

**Induction and modulation
of referred muscle pain
in humans**

Ph.D. - thesis

by

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1999

PREFACE

This thesis is based on experimental investigations carried out from 1996 to 1999 during my employment as research assistant at Laboratory for Experimental Pain Research, Aalborg University.

First of all, I would like to express my sincerest gratitude to Professor Lars Arendt-Nielsen for giving me the opportunity to work with basic pain research, and for all his support and supervision during the project. I am also greatly indebted to Associate Professor Thomas Graven-Nielsen, who has been a tremendous help in all aspects of this project. Professor Troels Staehelin Jensen is thanked for his invaluable help and comments, especially during manuscript processing.

The technical staff at SMI and especially Jesper Nielsen, Victor Grant, and Knud Larsen are acknowledge for always being helpful when technical and computer problems occurred during the project. Susanne Nielsen, Lone Daasbjerg, Anne S. Schmidt, Tina Korup, Charlotte Høeg Pedersen, and Tina Buus Andersen from the secretariats are recognized for, among other things, proof reading the manuscripts.

The Department of Neurosurgery at Aalborg Hospital is thanked for allowing parts of my investigations to take place there. The library at Aalborg University and the medical library at Aalborg Hospital are also thanked for assisting me throughout the project.

The work presented in this thesis has been supported by The Danish Cancer Society and The Danish National Research Foundation. Financial aid from The Obelske Family Foundation and Astra A/S, Denmark, is also kindly acknowledged.

Aalborg, July 1999

The thesis is based on the following four papers:

- I René J. Laursen, Thomas Graven-Nielsen, Troels S. Jensen, Lars Arendt-Nielsen: Quantification of local and referred pain in humans induced by intramuscular electrical stimulation.
European Journal of Pain 1997, 1: 105-113

- II René J. Laursen, Thomas Graven-Nielsen, Troels S. Jensen, Lars Arendt-Nielsen: Referred pain is dependent on sensory input from the periphery - a psychophysical study.
European Journal of Pain 1997, 1: 261-269

- III René J. Laursen, Thomas Graven-Nielsen, Troels S. Jensen, Lars Arendt-Nielsen: The effect of compression and regional anaesthetic block on referred pain intensity in humans.
Pain 1999, 80: 257-263

- IV René J. Laursen, Thomas Graven-Nielsen, Troels S. Jensen, Lars Arendt-Nielsen: The effect of differential and complete nerve block on experimental muscle pain in humans.
Muscle & Nerve 1999, 22: 1564-1570

LIST OF ABBREVIATIONS AND DEFINITIONS

Allodynia:	Pain due to a stimulus which does not normally provoke pain
Hyperalgesia:	An increased response to a normally painful stimulus
IMES:	Intramuscular electrical stimulation
IVRA:	Intravenous regional anesthesia
PT:	Pain threshold
Referred pain:	Pain felt at a site remote from the site of origin/stimulation
TA:	Tibial anterior muscle
Temporal summation:	Repeated stimuli resulting in an increased pain perception

CONTENTS

PREFACE	2
LIST OF ABBREVIATIONS AND DEFINITIONS	4
CONTENTS	5
1. INTRODUCTION	6
1.1 Background	6
1.2 Aim of the Ph.D. project	8
2. INDUCTION OF LOCAL AND REFERRED MUSCLE PAIN	9
2.1 Induction of muscle pain	9
2.2 Algogenic substances	9
2.3 Electrical stimulation	10
2.4 Summary	12
3. MANIFESTATION AND ASSESSMENT OF MUSCLE PAIN	13
3.1 Pain thresholds, pain intensity ratings, and pain area size	13
3.2 Appearance, quality, and distribution of local and referred muscle pain	15
3.3 Intra- and intersession variability	18
3.4 Summary	18
4. MODULATION OF LOCAL AND REFERRED MUSCLE PAIN	20
4.1 Nerve blocking techniques	20
4.2 Modulation of local muscle pain	22
4.3 Modulation of referred muscle pain	23
4.4 Summary	27
5. DISCUSSION	29
5.1 Neurophysiological mechanisms for referred pain	29
5.2 Summary	33
6. CONCLUSION	34
7. DANISH SUMMARY/DANSK SAMMENFATNING	36
8. TABLES	39
Table 8.1 Chronological review of intramuscular induced electrical muscle pain	39
Table 8.2 Review of experimentally induced referred muscle pain	42
Table 8.3 Review of the effect of anesthetizing experimentally and clinically referred pain	45
9. REFERENCES	48

