSP) and 0.1% (w/v) NaN_3 were mixed with 540 μL of CSF/Saline in a 96 deep well plate using a Gilson 215 liquid handler controlled by a Bruker Sample Track LIMS system.

NMR data acquisition and processing

^1H -NMR data was obtained using a Bruker 600 MHz AVANCE II spectrometer (Metabolic ProfilerTM) equipped with a 5mm TCI cryo probe and a z-gradient system; a Bruker

Efficient Sample Transfer (BEST) system was used in combination with a 120 μ L cryofit flow insert for direct infusion of the samples into the probe head. One-dimensional (1D) ^1H -NMR spectra were recorded at 300K using the first increment of a NOESY pulse sequence²³ with presaturation ($\gamma\text{B1} = 50\text{Hz}$) during a relaxation delay of 4 sec and a mixing time of 10 msec for efficient water suppression.⁴⁴ Thirty-two scans of 65536 points covering 12336 Hz were recorded and zero filled to 65536 complex points prior to Fourier transformation, an exponential window function was applied with a line-broadening factor of 1.0 Hz. The spectra were manually phase and baseline corrected and automatically referenced to the internal standard (TSP = 0.0 ppm). Phase offset artifacts of the residual water resonance were manually corrected using a polynomial of degree 5 least square fit filtering of the free induction decay (FID).⁷ In order to monitor proper filling of the NMR flow cell and for quality control one-dimensional gradient profiles⁵² along the z-axis were recorded for each sample prior and post data acquisition. Duration of 90 degree pulses were automatically calibrated for each individual sample using a homonuclear-gated nutation experiment⁵⁸ on the locked and shimmed samples after automatic tuning and matching of the probe head.

Multivariate data analysis of NMR spectroscopic data

A bucket table with a bucket size of 0.02 ppm was generated for the regions 10.0 – 5.2 and 4.4 – 0.2 ppm, respectively, using the AMIX (version 3.9.11, Bruker Biospin, Germany)⁹ software package. Bucket table and meta data were merged using Open Office Calc (version 3.3.0, <http://www.openoffice.org>). Variables were centered, and pareto scaled²¹ prior to statistical analysis using the SIMCA-P+ (version 12.0, Umetrics, Sweden) software package. For initial analysis and outlier detection, principal component analysis (PCA) was performed using 7 components.

For partial least squares-discriminant analysis (PLS-DA)³⁸ and orthogonal projection to latent structure discriminant analysis (OPLS-DA)⁶ data was normalized according to the sum of the total intensity of three spectral regions, namely 3.67-3.56, 1.85-1.50 and 1.10-0.70 ppm, respectively, and samples were categorized based on clinical data. The

low-field regions of citrate (2.71 to 2.65 ppm buckets) were excluded due to the presence of positional noise artifacts. Statistical models from supervised multivariate data analysis were validated by random permutation of the response variable and comparison of the goodness of fit (R²_Y and Q²_Y)^{25,54} as well as by analysis of variance of the cross-validated residuals (CV-ANOVA).¹² Relative changes in metabolites were identified based on coefficient plots and variable importance in the projection (VIP).⁵⁷ Identity of individual metabolites was confirmed based on NMR reference data from BioRefBase (Bruker Biospin, Germany). Metabolite concentrations were determined by deconvolution of representative signals using an in-house automation script applying Topspin mdcon line fitting algorithm (Bruker Biospin, Germany). Metabolite concentrations were obtained after normalization of absolute concentrations as determined from quantification according to internal standard TSP. Absolute concentrations were not corrected for differential attenuation of the signals caused by relaxation during the mixing time and rapid-pulsing saturation effects. For normalization the same normalization factors as for the bucket table were used. Individual metabolites were analyzed by independent t test with the Mann-Whitney U-test (R version 2.10.1, <http://www.r-project.org/>). To test the predictivity of the obtained OPLS-DA model for classification of CSF profiles, a misclassification table was calculated using a two-class response variable by assigning each observation to the nearest class. In addition predicted values of the response variable Y from the built OPLS-DA model were used to calculate a receiver operating characteristic (ROC) curve (R version 2.10.1, <http://www.r-project.org/>).

RESULTS

Data overview; unsupervised modeling

Clinical characteristics of the study cohort are summarized in table 7.1. ¹H-NMR spectra of CSF samples collected from 49 CRPS patients and 56 controls were included in the analysis. Initial principal component analysis (PCA) was used for outlier detection and

identification of associations with clinical parameters. After outlier removal, apparent clustering according to CRPS classification can be observed in the scores plot of the first two principal components of a PCA model (7 components explaining 91.2% of the variability), indicating a strong influence of CRPS on CSF metabolite profiles (supplemental figure 7.1). No further associations with gender or additional clinical parameters can be observed in the initial PCA analysis. In addition, a separate control based PCA model based on 9 components (explaining 92.1% of the variability) did not reveal associations according to gender or age (data not shown). Even though the CRPS and control clusters appear to be well separated in the scores plot of the initial PCA analysis, some of the samples were apparently allocated to the wrong cluster.

Supervised modeling, first round

To explore the discrimination of CRPS samples from control samples, supervised methods using CRPS classification as response variable were applied. This approach may

Table 7.1. Demographics and clinical characteristics of patients and controls

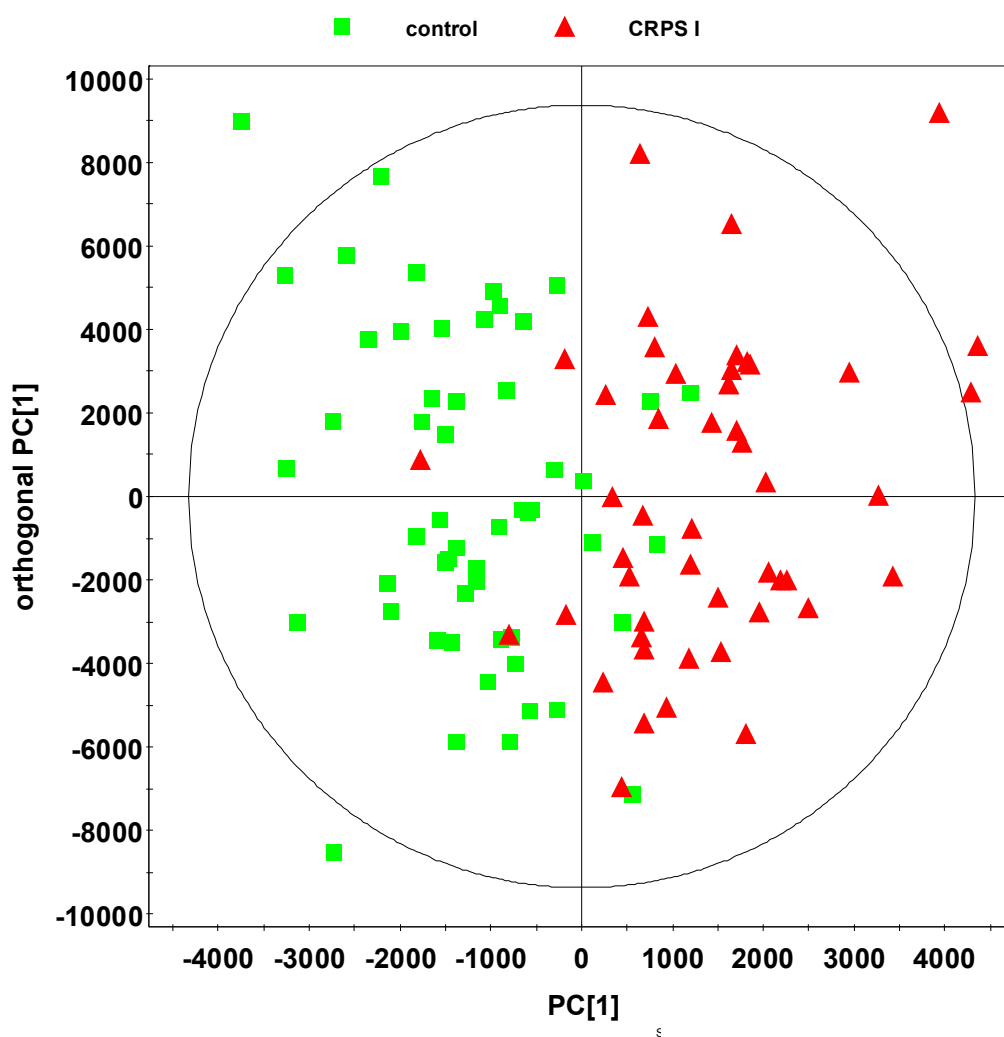
	CRPS (n = 49)	Controls (n = 56)
Age – mean (SD), years	43.3 (12.0)	60.6 (17.2)
Sex – male / female, no.	6 / 43	25 / 31
Disease duration – mean (SD), years	8.7 (5.7)	-
Extremities affected by CRPS – no. (%)		-
1	7 (14)	
2	6 (12)	
3	9 (18)	
4	27 (55)	
Extremities affected by dystonia – no. (%)		-
1	14 (29)	
2	8 (16)	
3	6 (12)	
4	21 (43)	
Content of CSF, mean (SD)		
White blood cell count	4 (4)	3 (7)
Red blood cell count	163 (240)	86 (200)

facilitate the process of finding the largest discrimination between the groups of interest, in contrast to unsupervised methods like PCA that only allows identifying the main sources of variability within a given data set. We built an initial PLS-DA two-class model using the class identity as response variable (Y). Cumulative explained variance (R^2Y) of 0.62 and cross validated predictive fraction (Q^2Y) of 0.53 were calculated for the model and the model validation plot showed intercepts of the R^2Y and Q^2Y regression lines with the vertical axis at 0.147 and -0.183, respectively, indicating a valid model. In the scores plot of the first two components the samples of CRPS patients and controls are clearly separated. However, two of the control samples and 9 of the CRPS samples are still located to the wrong cluster (supplemental figure 7.2).

Data normalization and second round of the supervised modeling

Detailed analysis of the loadings and coefficients for this model did not result in a specific metabolic pattern as discriminator, but indicated a general dilution effect for the CRPS type 1 cluster. We therefore decided to normalize all data prior to further analysis in order to compensate for this effect. Since major components like glucose can introduce a bias resulting in normalization artifacts when using total area normalization, we decided to exclude spectral regions of high abundant metabolites when computing the normalization coefficients of the individual spectra. From initial analysis we identified three spectral regions (3.67-3.56, 1.85-1.50 and 1.10-0.70 ppm) suitable to serve as reference region for normalization.^{8,10} To further refine the statistical model for investigation of CRPS-related effects, a second PLS-DA model was built after normalization and removal of the low field citrate regions. The positional noise of the low-field citrate resonances are correlated with the dilution effect most likely reflecting the citrate/ Ca^{2+} complexation equilibrium. As expected, the quality parameters of the new model did decrease in comparison to the initial model. A cumulative R^2Y of 0.47 and Q^2Y of 0.42 were observed with intercepts of the R^2Y and Q^2Y regression lines with the vertical axis at 0.12 and -0.11, respectively, in the model validation plot. Since the dilution artifact has been removed in this model the observed

Figure 7.1. Scores plot of first and orthogonal components from OPLS-DA model built using ^1H -NMR data of CSF from CRPS type 1 patients ($\blacktriangle$) and control subjects ($\blacksquare$), respectively, after outlier removal. PC[1] resolves the two classes explaining 57.4% of the covariance between the spectra with a cross validated predictive ability of 35.4%. The ellipse denotes the 95% significance limit of the model, as defined by Hotelling's t-test.

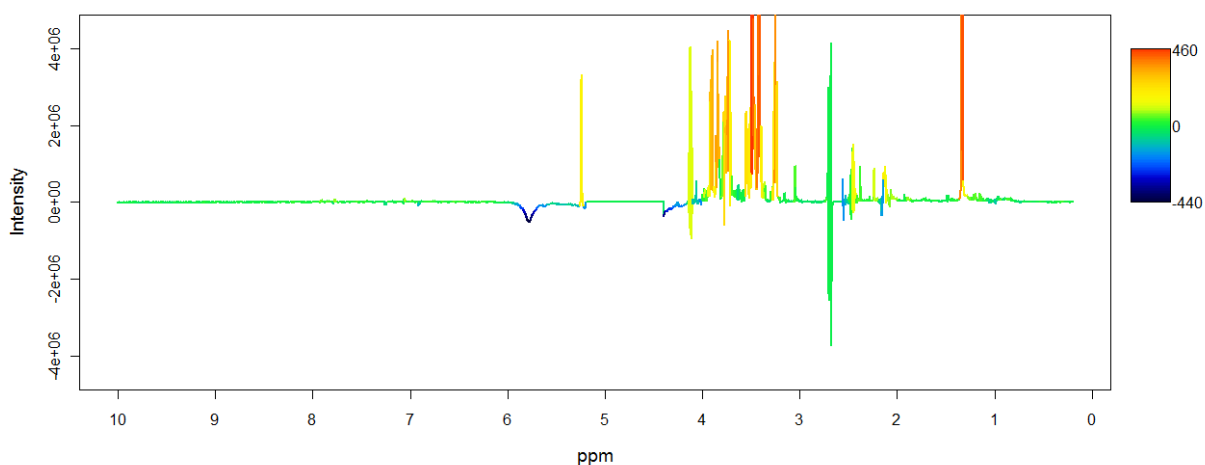


discrimination between CRPS samples and controls can now be attributed to physiological differences. The observed clusters are not completely separated, but show a very clear trend between the two groups (supplemental figure 7.3).

Identification and statistical validation of the metabolites

For further investigation of metabolic signatures of CRPS in CSF and identification of the metabolites contributing to this signature an OPLS-DA two class model was built using CRPS classification as response variable (Y). A cumulative R²_Y of 0.90 with a cross validated predictive ability Q²_Y of 0.84 was achieved using 3 orthogonal components. The reliability of the model was tested by analysis of variance testing of cross-validated predictive residuals (CV-ANOVA)¹² showing a fraction of total corrected SS of 76.0 with a p value of 6.81E-30, indicating a valid model. Samples from CRPS patients and controls are very well separated in the scores plot of the first and orthogonal component as shown in figure 7.1. Based on coefficients and loadings from this model the responsible metabolites for discrimination between the two groups were identified. In figure 7.2 the covariance plot colored by Y-relevant coefficients of the corresponding OPLS-DA model is shown. The coefficients with positive sign indicate spectral regions that show increased signal intensity in CRPS samples whereas coefficients with negative values represent regions with decreased signal intensity in CRPS or in other words indicate regions with increased signal intensity in controls. Identified metabolites from regions relevant for discrimination between the two classes were then quantified by spectral

Figure 7.2. Covariance plot colored by Y-relevant coefficients of OPLS-DA model built using ¹H-NMR data of CSF from CRPS type 1 patients and control subjects. Spectral regions with positive intensity are increased in CRPS type 1 as compared to control.



deconvolution and subjected to non-parametric significance tests by independent t test with the Mann-Whitney U-test as summarized in table 7.2.

Identification of the most relevant metabolites

From the set of identified discriminators, the most relevant metabolites were glucose, glutamine, 2-ketoisovalerate, and lactate which all showed increased concentrations and urea with a decreased concentration in CRPS subjects. However, typical ratios involving some of these metabolites like creatine/creatinine² and lactate/pyruvate did not show any significant correlation with CRPS. In figure 7.3 the bean plot for these metabolites is shown illustrating the concentration distribution for these metabolites in the respective groups. In addition we tested the predictivity of our OPLS-DA model for potential classification of unknown samples. Classification based on prediction of the response variable Y as summarized in table 7.3 showed a correct prediction of over 90%. The AUC of the corresponding ROC curve for the predicted class was 0.951 (figure 7.4) indicating excellent separation between the two groups within the underlying model.