- I** *Quantification of local and referred pain in humans induced by intramuscular electrical stimulation*
- II** *Referred pain is dependent on sensory input from the periphery - a psychophysical study*
- III** *The effect of compression and regional anaesthetic block on referred pain intensity in humans*
- IV** *The effect of differential and complete anaesthetic block on experimental muscle pain in humans*

1. INTRODUCTION

1.1 Introduction

Pain and relief of pain are a great challenge in medicine. Pain originating from deep somatic structures represents a major part of pain complaints in patients. Although muscle pain is an important factor in many disorders such as injuries, degenerative diseases, and cancer, the mechanisms underlying muscle pain are poorly understood. Obviously, this has led to various thoughts and theories related to etiology, pathogenesis, diagnosis, and treatment. In a survey, 14.4% of the American population indicated chronic pain related to the joints and the musculo-skeletal system (Magni et al. 1990). In a recent Danish study, including 1504 inhabitants between the age of 30 and 60, the prevalence of muscle pain was reported to be 36.9% in men and 64.7% in women (Drewes et al. 1995). In brief, muscle pain is a major clinical problem, and a further insight into the mechanism of muscle pain is considered necessary.

Paradoxically, a large amount of the research in experimental and clinical pain has been obtained from studies on cutaneous pain. Cutaneous pain varies in many ways from deep pain. It is described as a localized burning pain and is rarely (if ever) referred to other structures. On the contrary deep pain is often described as diffuse, dull pain usually referred to distant sites (Bonica 1990). A particularly interesting problem is the relationship between local pain and referred pain. Numerous studies in both humans and animals have shown that long-lasting pain causes pain-related phenomena such as referred pain, hyperalgesia, and allodynia (for review: Coderre et al. 1993).

Referred pain has been known and described throughout the century. In clinical practice, classical signs of viscerally referred pain are: 1) the radiating pain in arms, particularly the left, during angina pectoris; 2) McBurney's sign indicative of appendicitis; 3) epigastric pain from ulcers; 4) the pain of cholecystitis, which may radiate to the interscapular area, right scapula, or shoulder; and 5) pain from renal and urethral stones referred to the lower back. The referral of pain is also well known in deep pain, e.g., arthritis of the shoulder and hip to the areas around the elbow and

knee. During the past decades, a systematic attempt to chart referred musculoskeletal pain areas in humans has been made (Simons et al. 1998). Some of these findings have been reproduced in experimental studies in humans (Graven-Nielsen et al. 1997a-c, I - III).

Head initially used the term “referred pain” in 1893 (Head 1893), however, other clinicians have reported the phenomenon previously (for review: Bonica 1990). It has since then been used to describe pain perceived at a site adjacent to, or at a distance from the site of origin. The taxonomy committee of the International Association for the Study of Pain (IASP) has not made a definition of the term, however, several authors have defined it in different ways: *Pain resulting from stimulation of a somatic sensory nerve and referred to remote part of the distribution of that nerve or of the segmental sensory distribution with which it is connected* (Morley 1937); *Pain outside the boundaries of local pain* (Stohler et al. 1994); and *Pain that arises in a trigger point, but is felt at a distance remote from its source. The pattern of referred pain is reproducibly related to its site of origin. The distribution of referred trigger point pain rarely coincides with the distribution of a peripheral nerve or dermatomal segment* (Simons et al. 1998). In this thesis, the definition “pain felt at a site remote from the site of origin/stimulation” will be used.

Possible mechanisms responsible for referred pain have been investigated. In general, the theories suggested have evolved around the idea that nociceptive dorsal horn neurons receive convergent inputs from various tissues, thus higher centers cannot identify the actual input source. The mechanisms responsible for referred pain are undergoing intense research; however, a breakthrough, which would help controlling it, has still not taken place. Further basic research into all aspects of referred pain is needed. Clinical research and in particular research in pain are often confounded by many factors, which make it difficult to look at certain aspects of the disease. Experimental models, which can be standardized by using healthy subjects, are useful in basic research, because they allow a study without confounding factors (Arendt-Nielsen 1997a).

1.2 Aim of the project

In order to study the relationship between and properties of local and referred muscle pain in humans, an experimental pain model was developed and applied in various experiments. Intramuscular electrical stimulation (IMES) was chosen because of its ability to generate local and referred pain in an “on and off” manner.

Thus, the aim of this Ph.D. project was: 1) to develop and use a standardized intramuscular electrical experimental pain model, which could elicit local and referred muscle pain; 2) to assess temporal and spatial characteristics of the elicited local and referred pain; and 3) to modulate local and referred muscle pain by anesthetic techniques in order to examine underlying mechanisms for referred pain (Fig. 1.1).

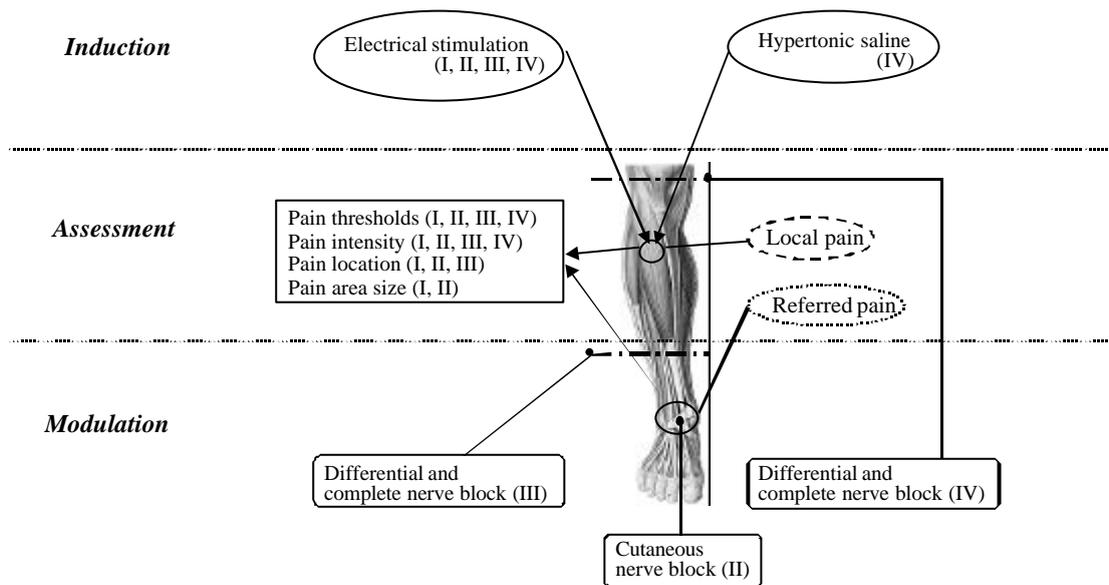


Figure 1.1. The Ph.D. study is comprised of four papers (I-IV). The induction, assessment and modulation of local (electrical stimulation and hypertonic saline) and referred (electrical stimulation) pain was performed by applying the stimuli to the anterior tibial muscle, except in study IV where the stimuli were applied to the brachioradial muscle, and the nerve block was applied to the upper arm. Pain characteristics were assessed in all studies. Referred pain was modulated in study II and III, while local pain was modulated in study IV.

2. INDUCTION OF LOCAL AND REFERRED MUSCLE PAIN

In this chapter methods to induce referred muscle pain for experimental purposes will be described, and emphasis will be put on the advantages and limits of IMES compared with the other methods presented.

2.1 Induction of muscle pain

Various methods can be used to induce experimental muscle pain. Usually, the methods are classified into two groups: 1) endogenous (without external stimuli) and 2) exogenous (external stimuli) methods (Svensson et al. 1995).