DISCUSSION

The clinical presentation of patients with CRPS is variable, reflecting the existence of several subtypes. One of such subtypes is characterized by predominant dystonia which tends to spread to other limbs, leaving patients severely disabled.⁵⁰ Though findings from different sources indicate involvement of the central nervous system in CRPS related dystonia,^{27,31,49} the pathobiology underlying this condition remains unclear. Thus, to enable a hypothesis free, unbiased approach to further enhance our understanding of the pathobiology of dystonia in CRPS we have applied exploratory metabolomics of CSF in these patients. We found a significantly different metabolic profile of CSF in CRPS patients as compared to controls. The differences are reflected already in the first two principle components of the PCA model which is an indication that the variance associated with CRPS is stronger than variance caused by such

Table 7.2. Changes of metabolite concentrations in CSF of CRPS type 1 patients compared to control

Metabolite	δ [ppm] ^a	VIP ^b	CRPS ^c	Control ^c	Fold change ^d	P value ^e
acetate	1.93	0.03	14.40±11.51	12.86±7.48	1.120	0.139
acetone	2.23	1.12	18.48±15.02	14.75±7.28	1.252	0.084
alanine	1.47, 1.49	0.02, 0.05	40.37±12.06	37.17±12.49	1.086	0.017
2-OH butyrate	0.89, 0.91,	0.23, 0.24	19.31±9.01	16.64±7.47	1.160	0.270
3-OH butyrate	1.19, 1.21	0.59, 0.53	9.09±17.75	7.45±8.50	1.221	0.443
choline	3.21	0.47	1.11±0.46	1.01±0.45	1.092	0.195
citrate	2.53, 2.55	1.09, 1.53	210.43±54.09	213.81±43.83	0.984	0.928
creatine	3.05, 3.95	0.54, 0.49	48.41±12.98	45.69±11.04	1.059	0.152
creatinine	3.05, 4.07	0.54, 0.58	75.80±14.67	68.01±13.96	1.115	0.029
formiate	8.47	0.18	11.45±6.59	11.08±4.72	1.034	0.431
glucose	3.41, 3.49, 5.23, 5.25	3.99, 4.25, 1.76, 1.77	3940.21±713.42	3390.03±661.74	1.162	3.0e-5
glutamine	2.13, 2.45	1.90, 1.38	593.15±99.22	504.00±87.18	1.177	7.7e-4
myo-inositol	3.63, 4.07	0.52, 0.58	163.23±28.10	148.04±29.50	1.103	0.040
3-OH isovalerate	1.27	0.78	3.45±1.42	3.61±1.00	0.956	0.488
2-ketoisovalerate	1.13	0.44	6.24±2.20	4.83±1.84	1.293	5.6e-4
lactate	1.33, 4.11, 4.13	4.08, 1.20, 1.17	1713.44±326.56	1521.37±258.10	1.126	4.7e-4
pyruvate	2.37	0.41	60.04±16.83	54.52± 15.43	1.101	0.022
tyrosine	7.21	0.27	6.21±2.92	5.15±2.09	1.204	0.043
urea	5.77, 5.79	4.05, 4.02	254.15±96.53	348.60±102.45	0.729	2.1e-4
valine	0.99, 1.05	0.75, 0.69	10.78±3.63	10.26±2.92	1.050	0.412
unknown 7.25 (d) / 6.91 (d)	6.91, 7.25	0.98, 0.44	6.60±14.14	9.54±13.15	0.692	0.121
creatine/creatinine	-	-	0.64±0.20	0.67±0.20	0.961	0.907
lactate/pyruvate	-	-	27.55±7.35	27.43±5.16	1.004	0.843

a. Mean value of corresponding bucket.

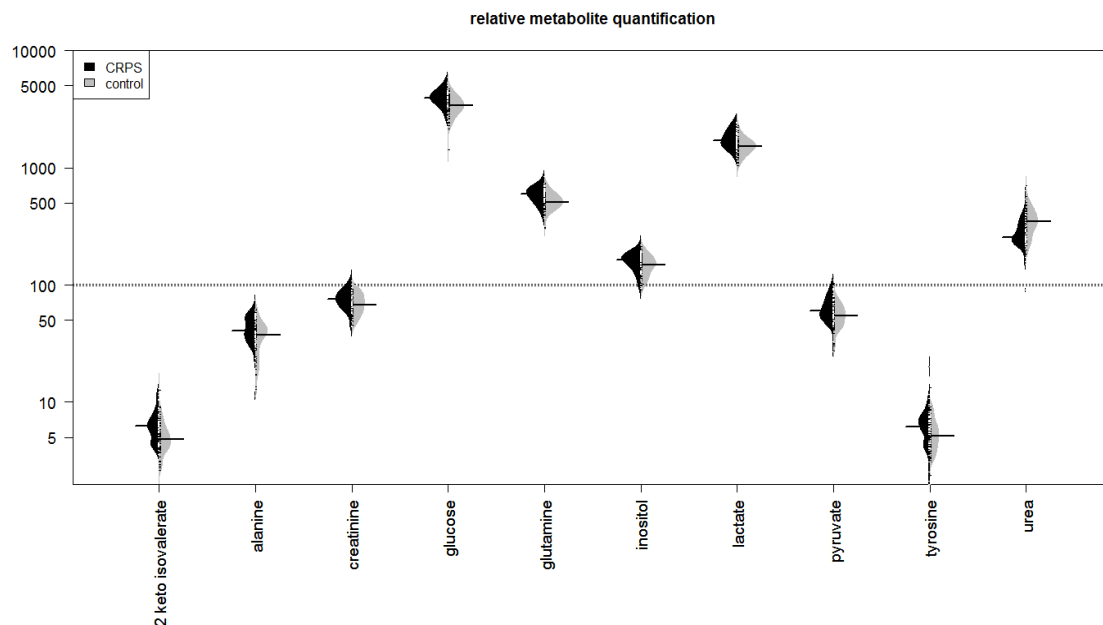
b. Importance of contribution values of buckets.

c. Median of concentrations in relative units ± SD.

d. Fold change was calculated by dividing the median concentration for each metabolite from CRPS patients by the median concentration of the same metabolite from control subjects.

e. P value was analyzed by independent t test with the Mann-Whitney U-test.

Figure 7.3. Bean plot for most significant ($p < 0.05$) metabolites in CSF responsible for discrimination between CRPS and control group. Black and grey areas represent concentration distributions for metabolites in CRPS and control subjects, respectively. Black bars indicate median concentration for the respective group and metabolite.



classical confounders as gender, age or individual differences. A supervised analysis generated a strong model pinpointing the metabolites, which contributed most significantly to the metabolic signature of patients with CRPS related dystonia. As figure 7.4 shows, the predictive power of the entire model is high, though individual metabolites contributing to this specific metabolic signature provide no simple explanation of potential mechanisms underpinning CRPS related dystonia.

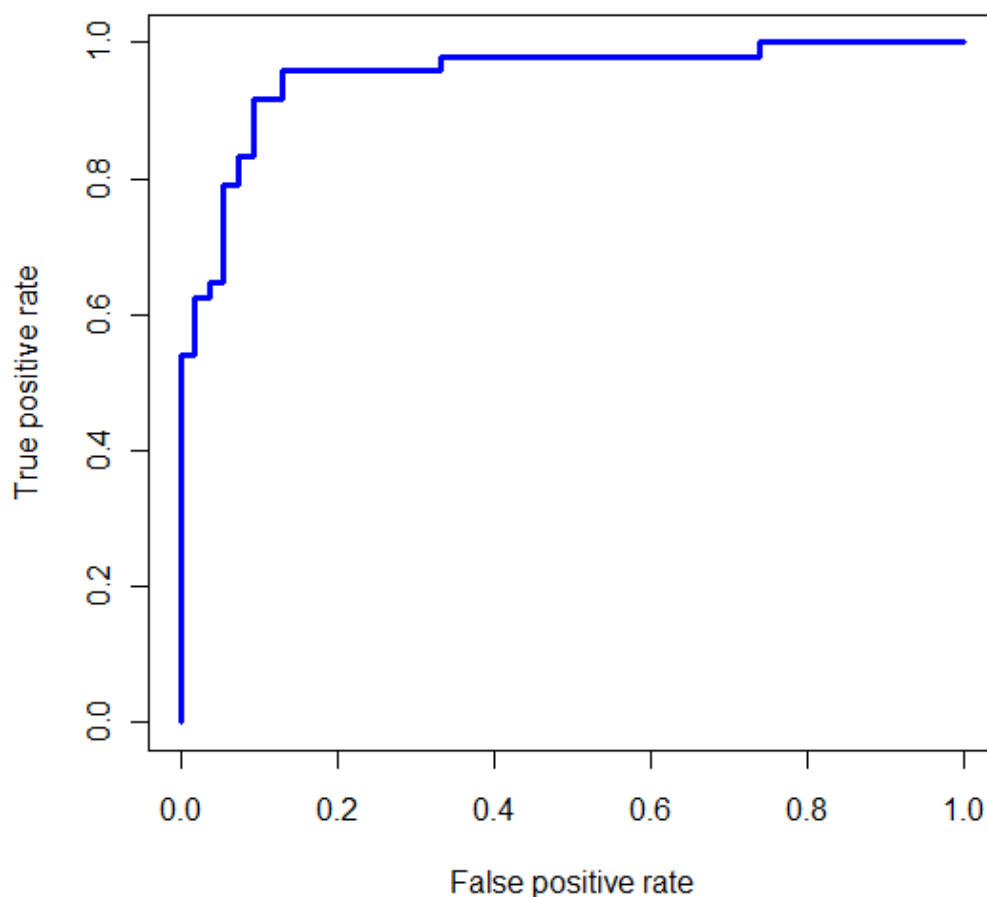
The very first question is whether this metabolic signature is a reflection of dystonia or CRPS. An overview of the available literature on dystonia hardly provides a ground for an unequivocal answer. Firstly, a range of the clinical conditions associated with dystonia is very broad going from neurodegenerative conditions to the diseases with a strong hereditary component such as dopa-responsive dystonia;⁵ as a consequence there is little consistency in the observations. Secondly, unlike our exploratory approach,

Table 7.3. Misclassification table for class prediction from OPLS-DA model built using 1H-NMR data of CSF from 48 CRPS type 1 patients and 54 control subjects.

	Members	Correct predicted	CRPS I	Control	No class (YPred < 0)
CRPS I	48	91.67%	44	4	0
Control	54	90.74%	5	49	0
No class	0		0	0	0
Total	102	91.18%	49	53	0
Fishers prob.	1.7e-018				

practically all previous attempts to address metabolic changes in dystonia used so-called targeted approach, where one or several metabolites were measured and selection of the metabolites was usually driven by the concepts existing in the field.^{24,42} Thus, we probably have to accept that at the current state of knowledge it is practically impossible to “distil” the pure metabolic effect of dystonia from CRPS one and description given in the title – “CRPS related dystonia” – appears as most logical one. Another key question is to which extent this characteristic profile reflects involvement of the CNS in CRPS. It is often taken for granted that concentrations of CSF metabolites are largely dependent on their rate of production in the brain. However, MRI studies of the patients with a chronic pain published so far show that such assumption could be debatable: the metabolic changes reported in such studies (e.g. decreased levels of N-acetylaspartate, changes of GABA or glutamate concentrations) are highly compartmentalized and rather speak against such a simple generalization.^{13,14,29} Besides, considering the chemical diversity of the CSF metabolites and complexity of the human metabolic network no simple generalization is possible. For example, there is experimental evidence showing a strong correlation between the rate of production of organic acids in CNS and their concentrations in CSF,¹⁵ but for such important constituent of CSF as glucose this correlation remains uncertain. We have shown that elevated levels of glucose are an important element of the CRPS-specific metabolic signature. Likewise, elevated levels of CSF glucose have been reported for drug naïve

Figure 7.4. ROC curve (AUC = 0.951) for predicted class from OPLS-DA model built using ^1H -NMR data of CSF from 48 CRPS type 1 patients and 54 control subjects.



schizophrenia patients, where authors link this effect to the brain-specific alteration of glucoregulatory mechanisms.¹⁶ Their interpretation is supported by the fact that there is little doubt about the central origin of schizophrenia. Earlier studies on CRPS, however, identified both central and peripheral mechanisms which may contribute to the clinical presentation of CRPS.²⁸ Hence, our finding of elevated glucose concentration in the CRPS group does not automatically imply an alteration of an underlying central process. The increased concentration of glutamine is another characteristic feature of the profile. In view of the importance of the glutamate-glutamine turnover for the entire CNS metabolism it seems only logical to interpret elevated levels of glutamine in CSF as a consequence of dysregulation in the brain glutamate-glutamine balance. However,

multiple observations showing that CSF glutamine levels are elevated in hepatic encephalopathy rule out such a simple interpretation, indicating the existence of peripheral factors which may affect the concentration of glutamine in CSF.⁵³

Table 7.2 includes one compound, namely urea, which most likely cannot be a product of the metabolic activity of human brain and there is little doubt about the direct correlation of its concentration in CSF and the concentration in the systemic circulation. Urea is a small, polar, nonelectrolyte molecule, the end product of the protein catabolism; thus from a biochemical point of view urea is not more than a waste product. The bulk of urea in the systemic circulation is produced in the liver as a result of the deamination of amino acids. It has been shown that urea is absorbed into CNS from the circulation^{19,22} which explains its presence in CSF.⁵⁶ Thus, the lower concentrations of urea in CSF of patients with CRPS most likely reflect a more systemic effect, which is associated with chronic disease, stress and disuse.^{20,40,48} All of these conditions apply to the patients investigated in this study. Moreover, the changes in energy metabolism encountered in these conditions are also associated with increased muscle protein degradation.¹¹ The muscle wasting that occurs in catabolic states results primarily from accelerated breakdown of the long-living myofibrillar proteins, such as actin and myosin. Unlike other proteins, actin and myosin contain a unique amino acid 3-methylhistidine which is released during the breakdown of muscles.⁴⁷ Interestingly, we recently found elevated levels of 3-methylhistidine in urine of chronic CRPS patients.⁴⁵ Our CSF findings therefore do not seem to provide evidence for the involvement of major primary metabolic derangements of the nervous system but instead corroborate with previous findings and underscore the important role of the catabolic state in chronic CRPS patients. In conditions associated with a catabolic state, including sepsis, trauma, burns, and major surgery, activation of ubiquitin-proteasome system has been demonstrated as the major pathway underlying muscle wasting.^{30,34,55} Moreover, the catabolic state in chronic patients with CRPS may lead to an impaired immune function³⁵ which not only increases the susceptibility to local infections and sepsis but also raises an important question whether a catabolic state may play a role in

the maintenance of inflammation, thus delaying the recovery of CRPS.⁴¹ Thus, in line with the care suggested for other inflammatory conditions,¹⁸ our findings indicate that appropriate nutrition assessment, intervention, and monitoring should be considered in patients with CRPS. Nutrition support alone however, is insufficient to prevent muscle loss during active inflammation and other interventions aiming to blunt the undesirable aspects of inflammation should be considered in the management of CRPS patients as well.¹⁸

Finally, our interpretation provides no final solution for the pathobiology of CRPS and some issues associated with this study limit the generalizability of our findings to other phases and subtypes of CRPS. The most important one is that this study included only patients with chronic CRPS and dystonia. However, since aberrant inflammation plays also a key role in acute CRPS, our findings suggest that future studies should evaluate whether early implementation of anti-inflammatory approaches and measures to maintain an optimal nutrition state may improve recovery of CRPS.

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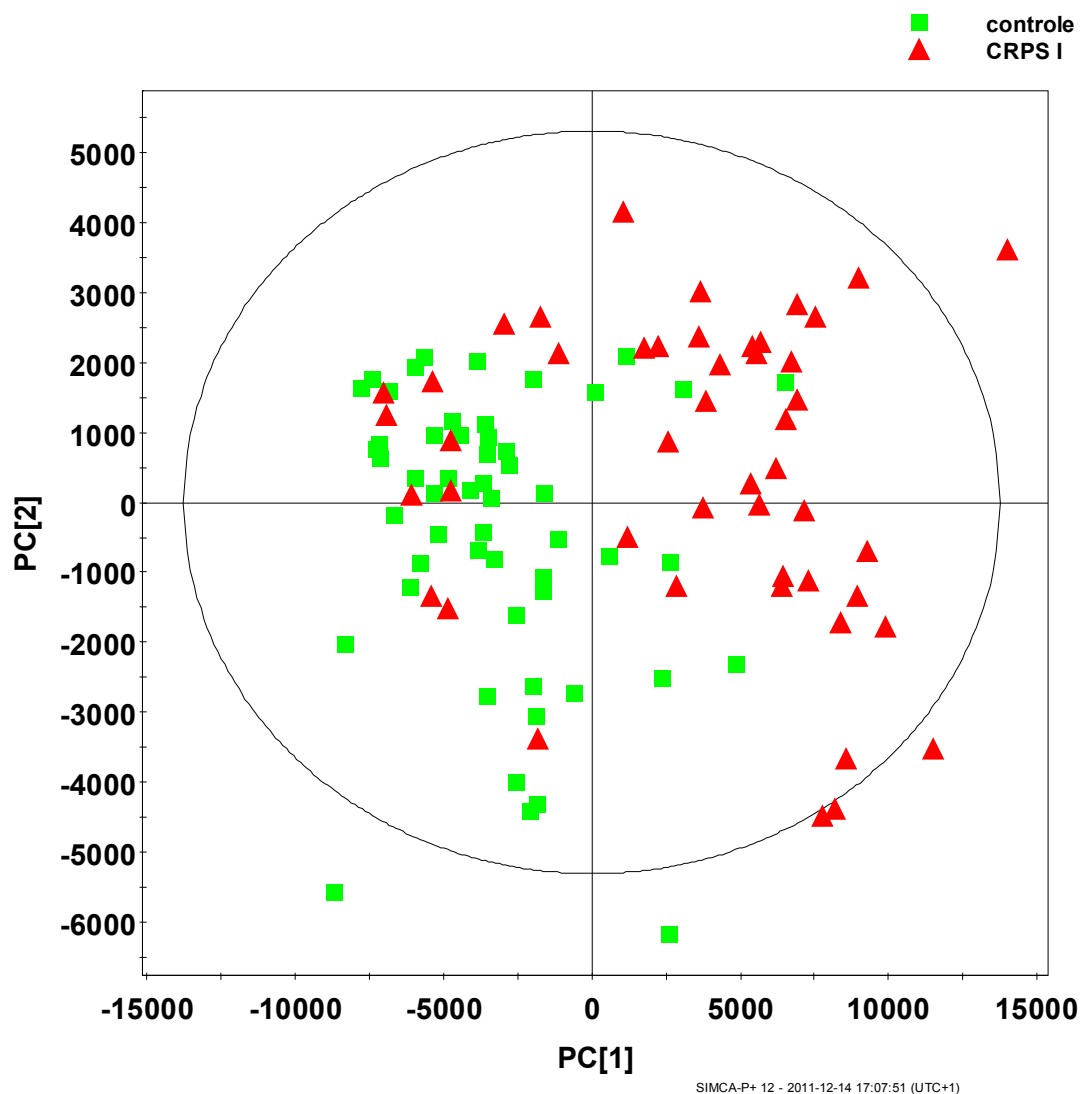
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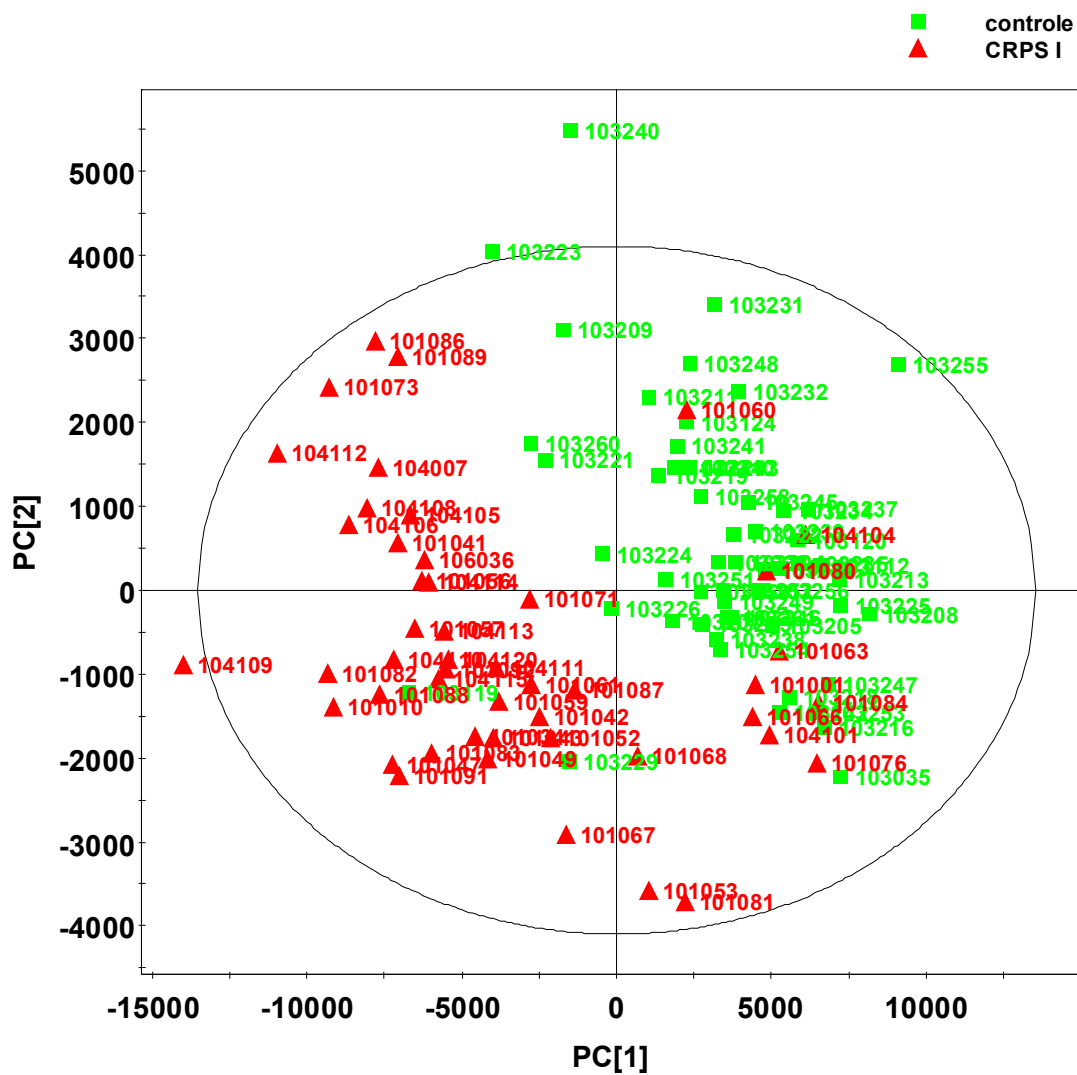
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SUPPLEMENTAL FIGURES

Supplemental figure 7.1. Scores plot of first two principal components from PCA model built after outlier removal. Data points are colored according to control (■) and CRPS (▲), respectively.

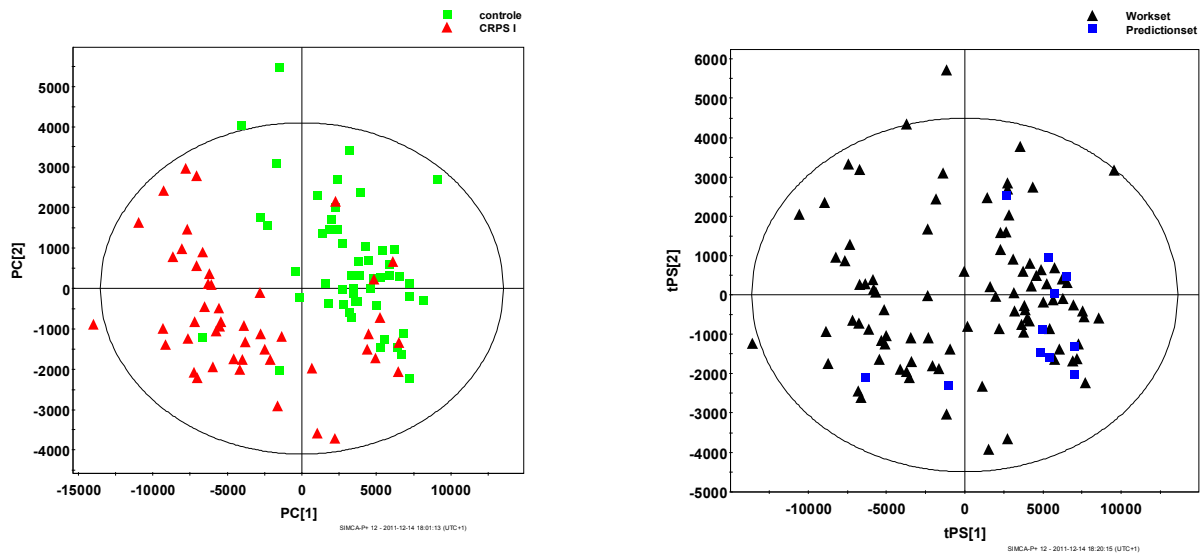


Supplemental figure 7.2. Scores plot of first two principal components from PLS-DA two-class model built after outlier removal. Data points are colored according to control (■) and CRPS I (▲), respectively.



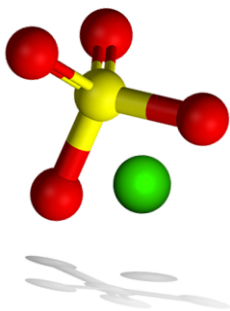
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Supplemental figure 7.3. Comparison of PLS-DA models before (left) and after (right) exclusion of apparently misclassified samples. In the plot on the right, the excluded samples are predicted using the corresponding two-class PLS-DA model.



Part IV.

Intramuscular Magnesium Sulphate Treatment in CRPS



Chapter 8

An Explanatory Study Evaluating the Muscle Relaxant Effects of Intramuscular Magnesium Sulphate for Dystonia in Complex Regional Pain Syndrome

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ABSTRACT

The treatment of dystonia related to complex regional pain syndrome (CRPS) remains unsatisfactory, raising the need of alternative targets for intervention. In dystonia pathological muscle changes may occur, which contribute to stiffness. Since magnesium sulphate (MG) may act as a muscle relaxant through its actions on the neuromuscular junction and muscle, we performed an explanatory study of the muscle relaxant effect and safety of intramuscular MG (IMMG) in CRPS patients with dystonia. In a double-blind randomized placebo-controlled crossover study 30 patients were assigned to 3-week treatments of IMMG and placebo. Treatments were separated by a one-week washout period. The daily dose of IMMG was 1000 mg in week 1, 1500 mg in week 2 and 2000 mg in week 3. The primary outcome measure was the difference in change in Burke-Fahn-Marsden (BFM) scores after 3 weeks of treatment between both interventions. Secondary outcomes involved severity of dystonia, myoclonus, tremor, pain and functional activity. Data of 22 patients available for the explanatory analysis revealed no significant differences between IMMG and placebo treatment in any of the outcomes. In conclusion, we found no indication of efficacy of IMMG in a daily dose of 2000 mg as a muscle relaxant in CRPS-related dystonia.

INTRODUCTION

Complex regional pain syndrome (CRPS) is characterized by various combinations of sensory, autonomic and motor disturbances, and its onset is usually triggered by a trauma to a limb.²² Dystonic postures are one of the most prevalent motor disturbances in CRPS occurring in 20% of the patients.^{10,13,38,49,51} Since these postures are frequently associated with severe disability,^{3,49} several therapeutic efforts have been undertaken to correct or improve these postural abnormalities.^{26,47,48,50} Systematic reviews on these efforts, however, have revealed a lack of evidence of symptomatic benefit of splints, oral muscle relaxants and botulinum toxin A in CRPS-related dystonia.³² Supported by findings from neurophysiological studies, which have highlighted the role of central disinhibition in this type of dystonia,^{37,39,46} several studies have examined the efficacy of intrathecal baclofen.^{47,48,50} Though this treatment may substantially decrease dystonia severity, the adverse event profile of this approach in CRPS is poor and in 30% of cases no benefit was established.⁵⁰ Given the current status of treatment options for dystonia in CRPS, alternative targets for intervention are needed.

Apart from central mechanisms involved in dystonia and spasticity, pathological changes may occur in muscle tissue and this may contribute to stiffness such that the range of motion around a joint is limited.⁴¹ Consequently, drugs that effectively reduce the influence of this factor may contribute to the treatment of dystonia in CRPS.

The regulation of muscle contraction is highly dependent on calcium fluxes in muscle tissue and at the neuromuscular junction.^{29,45} In turn, these calcium fluxes are controlled by magnesium,^{9,18,25,30,52-54} which is demonstrated by the relaxant property of parenterally administered magnesium sulphate (MG) in both smooth and striated muscle.^{21,33,36,44} Oral administration of magnesium salts, which are currently used in clinical practice, are not suitable for achieving efficient muscle relaxation as the systemic bioavailability of these salts is generally poor.^{8,34} Although most studies used intravenous regimens to evaluate the effect of MG, intramuscular injections of MG (IMMG) proved equally effective in studies on (pre)eclampsia and severe head injury using similar dose regimens.^{1,14,54} Additionally, in our experience, IM injections may be

preferred because CRPS patients are more susceptible to phlebitis during intravenous drug infusion.

This explanatory study was carried out to evaluate if there is supporting evidence of the muscle relaxant effects of IMMIG in patients with CRPS-related dystonia, which would justify exploration of alternative modes of administration.

MATERIALS AND METHODS

Patients

Subjects were male or female out-patients, aged 18 years or older and diagnosed with CRPS-related dystonia. Patients had to fulfill the diagnostic criteria for CRPS I established at the Orlando consensus conference in 1993, which were the criteria formally endorsed by the International Association for the Study of Pain at the time this study started²⁴ and suffer from tonic or intermittent dystonia resulting in slight disability or worse in one or more extremities for at least 1 year. Patients were excluded in case of satisfactory relief of symptoms with conventional treatments or intrathecal baclofen. Other exclusion criteria were a history of alcohol or drug abuse within the past year, a clinically significant psychiatric illness, pregnancy, breastfeeding, childbearing potential without using effective contraception, a history of poor compliance to medical regimens or study requirements or the suspicion of poor compliance, insufficient command and understanding of the Dutch language, involvement in legal proceedings (claiming compensation for CRPS I), impaired coagulation or renal function (i.e. serum creatinine below 10 or exceeding 80 $\mu\text{mol/l}$), hypermagnesaemia (i.e. total serum Mg exceeding 1.10 mmol/l) or requirement of diuretics. The study lasted from October 2009 to May 2012. Patient consent was obtained according to the Declaration of Helsinki and the study was approved by the Ethics Committee of the Leiden University Medical Center.

Study design

This was a randomized placebo-controlled crossover study conducted at the movement

disorders out-patients clinic of the Leiden University Medical Center. Patients, treating physicians and assessors were blinded for the sequence of treatments.

An independent pharmacist randomized patients over the two sequences of IMMIG and placebo in blocks of 4 using a computer generated randomization list. Each subject received two intramuscular treatments of 3 weeks each: magnesium sulphate 100 mg/ml solution and sodium chloride 0.9% (placebo). Both treatments were separated by a 1 week wash-out period, which was considered sufficient based on the pharmacokinetic data on magnesium sulphate.²⁰ Patients self-administered intramuscular solutions twice per day during each treatment period. Treatment started at a daily volume of 5 ml twice a day and was increased in week 2 and 3 to twice daily volumes of 7.5 ml and 10 ml, respectively. Syringes with 0.4 mm diameter needles were used for infusion. Solutions were injected in the buttocks and the lateral upper margins of both thighs in alternating sequence. Intramuscular injections of MG can be painful and potentially contribute to unblinding. To alleviate the pain of intramuscular injections and reduce the risk of unblinding, 2.5 gram of lidocaine-prilocaine 5% cream (EMLA®) was applied 120 minutes before injection at the site of puncture. After application of the cream the area was covered with a transparent film (Tegaderm®) prior to needle insertion to facilitate penetration through the various layers of the skin. This regimen has proved effective in decreasing both the superficial pain of needle puncture as well as the deeper pain of infiltration.^{5,15,17,42} Prior to the first injection, patients were instructed how to prepare and perform the injections properly. If needed - because of the occurrence of (probable) severe or serious adverse events or the development of hypermagnesaemia (i.e. total serum Mg level exceeding 1.10 mmol/l)- IMMIG was reduced to the previous dose or stopped. In case of (probable) severe or serious adverse events, concentrations of free-Mg were measured. An independent data monitoring committee reviewed unblinded data for patient safety. An investigator, not involved in the clinical assessment of patients, was exclusively informed on the presence of adverse events, the decision taken by the data monitoring committee and the development of hypermagnesaemia. Assessments were performed by another

investigator, who was blinded to these data and the allocated sequence of treatment. The study was registered in the Netherlands National Trial Register, number NTR 1873.

Outcome measures

Patients were assessed one week before the first treatment was initiated and during both treatment regimens at days 1, 8, 15 and 22 prior to dose adjustment. Assessments were performed 2 hours after the first of the 2 daily injections of study medication. The efficacy assessments of movement disorders included the Burke-Fahn-Marsden (BFM) dystonia rating scale,⁶ the Barry-Albright dystonia (BAD) rating scale,² the unified myoclonus rating scale (UMRS)¹² and the tremor research group rating scale (TRGRS).¹⁶ The primary outcome measure was the difference in change of the BFM-score over 3 weeks between IMMIG and placebo. On the same days safety measurements were performed, including medical history taking, physical and neurological examination, a 12-lead electrocardiogram and serum sampling of ionized calcium and magnesium, creatine kinase, blood urea nitrogen, creatinine and parameters of coagulation. Self-assessment of pain and dystonia was performed every day at 6 PM, starting 1 week before the first injection until the end of the study. Patients were asked to assess the severity of global pain not related to dystonia as well as the severity of dystonia separately on two different 11-point numeric rating scales. They used the McGill pain questionnaire to measure different aspects of pain.²³ In addition, patients rated the difficulty in performing activities every week, using the Radboud skills questionnaire²⁷ (if arms were involved) or the walking ability questionnaire³¹ (if legs were involved). At the end of each treatment the investigator assessed the change of dystonia from baseline on a clinical global impression (CGI) scale ranging from -3 (much worse) to +3 (much better). At the end of the study patients were asked to compare both treatment periods and express their preference using a patient preference questionnaire (PPQ), which consists of a 10 cm horizontal line ranging from -5 to +5. A score of 0 reflects no preference, whereas -5 or +5 expresses maximal preference for treatment period 1 and 2 respectively. Secondary outcome measures were the difference in change after 3 weeks

between interventions on measures other than the BFM scale. After the study, patients and investigator guessed which period IMMIG was administered to assess the adequacy of the blinding procedures.

Statistical analysis

Normality of the data was checked with the Shapiro-Wilk test and non-normally distributed data were transformed according to the shape of the distribution, and again tested for normality. Paired-samples t-tests were used to examine differences in change between both treatments for the normally distributed transformed and non-transformed data. For the remaining non-transformed data, Wilcoxon signed-rank tests were used to investigate the difference in effectiveness between IMMIG and placebo treatment. Chi square tests were used to examine differences between the number of patients who improved and did not improve on the CGI scale, and between the number of patients who preferred and did not prefer IMMIG treatment. The Chi square test was also used to examine the difference in the number of adverse events between IMMIG and placebo treatment. For the NRS and McGill Pain Questionnaire the scores of 7 consecutive days prior to the start of a treatment or dose adjustment were averaged. To evaluate the efficacy of IMMIG treatment only data of patients who received 3 weeks of IMMIG and 3 weeks of placebo treatment were analyzed. For the evaluation of safety of IMMIG all patients who were enrolled in the study were included.

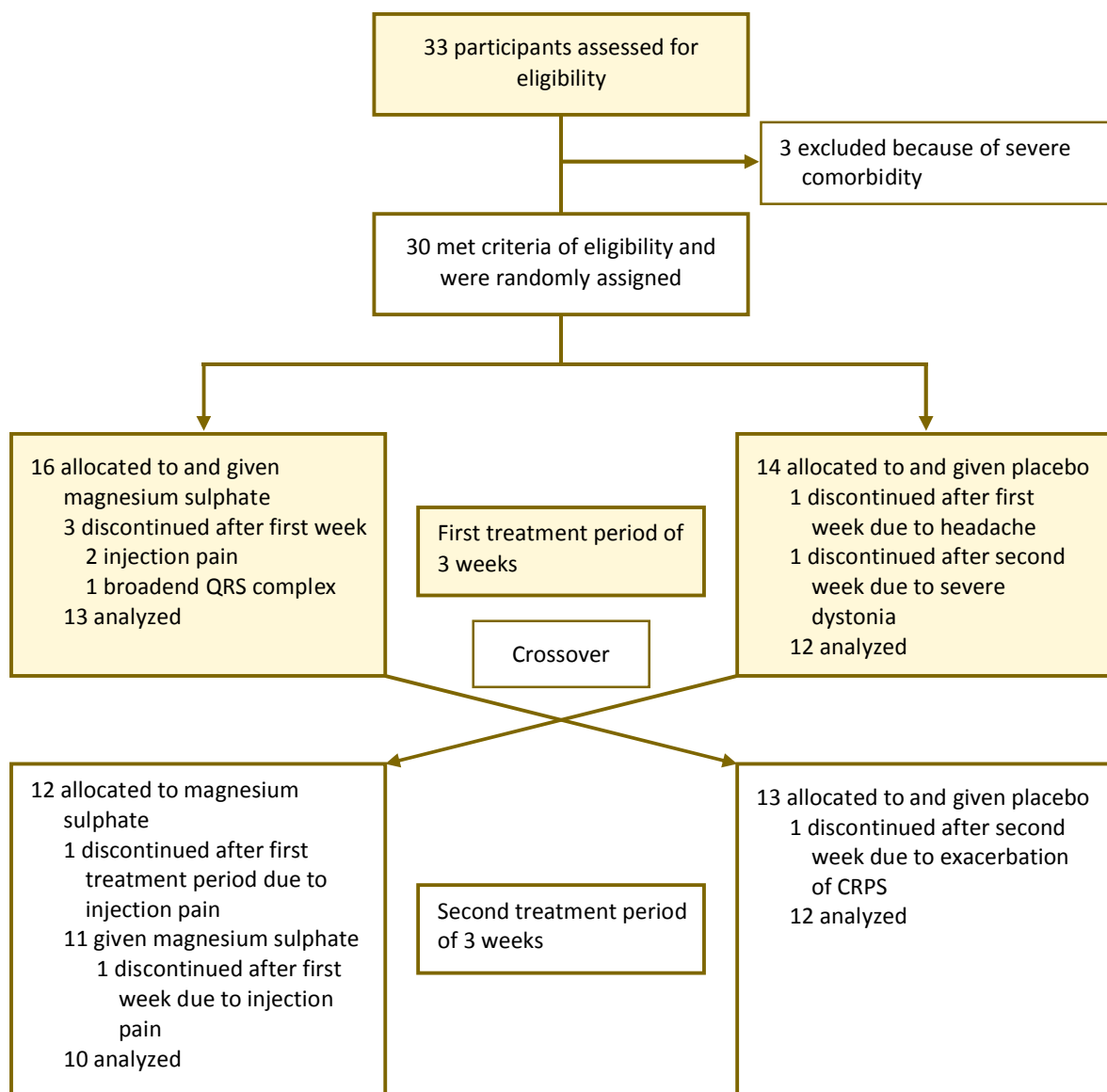
Significance was assumed at the 0.05 level. To find a statistically significant difference of $\geq 25\%$ in the reduction of BFM-scores between both treatments, 34 patients were required, assuming an alpha of 0.05, beta of 0.2, standard deviation of 12.5, and a correlation between measures of 0.6. To compensate for a potential drop-out (estimated as 15%), we aimed to recruit 40 patients. For all statistical tests, the SPSS[®] software package (version 17.0; SPSS Inc., Chicago, IL) was used.

RESULTS

Between October 2009 and April 2012, a convenience sample of 33 patients with CRPS-

related dystonia were evaluated for eligibility, of which 30 patients (28 women) participated in the study (figure 8.1). It was decided to stop the study prematurely, because of difficulty with recruitment and the high dropout rate (27%). Table 8.1 illustrates the demographic and baseline characteristics of all 30 participants. Sixteen patients were randomized to receive IMMIG in the first period, while 14 started with placebo. Five patients dropped out during the first treatment period due to adverse events, of which 3 had received IMMIG. One patient who was randomized to receive

Figure 8.1. Trial profile



placebo in the second period discontinued before this treatment was started. Two other patients, one in each tier, did not complete the second treatment period as a result of injection pain or exacerbation of CRPS.

Given the proof-of-concept nature of this study aiming to evaluate the muscle relaxant effect of MG on dystonia in CRPS, the efficacy of IMMIG was analyzed only for the 22 patients who completed the study. There was no significant effect of sequence (IMMG-placebo or placebo-IMMG) on the BFM measure baseline scores. Mann-Whitney *U* tests showed no significant differences in age, sex, inciting event, disease duration, dystonia

Table 8.1. Baseline characteristics of participants

Characteristics	Patients completing the study (<i>n</i> = 22)	Dropouts (<i>n</i> = 8)
Median (IQR) age (years)	40 (29 - 52)	42 (35 - 45)
Sex		
Male	1	1
Female	21	7
Inciting event (%)		
Fracture	4 (18)	1 (13)
Surgery	3 (14)	2 (25)
Soft tissue injury	10 (46)	5 (63)
Spontaneous	5 (23)	0 (0)
Median (IQR) duration of CRPS (years)	11.5 (6.0 - 16.0)	5.0 (2.5 - 8.5)
Median (IQR) dystonia severity		
Burke-Fahn-Marsden rating scale	24 (15 - 35)	18 (12 - 34)
Barry-Albright dystonia rating scale	8 (4 - 11)	6 (4 - 12)
Numeric rating scale	7 (6 - 8)	7 (6 - 8)
Number of affected extremities (%)		
1	4 (18)	2 (25)
2	7 (32)	2 (25)
3	5 (23)	2 (25)
4	6 (27)	2 (25)
Number of dystonic extremities (%)		
1	7 (32)	3 (38)
2	8 (36)	2 (25)
3	5 (23)	1 (13)
4	2 (9)	2 (25)
Median (IQR) heart rate (bpm)	72 (62 - 90)	68 (60 - 75)

CRPS = complex regional pain syndrome; IQR = interquartile range; bpm = beats per minute.

severity, heart rate and number of affected and dystonic extremities between patients who completed the study and dropouts (table 8.1).

Efficacy

There was no significant difference between the change in dystonia severity over three weeks of treatment with IMMIG and placebo (table 8.2). Concerning the secondary outcomes, only the NRS for pain and the functional section of the UMRS (table 8.2) showed a significant improvement after 3 weeks of IMMIG treatment in comparison with placebo ($p < 0.05$). Reflect and logarithm transformation was applied to data of the number of word count in the McGill pain questionnaire and the 'rising' section of the walking ability questionnaire. Logarithmic transformed data were used for the functional and action section of the UMRS and items 9-12 of the TRGRS. The proportion of patients improving on the CGI scale was significantly larger during IMMIG treatment ($11/22 = 50\%$) compared to placebo treatment ($3/22 = 14\%$; $\chi^2 = 5.13$, $df = 1$, $p = 0.02$). Thirteen patients preferred IMMIG with a median PPQ score of 4.0 (IQR 2.3 – 4.6), 6 patients preferred placebo with a median PPQ score of 4.7 (IQR 2.9 – 5.0) and 3 patients had no preference (McNemar test: $\chi^2_{(1)} = 1.895$; $P=0.169$). The preference for IMMIG or placebo treatment, though not significant, was based on the improvement of dystonia in all cases. The guess of the investigator and patient of which treatment was administered in which period was correct in 50% and 63%, respectively. Post-hoc analysis revealed no distinctive features in patients who improved $\geq 25\%$ in BFM-scores during IMMIG treatment compared to patients who failed to reach this level of improvement.

Safety

The mean (range) total magnesium concentration in blood remained within the normal range during both the IMMIG (0.90 [0.67 – 1.10] mmol/L) and placebo (0.84 [0.66 – 1.05] mmol/L) treatment. Blood levels of total magnesium increased significantly during IMMIG treatment (mean [SD] change of 0.11 [0.07]) compared with placebo treatment (mean [SD] change of 0.00 [0.06]; $p < 0.0001$).

Table 8.2. Summary of clinical outcome events

Outcome event	Range scale ^a	<i>n</i>	Intramuscular magnesium sulphate		Placebo		<i>P</i> value ^b
			<i>Baseline</i>	<i>After 3 weeks</i>	<i>Baseline</i>	<i>After 3 weeks</i>	
<i>Dystonia rating scales</i>							
Burke-Fahn-Marsden dystonia rating	<u>0</u> - 120	22	24.5 (14.7)	21.8 (15.4)	23.9 (14.1)	24.0 (15.9)	0.11 ^c
Barry-Albright dystonia rating	<u>0</u> - 32	22	7.9 (4.6)	6.8 (4.6)	7.6 (4.2)	7.6 (5.0)	0.05 ^c
Numeric rating	<u>0</u> - 10	22	7.1 (1.7)	6.2 (2.1)	7.1 (1.3)	7.3 (1.6)	0.26 ^c
<i>Pain rating scales</i>							
Numeric rating	<u>0</u> - 10	22	7.3 (1.6)	6.7 (2.1)	7.2 (1.5)	7.3 (1.6)	0.01 ^c
McGill number of words chosen	<u>0</u> - 20	22	13.4 (3.5)	12.0 (3.3)	13.2 (3.1)	13.0 (3.1)	0.05 ^e
McGill pain rating index	<u>0</u> - 63	22	27.4 (10.4)	23.3 (11.5)	26.5 (9.1)	26.2 (10.4)	0.11 ^c
<i>Unified myoclonus rating scale</i>							
Myoclonus at rest	<u>0</u> - 128	22	0.5 (1.5)	0.2 (0.9)	0.2 (0.9)	0.3 (0.9)	0.28 ^d
Myoclonus with action	<u>0</u> - 160	22	2.1 (3.7)	1.7 (3.3)	3.5 (4.2)	2.8 (4.5)	0.44 ^e
Functional effect of myoclonus	<u>0</u> - 20	22	6.5 (5.7)	5.6 (5.9)	6.5 (5.7)	6.5 (6.2)	0.04 ^e
<i>Tremor research group rating scale</i>							
Item 1 - 8	<u>0</u> - 76	22	2.1 (2.3)	1.7 (2.0)	2.3 (2.7)	2.3 (3.0)	0.38 ^d
Item 9 - 12	<u>0</u> - 28	22	1.9 (2.5)	1.1 (1.9)	2.3 (3.0)	2.0 (3.8)	0.14 ^e
<i>Functional rating</i>							
Radboud skills questionnaire (total)	<u>0</u> - 5	20	3.6 (0.9)	3.5 (1.1)	3.6 (0.9)	3.6 (1.0)	0.57 ^c
Walking ability questionnaire - indoor	<u>0</u> - 10	13	4.7 (3.0)	3.9 (3.2)	5.1 (2.4)	4.6 (2.9)	0.70 ^c
Walking ability questionnaire - outdoor	<u>0</u> - 10	10	5.7 (2.6)	4.8 (2.8)	6.1 (2.2)	6.2 (2.2)	0.21 ^c
Walking ability questionnaire - rising	<u>0</u> - 10	18	7.2 (3.1)	6.8 (3.2)	7.0 (2.8)	7.3 (3.0)	0.07 ^e

a. Best score is underlined

b. Change over 3-weeks IMMIG treatment versus change over 3-weeks placebo treatment

c. Paired samples t-test of non-transformed data

d. Wilcoxon signed rank test of non-transformed data

e. Paired samples t-test of transformed data with reflect and logarithm transformation for McGill number of words chosen and walking ability questionnaire on rising and logarithm transformation for section action and functional of the unified myoclonus rating scale and item 9-12 of the tremor research group rating scale; scores represent non-transformed data

Electrocardiographic monitoring did not reveal any serious arrhythmias, except for one patient who manifested with a broadened QRS complex pattern after the first week of the first treatment period (IMMG) and discontinued after this finding (figure 8.1). A causative relation between this arrhythmia and IMMG treatment was deemed unlikely as the blood concentration of total and ionized magnesium was normal.

The changes in heart rate during a treatment period were not significantly different between IMMG (mean change [SD] of 2 [9]) and placebo (mean change [SD] of 0 [13]; $p = 0.57$).

Thirty-eight adverse events (two severe, six moderate and thirty mild) were reported during IMMG treatment (table 8.3). Twenty-two adverse events (one severe, four moderate and seventeen mild) occurred during placebo treatment, including one serious adverse event of severe headache suggestive of subarachnoid hemorrhage. After in-patient hospitalization subarachnoid hemorrhage was ruled out, as were other serious causes of headache. There were significantly more patients, who reported adverse events during treatment with IMMG compared to placebo ($\chi^2 = 9.08$, $df = 1$, $p = 0.003$). Injection pain was the most frequently reported adverse event during both treatments, but was reported significantly more often during administration of IMMG compared with placebo ($n = 19$ versus $n=9$; $\chi^2 = 5.42$, $df = 1$, $p = 0.02$).

DISCUSSION

The treatment of CRPS-related dystonia remains unsatisfactory, raising the need of alternative targets for intervention.^{26,32,47,50} In dystonia and spasticity, pathological muscle changes may occur, which contribute to stiffness. Since MG may act as a muscle relaxant through its actions on the neuromuscular junction and involvement in terminating muscular contraction,^{21,33,36,44} we performed an explanatory study of the muscle relaxant effect and safety of IMMG in CRPS patients with dystonia.

Over the dose-escalation period of 3 weeks with doses up to 1000 mg b.i.d. we did not find evidence of efficacy of IMMG on dystonia in CRPS in 22 patients, who completed both treatment periods. There are several factors, which may explain this lack of

Table 8.3. Treatment-emergent adverse events

Adverse Event	IMMG (<i>n</i> = 27)	Placebo (<i>n</i> = 15)
Injection pain	19	9
Subcutaneous hematoma	7	-
Mild allergy	3	2
Itchy sensation at the site of injection	1	-
Edema	1	-
Flushing	1	1
Headache	1	3
Nausea	1	2
Impaired cardiac conduction	1	-
Sleepiness	1	-
Return of injection fluid	1	-
Hyperhidrosis	1	-
Exacerbation CRPS	-	1
Gingivitis	-	1
Hyperexcitation	-	1
Lightheadedness	-	1
Painful breasts	-	1
Total	38	22

CRPS = complex regional pain syndrome type I; IMMG = intramuscular magnesium sulphate

efficacy. First, in spite of efforts to enhance patient enrolment, we failed to meet the recruitment target after 2.5 years of patient inclusion. One explanation for this recruitment failure may be that only patients were used, who were refractory to conventional treatments or intrathecal baclofen. This selection bias hampers generalization of our results. However, since we took a worsening of CRPS symptoms as a result of repetitive IMMG injections into account, we considered it appropriate to restrict our selection to patients who had experienced no benefit of conventional treatments or intrathecal baclofen. Also, a relatively large number of patients dropped out because of adverse events.

However, given the associated patient burden of daily intramuscular injections, the aim of this explanatory study was not to find support for long-term administration of IMMIG in relieving dystonia severity, but to yield evidence of the efficacy of MG through a method of administration that ensures systemic delivery. If efficacy of MG would have been demonstrated, this would set a basis for the development of alternative modes of administration with larger patient acceptability. Second, the lack of efficacy of IMMIG may be related to the maximum daily dose of 2000 mg. Intravenous MG proved to be effective in reducing smooth muscle spasm^{21,28,40,43} with daily doses as low as 1.2 gram^{28,40} but to our knowledge, there are no studies, which evaluated the efficacy of IMMIG on striated muscle with a similar dose regimen. In a previous study the efficacy of MG in relaxing striatal muscle contraction was established in patients with severe tetanus, which may suggest an underlying mechanism different from CRPS-related dystonia.⁴⁴ However, in this study intravenous drug delivery was used, which together with continuous monitoring in a clinical setting allowed the administration of much higher daily doses of MG. In one of the aforementioned studies, the serum concentration of total magnesium was monitored during the first 24 hours after MG injection with concentrations exceeding 1.5 mmol/L in the first 20 minutes after injection.²⁸ Considering the pharmacokinetic properties of IMMIG, which include optimal bioavailability 1 to 3 hours after injection, all assessments and blood sampling were performed 2 hours after the first of the 2 daily injections of study medication. At this time of assessment, we expected the concentration of serum magnesium at its peak and consequently maximum efficacy of IMMIG. Using this approach, we found a relatively small increase of Mg blood levels in our patients. Administration of larger volumes of IMMIG solution would be needed to further raise the serum concentration of magnesium but this would have increased the amount of pain associated with the injections, which in turn would decrease patient acceptability and retention of patients in the study. Also, the short half-life time of parenterally administered MG necessitated repeated administration of IMMIG.²⁰ Continuous intravenous administration of MG would circumvent this problem. However, we preferred intramuscular administration,

as CRPS patients in our experience seem to be more susceptible to phlebitis during intravenous drug infusion at the site of injection. During IMM treatment a statistically significant improvement was found in pain severity as measured by an 11-point NRS. Magnesium treatment proved to be beneficial in other neuropathic pain conditions,^{4,35} probably by blocking NMDA-receptors in the dorsal horn.¹⁹ However, we consider the magnitude of change (here: 0.6) in NRS scores for pain in our study of insufficient clinical relevance, since it is much smaller than the 1.5 change in NRS (or 20% reduction) that is generally regarded as a minimal clinically important change in pain.¹¹ In a previous pilot study intravenous magnesium significantly reduced CRPS-related pain.⁷ The authors suggested that intravenous magnesium as a treatment in CRPS 1 should be further explored in a larger well-designed trial. The improvement of pain severity found in our patients supports this proposal.

In conclusion, this explanatory study revealed no evidence of a muscle relaxant effect of MG in CRPS-related dystonia. Although several limitations may impede the interpretation of our data, we feel there is insufficient support for new studies, evaluating the efficacy of other routes of MG administration in CRPS-related dystonia. Additional studies are needed to provide better insight into the structural and functional changes in muscle tissue that are associated with dystonia in CRPS which may contribute to the development of new management strategies of this disabling feature.

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Chapter 9

Summary and Conclusions

This thesis describes the results of studies on patients with CRPS which were conducted within the program of the national consortium “Trauma RElated Neuronal Dysfunction (TREND). The studies include trials on the intrathecal delivery of baclofen (*Part I*) and glycine (*Part II*), exploratory evaluations using mass spectrometry on urine and CSF (*Part III*) and an explanatory study, evaluating the muscle relaxant effect of magnesium sulphate in patients with CRPS-related dystonia (*Part IV*).

Chapter 1

Chapter 1 provides an overview of the broad clinical spectrum of CRPS. An update is given on the presumed molecular and neurophysiological basis of CRPS, including aberrant inflammation, vasomotor dysfunction and maladaptive neuroplasticity. Next, the history of the term CRPS-related dystonia is reviewed in short and controversial hypotheses about the underlying mechanism are addressed.

Chapter 2

The first part of this thesis is a sequel to a study which evaluated the efficacy of ITB on CRPS-related dystonia.¹ This study comprised a screening phase with a placebo-controlled dose-escalation study, followed by a one-year open label study. During the open label phase patients with CRPS-related dystonia received ITB in an ascending dose through an implanted pump. The severity of dystonia and global pain was assessed at baseline and every 3 months for the following year. ITB significantly improved both dystonia and global pain severity during the course of the year, especially in the first 6 months. Interestingly, this improvement of pain was only partly explained by a decrease of dystonia, suggesting a direct antinociceptive effect of baclofen.

The aim of the study described in *chapter 2* was therefore to analyze the differential effects of central gamma-aminobutyric acid B (GABA(B)) receptor stimulation on the ten pain qualities of the neuropathic pain scale (NPS). Forty-two CRPS patients with dystonia, receiving titrated doses of ITB treatment in an open design, were evaluated every three months over a period of one year. The efficacy of long-term ITB was

assessed on the ten different pain qualities of the NPS, global dystonia severity and changes in use of antinociceptive drugs. Using a linear mixed model analysis and controlling for global dystonia severity and the use of supplemental analgesics, we found a significant improvement in global intense pain, sharp pain, dull pain and deep pain over the first 6 months. After this period the scores levelled off despite further improvement of dystonia and continued ITB dose escalation. We conclude that GABA(B) receptor stimulation by ITB exerts differential antinociceptive effects on specific pain qualities in CRPS patients with dystonia.

Chapter 3

In a single-blind placebo-run-in dose-escalation study van Rijn *et al* used an external device for ITB delivery to select patients with CRPS-related dystonia for the implantation of a pump to deliver long-term continuous ITB.¹ During this screening phase patients had to improve at least 25% in global dystonia severity on a 10-point numeric rating scale on 2 consecutive days of ITB compared to placebo, in order to proceed to the pump implantation phase. Interestingly, 30% of the patients who received a pump never reached this improvement after one year of continuous ITB delivery. We noted that this decline in responsiveness to ITB was associated with a reduction of the infusion rate (IR) after the switch of drug delivery from the external to the implanted device. *Chapter 3* describes a double-blind crossover study in which we investigated the effect of varying the IR at a fixed daily dose on the efficacy and safety of ITB in patients with CRPS-related dystonia. Patients were eligible if they experienced no beneficial response to ITB on dystonia despite a minimum dose of 600 µg/day or because side effects limited dose-escalation. Patients were randomized to either slow (SIRD) or four-times faster infusion rate delivery (FIRD) for two weeks and were crossed over after a one week washout period. Outcome measures included changes in global dystonia and pain severity. We found no significant differences between the FIRD and the SIRD-group for any of the outcome measures, except for the frequency of adverse events which was significantly higher during FIRD. These adverse events mainly included supraspinal symptoms. FIRD

was preferred only by those patients who were included because side effects to ITB prevented dose escalation. Since increasing the IR at a fixed daily dose was not associated with improvement of dystonia or pain in the whole group of patients, we feel that further investigation is warranted in patients in whom side-effects prevent further dose-escalation.

Chapter 4

Chapter 4 describes the development of chorea after long-term administration of baclofen in 4 patients with CRPS-related dystonia. Worldwide, baclofen has been used for more than a century, initially in the treatment of epilepsy and since the 1960's as a first-choice drug in the treatment of spasticity.² To our knowledge baclofen-induced chorea (BIC) has only been described in 3 patients worldwide, which is a minimal fraction of all the subjects who used and still use baclofen.³⁻⁵ Remarkably, within a few years time we identified BIC in 4 CRPS patients, which is a relatively large number of people in a group of patients suffering from the same disease. Treatment with tiapride resolved BIC in these patients, suggesting that striatal dopaminergic hyperactivity underlies baclofen-induced chorea. Interestingly, BIC coincided to a large extent with body regions that were initially affected by dystonia, which supports, at least in part, the theory of central disinhibition underlying CRPS-related dystonia.

Chapter 5

Glycinergic neurotransmission plays an important inhibitory role in the processing of sensory and motor information. The aims of the study, described in *chapter 5*, were to evaluate the efficacy and safety of intrathecal glycine (ITG) administration in patients with CRPS-related movement disorders and pain. Nineteen patients were found eligible and randomized in a double-blind placebo-controlled crossover study. Patients were assigned to 4-week treatments of ITG and placebo. Treatments were separated by a one-week washout period. Safety was assessed by clinical evaluation, blood examinations and electrocardiograms. Efficacy measures involved pain, movement

disorders, activity and a global impression of perceived effect as evaluated by patient and clinician.

We found no significant differences between ITG and placebo treatment in any of the outcomes. We conclude that ITG is an ineffective treatment for pain or dystonia in CRPS, although the sample size was too small to draw definite conclusions regarding its efficacy.

Chapter 6

CRPS is characterized by various combinations of sensory, autonomic and motor disturbances, reflecting the involvement of multiple biological pathways that underlie exaggerated inflammation, vasomotor dysfunction, and maladaptive neuroplasticity. In time, the syndrome may spread to other extremities without a new trauma, especially in younger patients, which may suggest genetic predisposition. Mechanisms that play a role in this phase of the syndrome are largely unknown and even if such mechanisms are suggested, they are seldom based on solid biochemical data. The metabolome of urine provides an excellent insight into the actual biochemical status of an organism. In *chapter 6* we described the explorative analysis of urine, applying capillary electrophoresis time-of-flight mass spectrometry (CE-ToF-MS). This CE-ToF-MS method provides fast and stable metabolic profiles of urine samples. The mean intraday and interday CVs were <2% and <9% for migration times and peak areas, respectively, demonstrating robustness of the method. Using multivariate chemometric analysis, discrimination between urine samples from CRPS patients and controls was obtained, emphasizing differences in metabolic signatures between CRPS-diseased patients and controls. Several compounds, such as 3-methylhistidine, were responsible for discriminating the samples. 3-Methylhistidine is formed as a result of post-translational methylation of actin and myosin and considered a rather specific indication of catabolism of muscle tissue, a finding which has been associated with conditions like chronic physical stress, starvation, injury of muscle tissue, primary muscle diseases and

malignancy. We conclude that our results warrant further exploration of factors like physical stress and dietary intake in chronic CRPS patients.

Chapter 7

In contrast to urine, the metabolome of CSF primarily reflects the biochemical status of the central nervous system. Although, central inhibition is suggested to play a major role in the development of CRPS-related dystonia, the underpinning pathobiology is still unclear. *Chapter 7* involves an exploratory-based study, using ^1H -NMR spectroscopy, which aimed to search for metabolites in CSF that reflect common pathobiology underlying CRPS-related dystonia. CSF samples from 49 patients with CRPS-related dystonia and 56 controls were analyzed and the metabolic profiles of these groups were compared. Principal component analysis and partial least square discriminant analysis revealed significant separation between patients and controls. From the set of identified discriminators, the most relevant metabolites were 2-keto-isovalerate, alanine, creatinine, glucose, glutamine, myo-inositol, lactate, pyruvate and tyrosine which were increased in CRPS patients compared to controls and urea which was lower in CRPS patients. We hypothesize that the findings primarily reflect a catabolic state which likely is associated with inflammation. Our CSF findings therefore do not seem to provide evidence for the involvement of major primary metabolic derangements of the nervous system but instead corroborate with previous findings described in *chapter 6*.

Chapter 8

In previous chapters we described the results of studies which evaluated the efficacy of various centrally acting drugs in patients with CRPS-related dystonia. The unsatisfactory response of these agents raised the need of alternative targets for intervention. In dystonia pathological muscle changes may occur, which contribute to joint and muscle stiffness. Magnesium sulphate (MG) may act as a muscle relaxant through its actions on the neuromuscular junction and muscle. *Chapter 8* describes the results of an explanatory study in which we evaluated the muscle relaxant effect and safety of

intramuscular MG (IMMG) in CRPS patients with dystonia. In a double-blind randomized placebo-controlled crossover study 30 patients were assigned to 3-week treatments of IMMG and placebo. Treatments were separated by a one-week washout period. The daily dose of IMMG was 1000 mg in week 1, 1500 mg in week 2 and 2000 mg in week 3. The primary outcome measure was the difference in change in Burke-Fahn-Marsden (BFM) scores after 3 weeks of treatment between both interventions. Secondary outcomes involved severity of dystonia, myoclonus, tremor, pain and functional activity. Data of 22 patients available for the per protocol analysis, which revealed no significant differences between IMMG and placebo treatment in any of the outcomes. In conclusion, there is no indication of efficacy of IMMG in a daily dose of 2000 mg as a muscle relaxant in CRPS-related dystonia.

Concluding remarks

The pathobiology of CRPS-related pain has been under debate for many years and several causes have been forwarded. For many years the link between nociceptive neurons and postganglionic sympathetic activity was believed to play a pivotal role in the development and maintenance of CRPS-related pain. In the last decade accumulating evidence indicates major involvement of mechanisms of endogenous pain modulation at different levels of the neuroaxis.⁶ These mechanisms comprise 1) long-term potentiation and long-term depression of synaptic strength in the spinal dorsal horn, 2) deficient inhibition of spinal interneurons that utilize GABA, glycine or opioids, and provide feed-forward and feed-back inhibition to the spinothalamic and spinobulbar projection neurons and 3) impaired function of the descending bulbospinal pathways that inhibit or facilitate peripheral nociceptive input in the spinal dorsal horn.⁷⁻⁹ The activity of these pain modulatory systems is altered in CRPS, giving rise to a state of central sensitization and reorganization of somatotopic maps within the somatosensory cortex. These findings are based on studies which used tactile stimuli to evaluate the involvement of endogenous pain modulation in CRPS.^{6,10} We examined the efficacy of ITB on ten different qualities of spontaneous pain in CRPS patients and found a

significant improvement in severity of 4 pain qualities, which levelled off after 6 months of continuous ITB administration (*Chapter 2*). These results indicate that 1) GABA-B signalling pathways play a role in at least 4 distinctive pain qualities related to CRPS, 2) different processes may underpin the spectrum of pain components encountered in CRPS, and 3) continuous stimulation of GABA-B signalling pathways may induce desensitization. We also studied the efficacy of continuous ITG in CRPS patients and found no change in global pain sensation (*Chapter 5*). These results argue against a major involvement of spinal glycinergic signalling pathways in CRPS-related pain. However, the lack of efficacy of ITG may have resulted from an insufficient dose of ITG or compensatory increased reuptake of synaptic glycine into nerve terminals and adjacent glial cells.¹¹ A contribution of the spinal glycinergic system in the regulation of CRPS-related pain can therefore not be ruled out.

Silas Weir Mitchell thoroughly described the spectrum of pain experienced by his CRPS patients, but also recognized abnormal posturing as a distinctive feature in a subgroup of these patients.¹² It remains unclear if the abnormal postures described by Mitchell reflect contractures or dystonia. More than a century later Marsden *et al*¹³ described 5 patients who developed a movement disorder of the affected limb related to CRPS. Four of these patients suffered abnormal posturing described as sustained muscle spasms (3 patients) and dystonic posturing (1 patient). This study provides no insights with respect to the mechanism underlying the abnormal postures. In 1985 en 1986 Schott reported on a total 14 patients afflicted with various involuntary movement disorders - including focal and segmental dystonia - that developed after a physical trauma.^{14,15} Although the presence of signs and symptoms of CRPS were not reported in any of the cases, Schott's elaboration on the possible mechanisms involved in the development of movement disorders after a peripheral trauma is interesting. He suggested an interaction of the diencephalospinal dopaminergic motor system and central sensory mechanisms subserving pain to underlie these posttraumatic movement disorders.¹⁴ A few years later Jankovic and van der Linden proposed a cascade process triggered by a peripheral traumatic event starting with altered afferent input into the spinal cord or brain stem,

followed by reorganization of the local neuronal circuitry and finally enhancement of evoked and spontaneous motor output resulting in a hyperkinetic movement disorder.¹⁶ Using polyelectromyography they discovered synchronous bursts of activity in agonists and antagonists of the affected limb of patients with CRPS and dystonic posturing.¹⁶ This abnormal co-contraction of agonists and antagonists was, and still is, considered to be a fundamental characteristic of dystonia.¹⁷ In the following years several studies aimed to further clarify the pathophysiology of CRPS-related dystonia.¹⁸ The results of these studies point towards a major contribution of central processes, which largely correspond with mechanisms identified in other types of dystonia. For instance, the spontaneous spreading of dystonic posturing in many CRPS patients from one limb to the other¹⁹ is probably caused by maladaptive changes in the CNS and also found in other types of adult-onset dystonia.²⁰ In the latter types of dystonia, the process of aging is believed to enhance abnormal cortical excitability and consequently facilitate spreading of symptoms.²⁰ Neurophysiological and fMRI studies also indicate a major role of maladaptive neuroplasticity in the development and maintenance of CRPS-related dystonia.^{21,22} In primary dystonia deficient inhibition occurring at different levels of the neuraxis is also presumed to be a major pathophysiological feature.^{23,24} Three interrelated neurophysiological abnormalities have been identified in primary dystonia: 1) impaired inhibition (including surround inhibition), 2) abnormal cortical plasticity (both in affected and unaffected body regions), and 3) sensory processing dysfunction.²⁵ The efficacy of continuous ITB administration on dystonia severity in the majority of CRPS patients¹ supports the role of GABA(B) receptor signalling pathways in the pathobiology of CRPS-related dystonia. Interestingly, a subset of patients with CRPS-related dystonia did not improve on continuous ITB, regardless of the infusion rate of ITB (*chapter 3*), but some of these patients developed baclofen-induced chorea, which to a large extent coincided with body regions that were initially affected by dystonia (*chapter 4*). Treatment with tiapride resolved the chorea, suggesting that striatal dopaminergic hyperactivity underlies baclofen-induced chorea. These findings argue against a major role of GABA(B) receptor signalling pathways in dystonia in these

patients, but make a compelling case that in subtypes of CRPS, a combination of changes in cerebral circuitry and exposure to ITB may contribute to increased striatal dopaminergic hyperactivity leading to the development of chorea.

Similar to GABA, glycine is a major central inhibitory neurotransmitter, especially at the level of the spinal cord. However, we found no effect of ITG on pain and dystonia in patients with CRPS (*chapter 5*). The involvement of glycinergic signalling pathways in pain and dystonia in CRPS, however, cannot be ruled out completely for reasons mentioned above.

In spite of growing insights into the central mechanisms involved in CRPS, many aspects of processes underpinning the development and progression of CRPS-related pain and dystonia are still unclear. Several issues indicate that aside central mechanisms, peripheral mechanisms may also play a role in CRPS-related pain and dystonia. In other neuropathic pain syndromes, distinctive central and peripheral mechanisms have been identified to underlie the development of different pain qualities.²⁶ In a recent study van Rooijen *et al* found muscle hyperalgesia to be the most prominent sensory abnormality in CRPS patients. Moreover, muscle hyperalgesia was most pronounced in CRPS patients who also suffered dystonia. The authors hypothesized that sensitization of neuronal networks involved in mediating muscle nociception may play an important role in CRPS-related dystonia.²⁷ The question remains, however, if the primary neuronal changes underpinning muscle hyperalgesia in these patients are mediated at a central or peripheral level.

To identify metabolic profiles, which reflect systemic and central processes associated with the development of CRPS and CRPS-related dystonia, we examined urine (*chapter 6*) and CSF (*chapter 7*) of CRPS patients using two different metabolomics approaches. The urinary metabolome was indicative of increased muscle proteolysis, a catabolic state which is associated with conditions such as chronic physical stress and starvation. Using ¹H-NMR spectroscopy on CSF we aimed to gain more insight into central biochemical processes associated with CRPS-related dystonia, but interestingly found a metabolic profile which, similar to the urinary metabolome, corresponded largely with a

condition of muscle tissue catabolism. Such a catabolic state may impede the normal immune function and consequently play a role in the maintenance of inflammation, thus delaying the recovery of CRPS.

Pathological changes in muscle tissue may also contribute to stiffness such that the range of motion around a joint is limited.²⁸ Together with the limited efficacy and poor safety profile of intrathecal treatments investigated in CRPS patients, this feature stimulated research into muscle relaxants. We considered magnesium sulphate a good candidate drug, but in an explanatory study found no evidence of a muscle relaxant effect of magnesium sulphate in CRPS-related dystonia (*chapter 8*).

Future perspectives

In this thesis we aimed to obtain better understanding of the pathophysiology underpinning dystonia in CRPS. Though our studies generated new insights on the central and peripheral processes underpinning this movement disorder, many new questions arose, which need to be addressed in future studies.

First of all, molecular and neurophysiological aspects underpinning the different pain qualities found in CRPS patients need further exploration in order to develop more efficacious treatments. Current understanding of pathophysiological mechanisms of similar pain qualities found in other neuropathic pain syndromes may add to our knowledge on the pathophysiology of CRPS-related pain and provide a basis for future diagnostic and therapeutic CRPS studies.²⁹

In order to gain better insight into the central pathobiology involved in CRPS-related dystonia, several techniques are currently available and potentially may provide new insights. In vivo proton MR spectroscopy of the brain can be used for identification of different compound spectra - including excitatory and inhibitory neurotransmitters - in predefined regions of interest.³⁰⁻³² Another technique involves pharmacologic magnetic resonance imaging, which uses receptor specific ligands to i) characterize brain circuitry specific to neurotransmitter systems; ii) measure parameters reflecting neurotransmitter release and binding associated with the pharmacokinetics and/or the

pharmacodynamics of drugs; and iii) measure up- and down-regulation of receptors in specific disease states.³³

Knowledge on the pathobiology involved in CRPS-related dystonia is also a prerequisite for the development of more efficacious treatment. ITB may result in improvement of dystonia in CRPS patients but this treatment fails in 30% of cases and is associated with important adverse events.¹ Though the results of ITB underscore the role of GABA-B receptor signalling pathways in dystonia of patients with CRPS, the large number of adverse events associated with intrathecal delivery in these patients raises the need for GABA-B receptor agonists with a better blood-brain barrier passage.

We also recommend further exploration of neurophysiological aspects of CRPS-related dystonia. Transcranial magnetic stimulation (TMS) is a non-invasive technique, which allows the assessment of sensorimotor integration, cortical motor representation and excitability of the motor system, i.e. motor threshold, intracortical inhibition and facilitation. Several studies have employed TMS in CRPS patients and found abnormal cortical motor representation,³⁴ bilateral motor cortex disinhibition,³⁵ and normal sensorimotor interaction at a cortical level.³⁶ Although motor signs were reported in many of the 47 patients described in these studies,³⁴⁻³⁶ dystonia was described in only one patient. Hence, it would be interesting to know whether the same TMS results are found in patients with CRPS-related dystonia. When comparing CRPS patients with and without dystonia, questions have to be answered such as: Are maladaptive changes in cortical sensorimotor areas more prominent in patients with dystonia? Confirmative answers will support the hypothesis of a strong contribution of central processes in CRPS-related dystonia, while negative results would favor a more important role of peripheral processes. Additionally, TMS studies may contribute to our understanding of differential mechanism involved in more typical mobile dystonia as compared to fixed dystonia in patients with CRPS.³⁷

Transcranial direct current stimulation modulates cortical excitability and may also provide a therapeutic alternative. Repetitive transcranial magnetic stimulation proved to be efficacious as an add-on to pharmacological therapy in patients with CRPS.^{38,39} The

therapeutic potential of TMS varies between movement disorders not related to CRPS, with no improvement in patients with writer's cramp⁴⁰ and good outcome in patients with blepharospasm.⁴¹ Possibly, the area of stimulation is of importance as the motor cortex was stimulated in the writer's cramp study and the anterior cingulate cortex was stimulated in the blepharospasm study.

The involvement of psychological factors in the onset and maintenance of CRPS-related dystonia is still under debate. However, CRPS patients do not exhibit particular psychological traits or a unique psychological profile before or around the period of disease onset, which would make them susceptible to develop and maintain abnormal postures.⁴² Furthermore, the efficacy of ITB in patients with CRPS-related dystonia was established in a single-blind placebo-run-in dose-escalation study¹ and in several trials we found no detectable placebo response (*chapters 3, 5 and 8*). Hence we consider a psychogenic cause as a sole explanation for CRPS-related dystonia unlikely. Compelling evidence indicates that aberrant reorganisation of sensory and motor networks may occur in patients with CRPS.^{6,10,21} Such changes are considered to be responsible for delayed recognition of hand laterality,^{43,44} impaired self-perception of the affected limb,⁴⁵ distorted body image and body perception disturbances in CRPS.⁴⁶ Planning and execution of movement depend on mapping of sensory input onto representations of intended movement.⁴⁷ Functional changes of sensory and motor networks may also play a role in the development and maintenance of movement disorders in CRPS. Interestingly, abnormal processing in non-motor brain regions is already considered to play a major role in the development of primary dystonia.⁴⁸ Possibly, these insights may contribute to a better understanding of currently so-called psychogenic or functional movement disorders. Although the aforementioned arguments support a somatic explanation as underlying cause of CRPS-related dystonia, the discussion in the literature is far from settled; the controversy as to whether the condition originates from a somatic or psychogenic cause (or both) is still ongoing. Many researchers hold the view that the CRPS-related dystonia – 'fixed dystonia' in particular - is a psychogenic movement disorder.^{49,50}

Future studies are also warranted to examine the role of peripheral processes, particularly muscle energy metabolism, in the development and maintenance of CRPS-related dystonia.⁵¹ Especially studies using MR spectroscopy may provide useful data on the energy status of muscle tissue in CRPS patients with dystonia and together with neurophysiological studies may clarify the role of the contractile apparatus in the development of abnormal posturing (not related to contracture) in CRPS patients. Is abnormal posturing caused by abnormal co-contraction of agonists and antagonists, which we define as dystonia, or the result of deficient decoupling of myosin/actin filaments, e.g. because of low ATP production, or is there another underlying process? In a recent electromyographic study Bank *et al* demonstrated that abnormal posturing in chronic CRPS patients - at least in those patients with some active range of motion (AROM) - was not associated with sustained muscle contractions in the affected limb and the AROM was not restricted because of excessive agonist-antagonist co-contraction.⁵²

These findings as well as those of studies presented in this thesis, underline that in different CRPS patients or during different stages of the disease, different mechanisms may contribute to the development or maintenance of abnormal postures in these patients. We therefore suggest just using the term 'abnormal posturing' in CRPS patients, at least until the role of muscular mechanisms has been clarified. The lack of evidence of a muscle relaxant effect of magnesium sulphate, which we found in an explanatory study, does not invalidate this recommendation as only a small component of the contractile system is targeted by magnesium sulphate and pharmacokinetic limitations may be responsible for the lack of efficacy.

New studies are also warranted to answer questions such as: Are the findings in our mass-spectrometry studies, which hint at increased muscle protein breakdown, just the result of chronic immobilization or malnourishment secondary to the disease burden that CRPS patients experience, or are they closely related to intrinsic pathophysiological processes? Does muscle proteolysis manifest in the later stages of the disease or is it already present in the early phase? These questions need to be addressed in the near

future, not only as muscle protein breakdown negatively affects the human immune status but also because it may be reversed by adequate nutritional care and feeding. It is most likely that different processes at the peripheral and central level influence each other. The interaction of these processes is probably responsible for the heterogeneous clinical spectrum of CRPS and the onset and progress CRPS symptoms. To gain more insight into this interplay of central and peripheral processes, we recommend future studies to evaluate for each subject both changes in central and peripheral processing.

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Chapter 10

Samenvatting en Conclusies

Dit proefschrift beschrijft de resultaten van studies bij patiënten met complex regionaal pijnsyndroom (CRPS) die werden uitgevoerd binnen het programma van het landelijk consortium “Trauma RElated Neuronal Dysfunction” (TREND). De studies betreffen onderzoeken met intrathecale toediening van baclofen (*Deel I*) en glycine (*Deel II*), exploratieve evaluaties met behulp van massa-spectrometrie van urine en hersenvocht (*Deel III*) en een verklarende studie, waarin het spierverslappende effect van magnesiumsulfaat is onderzocht bij patiënten met dystonie gerelateerd aan CRPS (*Deel IV*).

Hoofdstuk 1

Hoofdstuk 1 geeft een overzicht van het uitgebreide scala aan klinische verschijnselen die bij CRPS wordt gezien. Er wordt een update gegeven over de veronderstelde moleculaire en neurofysiologische basis van CRPS, waaronder buitensporige ontstekingsreactie, vasomotorisch disfunctioneren en maladaptieve neuroplasticiteit. Vervolgens wordt in het kort een terugblik gegeven op de terminologie die door de jaren heen voor CRPS-gerelateerde dystonie is gebruikt en controversiële hypothesen over het onderliggende mechanisme van deze vorm van dystonie worden aangekaart.

Hoofdstuk 2

Het eerste deel van dit proefschrift is een vervolg op een studie, waarin de effectiviteit van intrathecaal baclofen (ITB) op CRPS-gerelateerde dystonie is onderzocht.¹ Deze studie bestond uit een placebogecontroleerde screeningsfase met opklimmende dosis, gevolgd door een ‘open label’ studie voor de duur van één jaar. Tijdens de ‘open label’ fase kregen patiënten met CRPS-gerelateerde dystonie via een geleidelijke dosisopbouw ITB toegediend met behulp van een geïmplanteerde pomp. De ernst van de dystonie en de globale pijn werd beoordeeld vlak voordat ITB gestart werd en vervolgens om de 3 maanden voor de duur van één jaar. ITB resulteerde in de loop van het jaar in significante verbetering van zowel de ernst van dystonie als van globale pijn, vooral tijdens de eerste 6 maanden. Interessant is, dat verbetering van pijn slechts ten dele

verklaard kon worden door vermindering van de dystonie, wat wijst op een direct antinociceptieve werking van baclofen.

Het doel van de studie beschreven in *hoofdstuk 2* was om het gedifferentieerde effect van centraal gamma-aminoboterzuur B (GABA(B)) receptorstimulatie te analyseren op de tien pijnkwaliteiten van de neuropathische pijnschaal (NPS). Tweeënveertig CRPS-patiënten met dystonie die ongeblindeerd in getitreerde doses ITB kregen, werden iedere 3 maanden geëvalueerd voor de duur van één jaar. De effectiviteit van langdurig toegediend ITB werd beoordeeld op de tien verschillende pijnkwaliteiten van de NPS, op globale ernst van dystonie en op veranderingen in gebruik van pijnmedicatie. Aan de hand van 'linear mixed model' analyse met correctie voor globale ernst van dystonie en gebruik van aanvullende analgetica, vonden we significante verbetering van globale intense pijn, scherpe pijn, zeurende pijn en diepe pijn gedurende de eerste 6 maanden. Na deze periode vlakten de scores af ondanks verdere verbetering van dystonie en aanhoudende verhoging van de ITB dosis. Onze conclusie is, dat GABA(B) receptorstimulatie via ITB gedifferentieerde antinociceptieve effecten genereert op specifieke pijnkwaliteiten bij CRPS-patiënten met dystonie.

Hoofdstuk 3

Van Rijn *et al* hadden in een enkelblinde placebo-inloop studie met opklimmende dosis een extern systeem gebruikt om ITB toe te dienen om zo patiënten met CRPS-gerelateerde dystonie te selecteren voor implantatie van een pomp voor langdurige continue ITB-administratie.¹ Tijdens de screeningsfase moesten patiënten op 2 achtereenvolgende dagen van ITB-toediening ten opzichte van placebo tenminste 25% verbeteren in globale ernst van dystonie op een numerieke schaal die loopt van 0 tot 10, om door te kunnen gaan naar de fase van pompimplantatie. Interessant genoeg bleken 30% van de patiënten die een pomp geïmplantéerd kregen nooit deze verbetering te halen tijdens een jaar van continue ITB-toediening. Het viel ons op, dat deze afname in reactiviteit op ITB was geassocieerd met een afname in infusiesnelheid (IS) na omschakeling van toediening met een externe naar een geïmplantéerde pomp.

Hoofdstuk 3 beschrijft een dubbelblinde cross-over studie, waarin we het effect van variabele infusiesnelheden bij een constant gehouden dagdosis onderzochten op effectiviteit en veiligheid van ITB bij patiënten met CRPS-gerelateerde dystonie. Patiënten waren geschikt voor studiedeelname als zij geen gunstig effect van ITB ervaarden op dystonie bij een dosis van tenminste 600 µg/dag of omdat bijwerkingen dosisverhoging verhinderden. Patiënten werden gerandomiseerd naar een langzame (LIS) of een 4 keer snellere infusiesnelheid (SIS) voor de duur van 2 weken en continueerden vervolgens na 1 week 'washout' met de andere IS. Veranderingen in globale ernst van dystonie en pijn golden als uitkomstmaten. We vonden voor geen enkele uitkomstmaat significante verschillen tussen de SIS en de LIS-groep, behalve voor het aantal nadelige effecten die significant hoger was tijdens de SIS-behandeling. Deze nadelige effecten betroffen voornamelijk supraspinale symptomen. SIS kreeg de voorkeur van alle patiënten die waren geïnccludeerd, omdat bijwerkingen verhoging van de ITB-dosis hadden verhinderd. Hoewel verhoging van de IS bij een constant gehouden dagdosis niet was geassocieerd met verbetering van dystonie of pijn bij de groep van patiënten als geheel, menen we dat verder onderzoek gerechtvaardigd is bij patiënten die dosisverhoging vanwege bijwerkingen niet tolereren.

Hoofdstuk 4

Hoofdstuk 4 beschrijft de ontwikkeling van chorea na langdurige administratie van baclofen bij 4 patiënten met CRPS-gerelateerde dystonie. Wereldwijd wordt baclofen al meer dan 100 jaar gebruikt, aanvankelijk als behandeling tegen epilepsie en sinds de jaren 60 als eerste keuze middel bij de behandeling van spasticiteit.² Voor zover ons bekend, is baclofen-geïnduceerde chorea (BIC) wereldwijd ooit eerder beschreven bij slechts 3 patiënten, wat een zeer beperkte fractie is van alle mensen die baclofen hebben gebruikt of nog steeds gebruiken.³⁻⁵ Het is daarom opvallend, dat wij binnen een beperkt aantal jaren 4 CRPS patiënten hebben geïdentificeerd, die BIC hebben ontwikkeld, wat een relatief groot aantal is van de totale populatie mensen die leidt aan dezelfde ziekte. Behandeling met tiapride zorgde voor verdwijnen van de BIC bij deze

mensen. Dit maakt aannemelijk, dat striatale dopaminerge hyperactiviteit ten grondslag ligt aan de BIC. Een interessante bevinding is, dat de verdeling van BIC in belangrijke mate correspondeerde met de distributie van dystonie, waarmee in ieder geval ten dele de theorie van centrale disinhibitie als onderliggend mechanisme van CRPS-gerelateerde dystonie wordt ondersteund.

Hoofdstuk 5

Glycinerge neurotransmissie speelt een belangrijke inhiberende rol bij de verwerking van sensibele en motorische informatie. Het doel van de studie beschreven in *hoofdstuk 5* was om de effectiviteit en veiligheid van intrathecaal glycine (ITG) bij patiënten met CRPS-gerelateerde bewegingsstoornissen en pijn te evalueren. Negentien patiënten werden geschikt bevonden om deel te nemen aan de studie en werden gerandomiseerd in een dubbelblinde placebogecontroleerde cross-over studie. Aan patiënten werd 4 weken behandeling met ITG en 4 weken placebo toegewezen. Beide behandelingen werden gescheiden door een washout periode van 1 week. Veiligheid werd beoordeeld aan de hand van klinische evaluatie, bloedonderzoeken en electrocardiografie. Effectiviteitsmaten bestonden uit pijn, bewegingsstoornissen, activiteit en een globale inschatting van het doorgemaakte effect door patiënt en arts.

We vonden voor geen enkele uitkomstmaat significante verschillen tussen ITG- en placebobehandeling. Onze conclusie is, dat ITG niet effectief is tegen pijn of dystonie bij CRPS, hoewel het aantal studiedeelnemers te klein was om definitieve conclusies te trekken ten aanzien van de mate van effectiviteit.

Hoofdstuk 6

CRPS wordt gekarakteriseerd door diverse combinaties van sensibele, autonome en motorische afwijkingen, welke een weergave vormen van betrokkenheid van meerdere biologische processen die ten grondslag liggen aan disproportionele ontstekingsreactie, vasomotorische dysfunctie en maladaptieve neuroplasticiteit. In de loop van de tijd kan het syndroom uitbreiden naar andere ledematen zonder voorafgaand nieuw trauma,

vooral bij jongere patiënten, wat een genetische predispositie zou kunnen veronderstellen. Mechanismen die in deze fase van het syndroom een rol spelen zijn grotendeels onbekend en als er al een bepaalde rol voor deze mechanismen wordt verondersteld, dan is dit zelden gebaseerd op zuivere biochemische data-extractie. Het metabooloom van urine geeft uitstekend inzicht in de actuele biochemische toestand van een organisme. In *hoofdstuk 6* beschrijven we de exploratieve analyse van urine, uitgevoerd door middel van capillaire electroforese ‘time-of-flight’ massa-spectrometrie (CE-ToF-MS). Deze CE-ToF-MS methode genereert snelle en stabiele metabole profielen van urinemonsters. De gemiddelde ‘intraday’ en ‘interday’ CV’s waren respectievelijk <2% en <9% voor migratietijd en piekgebieden, waarmee het robuuste karakter van de techniek is aangetoond. Met behulp van multivariate chemometrische analyse konden urinemonsters van CRPS-patiënten en controles van elkaar worden onderscheiden en daarmee de verschillen in metabole profielen tussen CRPS-patiënten en controles worden uitgelicht. Verscheidene substraten, waaronder 3-methylhistidine, lagen ten grondslag aan de verschillen tussen de monsters van beide groepen mensen. 3-Methylhistidine is het product van posttranslationale methylering van actine en myosine en wordt beschouwd als een tamelijk specifieke weergave van katabolisme van spierweefsel, een bevinding die is geassocieerd met condities zoals chronische fysieke stress, ondervoeding, schade aan spierweefsel, primaire spierziekten en maligniteit. We concluderen, dat onze resultaten verdere exploratie legitimeren naar factoren zoals fysieke spanningen en voedselconsumptie bij patiënten met chronisch CRPS.

Hoofdstuk 7

In tegenstelling tot urine vormt het metabooloom van hersenvocht voornamelijk een afspiegeling van de biochemische status van het centrale zenuwstelsel. Hoewel verondersteld wordt, dat centrale inhibitie een belangrijke rol speelt bij de ontwikkeling van CRPS-gerelateerde dystonie, is de onderliggende pathobiologie nog steeds niet opgehelderd. *Hoofdstuk 7* beslaat een studie met een exploratieve basis, waarin gebruik gemaakt wordt van ^1H -NMR spectroscopie met als doel om metabolieten in hersenvocht

aan te tonen die een reflectie vormen van de gezamenlijke pathobiologie van CRPS-gerelateerde dystonie. Monsters van hersenvocht van 49 patiënten met CRPS-gerelateerde dystonie en 56 controles werden geanalyseerd en de metabole profielen van beide groepen werden met elkaar vergeleken. Principale componentanalyse en partiële kleinste kwadraten discriminante analyse toonden significante separatie tussen patiënten en controles. Van de set van geïdentificeerde onderscheidende metaboliëten waren de meest relevanten: verhoogde concentraties van 2-keto-isovaleraat, alanine, creatinine, glucose, glutamine, myo-inositol, lactaat, pyruvaat en tyrosine bij CRPS-patiënten ten opzichte van controles en verlaagde concentratie van ureum bij patiënten met CRPS. Onze hypothese is, dat deze bevindingen voornamelijk een katabole conditie representeren die waarschijnlijk geassocieerd is met ontsteking. Onze bevindingen in hersenvocht leveren dus geen bewijs voor de betrokkenheid van een belangrijke primaire metabole ontregeling in het centrale zenuwstelsel, maar sluiten eerder aan op de bevindingen zoals beschreven in *hoofdstuk 6*.

Hoofdstuk 8

In voorgaande hoofdstukken beschreven we de resultaten van studies, waarin de effectiviteit van diverse centraal-opererende middelen bij patiënten met CRPS-gerelateerde dystonie werd geëvalueerd. Het onbevredigende resultaat van deze middelen ondersteunde de focus op alternatieve aangrijpingspunten voor nieuwe interventies. Bij dystonie kunnen pathologische spierveranderingen optreden die bijdragen aan stijfheid van gewrichten en spieren. Magnesiumsulfaat (MG) zou als spierverslapper kunnen acteren door aan te grijpen op neuromusculaire structuren of structuren binnen de spier zelf. *Hoofdstuk 8* beschrijft de resultaten van een verklarende studie, waarin we het spierverslappende effect en de veiligheid van intramusculair MG (IMMG) evalueerden bij CRPS-patiënten met dystonie. In een dubbelblinde gerandomiseerde placebogecontroleerde cross-over studie werd aan 30 patiënten een behandeling van 3 weken met IMMG en placebo toegewezen. Beide behandelingen werden gescheiden door een washout periode van een week. De dagdosis IMMG was

1000 mg in week 1, 1500 mg in week 2 en 2000 mg in week 3. De primaire uitkomstmaat was het verschil in verandering van de Burke-Fahn-Marsden (BFM) scores gedurende 3 weken van behandeling tussen beide interventies. Secundaire uitkomstmaten betroffen ernst in dystonie, myoclonus, tremor, pijn en functionele activiteit. Data van 22 patiënten waren beschikbaar voor analyse 'per protocol' en onthulden voor geen van de uitkomstmaten significante verschillen tussen IMMIG- en placebobehandeling. De conclusie is, dat er geen aanwijzingen zijn voor een spierverslappend effect van IMMIG bij een dagelijkse dosis oplopend tot 2000 mg op CRPS-gerelateerde dystonie.

Concluderende opmerkingen

Over de pathobiologie van pijn gerelateerd aan CRPS wordt al vele jaren gediscussieerd en verscheidene oorzaken zijn al geopperd. Gedurende lange tijd werd aangenomen, dat de connectie tussen nociceptieve neuronen en postganglionaire sympathische activiteit essentieel was in de ontwikkeling en aanhouden van CRPS-gerelateerde pijn. In de laatste 10 jaar kwam er een opeenstapeling van bewijs voor betrokkenheid van mechanismen van endogene pijnmodulatie op verschillende niveaus van het centrale zenuwstelsel.⁶ Deze mechanismen omvatten 1) langdurige potentiatie en langdurige onderdrukking van synapssterkte in de achterhoorn van het ruggenmerg, 2) gestoorde inhibitie van spinale interneuronen die gebruik maken van GABA, glycine of opioïden en zorgen voor ‘feed-forward’ en ‘feed-back’ remming naar spinothalaam en spinobulbair projecterende neuronen en 3) gestoorde functie van afdalende bulbospinale banen die perifere nociceptieve input in de achterhoorn van het ruggenmerg inhiberen of stimuleren.⁷⁻⁹ De activiteit van deze pijnmodulerende systemen is veranderd bij CRPS resulterend in centrale sensitisatie en reorganisatie van de somatotopische atlas binnen de somatosensorische cortex. Deze bevindingen zijn gebaseerd op studies, waarin gebruik is gemaakt van tactiele stimuli om de betrokkenheid van endogene pijnmodulatie bij CRPS te evalueren.^{6,10} Wij onderzochten de effectiviteit van ITB op 10 verschillende kwaliteiten van spontane pijn bij patiënten met CRPS en vonden een significante verbetering in ernst van 4 pijnkwaliteiten, die stagneerde na 6 maanden continue administratie van ITB (*Hoofdstuk 2*). Deze resultaten laten zien, dat 1) GABA(B)-erge zenuwbanen betrokken zijn bij tenminste 4 verschillende pijnkwaliteiten gerelateerd aan CRPS, 2) verschillende processen ten grondslag kunnen liggen aan het spectrum van pijncomponenten die worden gevonden bij CRPS en 3) continue stimulatie van GABA(B)-modulerende banen desensitisatie kan induceren. We bestudeerden ook de effectiviteit van continue ITB-toediening bij CRPS-patiënten en vonden geen verandering in globale pijnsensatie (*Hoofdstuk 5*). Deze resultaten pleiten tegen een grote rol voor spinale glycinerge modulerende banen bij CRPS-gerelateerde pijn. Echter de tekortkoming in effectiviteit van ITG zou te maken kunnen hebben met een

onvoldoende dosis ITG of compensatoir verhoogde heropname van glycine vanuit de synaps in zenuwuiteinden en aangelegene gliale cellen.¹¹ Een bijdrage van het spinale glycinerge systeem aan de regulatie van CRPS-gerelateerde pijn kan daarom niet worden uitgesloten.

Silas Weir Mitchell gaf een zorgvuldige beschrijving van het scala aan pijnsoorten die door zijn patiënten met CRPS werd ervaren, maar herkende daarbij opvallende standsafwijkingen bij een deel van deze patiënten.¹² Het blijft onduidelijk of de afwijkende standen die Mitchell beschreef een uiting waren van contracturen of dystonie. Meer dan 100 jaar later beschreven Marsden *et al*¹³ 5 patiënten die een bewegingsstoornis ontwikkelden aan een ledemaat met verschijnselen van CRPS. Bij 4 van deze patiënten werd de standsafwijking omschreven als aanhoudende spierspasmen (3 patiënten) en een dystone stand (1 patiënt). De studie leverde geen inzicht over het mechanisme die ten grondslag ligt aan de afwijkende standen. In 1985 en 1986 beschreef Schott een totaal van 14 patiënten met verschillende ongewilde bewegingen, waaronder focale en segmentale dystonie die waren ontstaan in aansluiting op een fysiek trauma.^{14,15} Hoewel de aanwezigheid van CRPS-symptomen (anamnestisch en objectiveerbaar) bij geen enkele casus werd beschreven, is de uiteenzetting van Schott over de mechanismen die mogelijk betrokken zijn bij de ontwikkeling van de ongewilde bewegingen na een perifeer trauma interessant. Hij suggereerde, dat een interactie van het diencefalospinale dopaminerge motorsysteem en centrale sensibele mechanismen, gerelateerd aan pijn, ten grondslag ligt aan deze posttraumatische bewegingsstoornissen.¹⁴ Enkele jaren later lanceerden Jankovic en van der Linden de hypothese van een cascade van processen getriggered door een perifere traumatische gebeurtenis, beginnend met veranderde afferente input in ruggenmerg of hersenstam, gevolgd door reorganisatie het lokale neuronale circuit en uiteindelijk versterking van opgewekte of spontane motore output resulterend in een hyperkinetische bewegingsstoornis.¹⁶ Met behulp van polyelectromyografie ontdekten zij synchrone ontladingen van activiteit in agonist- en antagonistspieren van de aangedane ledemaat van patiënten met CRPS en dystone standsafwijkingen.¹⁶ Deze

abnormale co-contractie van agonisten en antagonisten werd, en wordt nog steeds, beschouwd als een fundamentele eigenschap van dystonie.¹⁷ In de hieropvolgende jaren was het doel in verschillende studies om de pathofysiologie van CRPS-gerelateerde dystonie verder op te helderen.¹⁸ De resultaten van deze studies wijzen op belangrijke betrokkenheid van centrale processen die grotendeels corresponderen met mechanismen die ook geïdentificeerd zijn bij andere typen dystonie. Bijvoorbeeld de spontane uitbreiding van dystone standen bij veel CRPS-patiënten van de ene extremiteit naar de andere¹⁹ wordt waarschijnlijk veroorzaakt door maladaptieve veranderingen in het centrale zenuwstelsel en ook gevonden bij andere typen dystonie die op volwassen leeftijd ontstaan.²⁰ Voor wat betreft de laatstgenoemde vormen van dystonie wordt verondersteld, dat het proces van veroudering abnormale corticale prikkelbaarheid versterkt en daarmee verspreiding van symptomen faciliteert.²⁰ Neurofysiologische en fMRI-studies laten ook een belangrijke rol zien voor maladaptieve neuroplasticiteit bij de ontwikkeling en aanhouden van CRPS-gerelateerde dystonie.^{21,22} Bij primaire dystonie wordt er ook vanuit gegaan, dat deficiënte inhibitie binnen verschillende niveaus van het ruggenmerg een belangrijk pathofysiologisch fenomeen is.^{23,24} Drie aan elkaar gerelateerde neurofysiologische abnormaliteiten zijn geïdentificeerd bij primaire dystonie: 1) gestoorde inhibitie, waaronder uitgebreidere inhibitie in de omgeving of 'surround inhibition', 2) abnormale corticale plasticiteit, zowel betreffende aangedane als niet-aangedane lichaamsgebieden en 3) dysfunctie van sensibele signaalverwerking.²⁵ De effectiviteit van continue ITB-toediening op de ernst van dystonie bij het merendeel van patiënten met CRPS¹ ondersteunt de rol van GABA(B)-receptormodulerende banen binnen de pathobiologie van CRPS-gerelateerde dystonie. Het is interessant om te zien, dat een deel van patiënten met CRPS-gerelateerde dystonie geen verbetering toonde op continue ITB-administratie, onafhankelijk van de infusiesnelheid (*Hoofdstuk 3*). Echter enkelen van deze patiënten ontwikkelden wel baclofen-geïnduceerde chorea, waarvan de verdeling in grote mate correspondeerde met lichaamsregio's, waarin aanvankelijk dystonie was ontstaan (*Hoofdstuk 4*). Met behandeling met tiapride verdween de chorea. Hiermee kan

verondersteld worden, dat striatale dopaminerge hyperactiviteit ten grondslag ligt aan baclofen-geïnduceerde chorea. Deze bevindingen pleiten tegen belangrijke betrokkenheid van GABA(B)-receptormodulerende banen bij de totstandkoming van dystonie bij deze patiënten, maar maken het wel aannemelijk, dat bij bepaalde subtypen CRPS een combinatie van veranderingen in cerebrale circuits en blootstelling aan ITB zou kunnen bijdragen aan toegenomen striatale dopaminerge hyperactiviteit resulterend in de ontwikkeling van chorea.

Net als GABA is glycine een belangrijke centraal remmende neurotransmitter, voornamelijk op het niveau van ruggenmerg. Echter we vonden geen effect van ITG op pijn en dystonie bij patiënten met CRPS (*Hoofdstuk 5*). De betrokkenheid van glycinerge modulerende zenuwbanen bij CRPS-gerelateerde pijn en dystonie kan echter niet volledig worden uitgesloten op basis van argumenten die al eerder genoemd zijn.

Ondanks het groeiende inzicht in de centrale mechanismen die een rol spelen bij CRPS, blijven veel aspecten van processen die ten grondslag liggen aan de ontwikkeling en progressie van CRPS-gerelateerde pijn en dystonie nog steeds onduidelijk. Verschillende bevindingen wijzen erop, dat naast centrale mechanismen ook perifere mechanismen betrokken zijn bij pijn en dystonie gerelateerd aan CRPS. Bij andere neuropathische pijnsyndromen zijn al diverse centrale en perifere mechanismen onderliggend aan verschillende pijnkwaliteiten aangetoond.²⁶ In een recente studie van van Rooijen *et al* werd spierhyperalgesie als belangrijkste sensibele afwijking gevonden bij patiënten met CRPS. Bovendien bleek spierhyperalgesie het meest op de voorgrond te staan bij CRPS-patiënten die ook leden aan dystonie. De auteurs stelden de hypothese, dat sensitisatie van neuronale netwerken betrokken bij de verwerking van spiernociceptie een voorname rol speelt bij CRPS-gerelateerde dystonie.²⁷ De vraag blijft echter staan of de primaire neuronale veranderingen die spierhyperalgesie veroorzaken worden gemedieerd op een centraal of perifeer niveau.

Om metabole profielen in kaart te brengen die een weergave vormen van systemische en centrale processen, geassocieerd met ontwikkeling van CRPS en CRPS-gerelateerde dystonie, onderzochten we urine (*Hoofdstuk 6*) en hersenvocht (*Hoofdstuk 7*) van CRPS

patiënten aan de hand van 2 verschillende metabolomics-technieken. Het metaboloom van urine leverde aanwijzingen voor een toegenomen proteolyse in spierweefsel, een katabole toestand die wordt gezien bij condities als chronische fysieke stress en verhongering. Door middel van ^1H -NMR spectroscopie onderzoek op hersenvocht beoogden we meer inzicht te krijgen in centrale biochemische processen die geassocieerd zijn met CRPS-gerelateerde dystonie, maar interessant genoeg vonden een metabool profiel die vergelijkbaar is met het eerder beschreven metaboloom in urine en grotendeels correspondeert met een toestand van spierkatabolisme. Een dergelijke katabole toestand kan de normale immuunfunctie negatief beïnvloeden en daarmee een belangrijke rol spelen bij het in standhouden van inflammatie, waarmee herstel van CRPS vertraagd wordt.

Pathologische veranderingen in spierweefsel kunnen ook een bijdrage leveren aan stijfheid van spieren, waardoor de bewegingsuitslag van een gewricht beperkt wordt.²⁸ Samen met de beperkte effectiviteit en het povere veiligheidsprofiel van intrathecale behandelingen bij patiënten met CRPS vormde dit een basis om het effect van spierrelaxerende middelen te onderzoeken. We beschouwden magnesiumsulfaat een goede kandidaat, maar vonden in een verklarende studie geen bewijs voor een spierverslappend effect van magnesiumsulfaat op CRPS-gerelateerde dystonie.

Toekomstperspectieven

In dit promotie-onderzoek hadden we als doel om de pathofysiologie die ten grondslag ligt aan dystonie bij CRPS op te helderen. Hoewel onze studies nieuwe inzichten hebben gegenereerd in de centrale en perifere processen die deze bewegingsstoornis veroorzaken, zijn er veel nieuwe vragen ontstaan die in toekomstige studies aandacht verdienen.

In de eerste plaats moleculaire en neurofysiologische aspecten die de oorsprong vormen van verschillende pijnkwaliteiten dienen verder onderzocht te worden om vervolgens meer effectieve behandelingen te kunnen ontwikkelen. De huidige kennis over pathofysiologische mechanismen van vergelijkbare pijnkwaliteiten bij andere neuropathische pijnsyndromen kan een bijdrage leveren in ons begrip over de pathofysiologie van CRPS-gerelateerde pijn en een basis vormen voor toekomstige diagnostische en therapeutische studies.²⁹

Om meer inzicht te krijgen in de betrokkenheid van centrale pathobiologie bij CRPS-gerelateerde dystonie, zijn er momenteel diverse potentiële technieken beschikbaar. In vivo proton MR-spectroscopie van de hersenen kan worden gebruikt om de verschillende spectra van bestanddelen, zoals excitatoire en inhibitoire neurotransmitters, in vooraf bepaalde gebieden in kaart te brengen.³⁰⁻³² Een andere techniek betreft farmacologische MRI die gebruik maakt van receptorspecifieke liganden om 1) cerebrale circuits die geassocieerd zijn met specifieke neurotransmittersystemen te karakteriseren, 2) parameters vast te leggen die een reflectie zijn van het vrijkomen en de binding van neurotransmitters en die geassocieerd zijn met farmacokinetiek en/of farmacodynamiek van medicijnen en 3) meting van up- en downregulatie van receptoren bij specifieke aandoeningen.³³

Kennis over de pathobiologie van CRPS-gerelateerde dystonie is ook een vereiste om meer effectieve behandelingen te kunnen ontwikkelen. ITB kan verbetering geven van dystonie bij CRPS-patiënten, maar deze behandeling blijkt bij 30% van de patiënten niet te werken en geassocieerd te zijn met belangrijke nadelige effecten.¹ Hoewel de resultaten van ITB de betrokkenheid van GABA-receptormodulatie bij CRPS-gerelateerde

dystonie onderstrepen, maakt het voorkomen van het grote aantal nadelige effecten door intrathecale behandeling bij deze patiënten de ontwikkeling van GABA(B)-receptoragonisten met een betere bloed-hersenbarrière gewenst.

We bevelen ook verdere exploratie aan van neurofysiologische aspecten van CRPS-gerelateerde dystonie. Transcraniële magnetische stimulatie (TMS) is een niet-invasieve techniek die beoordeling van sensorimotorische integratie, corticale motorrepresentatie en prikkelbaarheid van het motorsysteem, ook wel drempel naar motorische activiteit, intracorticale inhibitie en facilitatie mogelijk maakt. Verscheidene studies hebben TMS toegepast op CRPS-patiënten en vonden abnormale corticale motorrepresentatie,³⁴ bilaterale motor cortex disinhibitie³⁵ en normale sensorimotorische interactie op corticaal niveau.³⁶ Hoewel motorische verschijnselen zijn beschreven bij veel van de 47 patiënten in deze studies,³⁴⁻³⁶ werd slechts bij 1 patiënt melding gemaakt van dystonie. Het zou derhalve interessant zijn om te weten of dezelfde TMS-resultaten ook worden gevonden bij patiënten met CRPS-gerelateerde dystonie. Als we CRPS-patiënten met en zonder dystonie met elkaar vergelijken, zullen vragen moeten worden beantwoord, zoals zijn maladaptieve veranderingen in corticale sensorimotorische gebieden meer prominent aanwezig bij patiënten met dystonie? Resultaten die dit bevestigen zullen de hypothese ondersteunen, dat er een sterke bijdrage is van centrale processen bij CRPS-gerelateerde dystonie, terwijl negatieve resultaten daar en tegen eerder zouden wijzen op een belangrijke rol voor perifere processen. Daarbij kunnen TMS-studies helpen bij ons begrip over verschillende mechanismen die betrokken zijn bij de meer voorkomende mobiele dystonie in vergelijking tot de gefixeerde dystonie die wordt gezien bij patiënten met CRPS.³⁷

Transcraniële directe stroomstimulatie moduleert corticale prikkelbaarheid en kan ook een therapeutisch alternatief vormen. Repetitieve transcraniële magnetische stimulatie is effectief gebleken als toevoeging aan farmacologische behandeling van patiënten met CRPS.^{38,39} De therapeutische potentie van TMS varieert tussen bewegingsstoornissen die niet zijn gerelateerd aan CRPS met geen verbetering bij patiënten met schrijverskramp⁴⁰ en goed effect bij patiënten met blefarospasme.⁴¹ Mogelijk is het gebied, dat

gestimuleerd wordt hierin van belang, aangezien motorcortex werd gestimuleerd in de studie met schrijverskramp en de anterieure cortex cinguli in de blefarospasmestudie. De invloed van psychologische factoren op aanvang en aanhouden van CRPS-gerelateerde dystonie is nog steeds onderdeel van discussie. Echter patiënten met CRPS blijken geen specifieke psychologische kenmerken te hebben of een uniek psychologisch profiel voorafgaand of rond aanvang van de ziekte die hen kwetsbaarder zou maken om standsafwijkingen te ontwikkelen en onderhouden.⁴² Bovendien werd de effectiviteit van ITB bij patiënten met CRPS-gerelateerde dystonie aangetoond in een enkelblinde placebo-inloop dosisopbouw studie¹ en in de diverse trials van ons promotie-onderzoek vonden we geen aantoonbaar placeborespons (*Hoofdstukken 3, 5 and 8*). Derhalve beschouwen we een psychogene oorzaak als enige verklaring van CRPS-gerelateerde dystonie onwaarschijnlijk. Er is groeiend bewijs, dat bij patiënten met CRPS sprake is van aberrante reorganisatie van sensibele en motorische netwerken.^{6,10,21} Zulke veranderingen worden beschouwd de oorzaak te zijn voor vertraagde herkenning van handdominantie,^{43,44} gestoorde zelfperceptie van de aangedane ledemaat,⁴⁵ een afwijkend lichaamsschema en abnormale lichaamsperceptie bij CRPS.⁴⁶ Planning en uitvoering van beweging is afhankelijk van de afstemming van sensibele input op representaties van de intentionele beweging.⁴⁷ Functionele veranderingen van sensibele en motorische netwerken kunnen ook een rol spelen in de ontwikkeling en handhaving van bewegingsstoornissen bij CRPS. Interessant om te vermelden is, dat abnormale verwerking in non-motore gebieden van de hersenen al een belangrijke rol wordt toebedeeld bij de ontwikkeling van primaire dystonie.⁴⁸ Mogelijk leiden deze inzichten tot een beter begrip over de zogenaamde psychogene of functionele bewegingsstoornissen. Hoewel de eerdergenoemde argumenten een somatische verklaring als onderliggende oorzaak voor CRPS-gerelateerde dystonie ondersteunen, is de discussie in de literatuur nog ver van beëindigd. De controverse of een somatische of psychogene oorsprong (of beiden) ten grondslag ligt aan deze bewegingsstoornis is nog steeds actueel. Veel onderzoekers zijn van mening, dat CRPS-gerelateerde dystonie, met name gefixeerde dystonie, een psychogene bewegingsstoornis is.^{49,50}

Toekomstige studies zijn ook noodzakelijk om de rol van perifere processen, voornamelijk energiemetabolisme in spierweefsel te onderzoeken bij het tot stand komen en aanhouden van CPRS-gerelateerde dystonie.⁵¹ Vooral studies die gebruik maken van MR-spectroscopie kunnen nuttige informatie leveren over de energiestatus van spierweefsel bij CRPS-patiënten met dystonie en samen met neurofysiologische studies opheldering geven over de rol van het contractiele apparaat bij de totstandkoming van abnormale houdingen (niet gerelateerd aan contractuur) bij patiënten met CRPS. Worden deze standsafwijkingen veroorzaakt door abnormale co-contractie van agonisten en antagonist die we definiëren als dystonie of zijn deze het resultaat van deficiënte koppeling van myosine/actinefilamenten, bijvoorbeeld door een lage ATP-productie, of is er een ander onderliggend proces? In een recente electromyografische studie demonstreerden Bank *et al*, dat abnormale standen bij chronische CRPS-patiënten, in ieder geval bij patiënten die enige actieve bewegingsuitslag hadden, niet geassocieerd waren met aanhoudende spiercontracties in de aangedane extremiteit en dat de actieve bewegingsuitslag niet gelimiteerd was vanwege excessieve agonist-antagonist spiercontractie.⁵²

Deze bevindingen, samen met de studies die in dit proefschrift gepresenteerd staan, ondersteunen, dat bij verschillende CRPS-patiënten of in verschillende stadia van de ziekte verschillende pathofysiologische mechanismen ten grondslag liggen aan de ontwikkeling en handhaving van standsafwijking bij deze patiënten. We stellen daarom voor om ons te beperken tot het gebruik van de term “abnormale houding” bij patiënten met CRPS, in ieder geval tot de rol van processen in spierweefsel is opgehelderd. Het gebrek aan bewijs van een spierverslappend effect van magnesiumsulfaat die we vonden in een verklarende studie doet geen afbreuk aan deze aanbeveling, aangezien magnesiumsulfaat zich slechts richt op een klein onderdeel van het contractiele apparaat en farmacokinetische beperkingen verantwoordelijk kunnen zijn voor het ontbreken van effectiviteit.

Nieuwe studies zijn ook noodzakelijk om vragen te beantwoorden als: zijn de bevindingen in onze massa-spectrometriestudies die wijzen op een verhoogde

eiwitafbraak in spierweefsel alleen maar het gevolg van chronische immobilisatie of ondervoeding secundair aan de ziektelast die CRPS-patiënten ervaren of zijn deze nauw verwant aan intrinsieke pathofysiologische processen? treedt spierproteolyse pas in latere stadia van de ziekte op of is hier al sprake van in een vroege fase? Deze vragen moeten in de nabije toekomst worden aangekaart, niet alleen omdat afbraak van spiereiwitten het immuunsysteem negatief beïnvloeden, maar ook omdat het wellicht gecorrigeerd zou kunnen worden met een adequaat voedingsbeleid.

Het is zeer waarschijnlijk, dat verschillende processen op perifeer en centraal niveau elkaar beïnvloeden. De interactie van deze processen is waarschijnlijk verantwoordelijk voor het heterogene klinische spectrum van CRPS en de aanvang en progressie van CRPS-symptomen. Om meer inzicht te krijgen in dit samenspel van centrale en perifere processen adviseren we in toekomstige studies voor ieder individu zowel verandering in centrale als perifere procesverwerking te evalueren.

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Curriculum vitae

Anton Adriaan van der Plas (given name: Anne) was born in Leiderdorp, the Netherlands, on September 10, 1974. He completed the pre-university secondary education at the Visser 't Hooft Lyceum in Leiden in 1992 and started the study of medicine at the University of Leiden in the same year. In 1997 and 1998 he performed a graduation project, called "accelerometric assessment of levodopa-induced dyskinesias in Parkinson's disease" under the supervision of prof.dr. J.J. van Hilten. In 2000 he obtained the degree of medical doctor. Between 2000 and 2004 he was a clinical trainee at the department of neurosurgery (Leiden University Medical Center and Erasmus Medical Center) and neurocritical care (Erasmus Medical Center). He started as a neurology resident at the Leiden University Medical Center in 2004 and received his degree as a neurologist in 2012. The period of April 2007 to April 2010 was reserved for this PhD project. In 2011 he was awarded a grant by the Princess Beatrix Foundation for a fellowship "Neurological Movement Disorders", which he performed in the period between August 2012 and August 2013 at the Leiden University Medical Center. Since August 2012 he works as a clinical researcher and neurology consultant in Topaz Huntington Centre Overduin in Katwijk aan Zee.