Human endogenous methods are characterized by high response rate and are suitable to study general pain states. However, they have the disadvantage of involving several/all muscle groups in the region investigated, and often pain from other somatic tissues cannot be excluded (Newham et al. 1994; Svensson et al. 1995). Finally, endogenous methods are not known for generating referred pain. A further description of these methods is therefore omitted.

2.2 Algogenic substances

A number of exogenous methods have been used to induce experimental human muscle pain (Table 8.2). The most used and accepted method is intramuscular infusion of hypertonic saline (5%). Kellgren and Lewis introduced the method in 1938 (Kellgren 1938; Lewis 1938), and since then intramuscular infusion of hypertonic saline has been used extensively (Kellgren 1939; Inman et al. 1944; Feinstein et al. 1954; Hockaday et al. 1967; Meadows 1970; Vecchiet et al. 1988; Vecchiet et al. 1990a; Vecchiet et al. 1993; Graven-Nielsen et al. 1997a-c; Graven-Nielsen et al. 1998a). A variety of parameters have been shown to correlate to the infusion of hypertonic saline (e.g. saline concentrations, infusion rate and pressure, and amount of saline infused) (Vecchiet et al. 1993; Graven-Nielsen et al. 1997a-d). In spite of this, the mechanisms responsible for the excitation of pain shortly after the infusion are still unknown, however, a direct excitation of afferents due to osmotic difference has been proposed (Graven-Nielsen et al. 1997d). Saline induced muscle pain is characterized by a drilling, taut, and tight local pain appearing after

approximately 20 s, and it lasts for several minutes depending on concentration and volume of infusion (Kellgren 1938; Hockaday et al. 1967; Graven-Nielsen et al. 1997a+d). Referred pain is felt in structures at a distance from the infusion site and appears with a delay of 20 s compared with local pain (Graven-Nielsen et al. 1997b). This pain is characterized as diffuse and unpleasant (Graven-Nielsen et al. 1997a). In some studies muscular hyperalgesia after saline-induced pain has been demonstrated to various stimuli (Vecchiet et al. 1988; Vecchiet et al. 1993), while others have failed (Arendt-Nielsen et al. 1997b; Graven-Nielsen et al. 1998a). Besides studies looking at characteristics of pain induced by hypertonic saline, clinical studies have used hypertonic saline to induce muscle pain in fibromyalgia patients (Sørensen et al. 1998; Graven-Nielsen et al. 1999) and in patients suffering from whip-lash syndrome (Johansen et al. 1999). Saline has several advantages: It is easy and safe to use, and it generates local and referred muscle pain in many subjects (40-85%) (Graven-Nielsen et al. 1997a-c). The disadvantages of this muscle pain model is the relatively long-lasting pain (several minutes) after a bolus infusion (Graven-Nielsen et al. 1997a-d; IV). Also, it is not possible to generate pain at the exactly same site repeatedly so the infusion needle has to be moved (Graven-Nielsen et al. 1997b).

In recent years other algogenic substances have been tested as muscle pain models. Bradykinin (Kantor et al. 1967; Pedersen Bjergaard et al. 1989; Jensen et al. 1990; Babenko et al. 1999a-b), serotonin (Jensen et al. 1990; Babenko et al. 1999a-b), capsaicin (Marchettini et al. 1996), and substance P (Jensen et al. 1990; Babenko et al. 1999a-b) have been used separately or in combination to induced muscle pain. This has been a promising hyperalgesia model. It permits an analysis of the underlying pharmacology, but drugs are expensive, and referred pain is rarely generated. However, one study has shown that some subjects experienced a consistent appearance of referred pain when a combination of bradykinin and serotonin was used (Babenko et al. 1999a).

2.3 Electrical stimulation

Electrical stimulation of muscle tissue has been used in various experimental and clinical settings (for review: see Table 8.1). It has been used to assess the

somatosensory sensibility by determination of various thresholds (e.g., sensation detection, pain). For instance, Vecchiet and co-workers found a significantly lower pain threshold in muscle, subcutis, and skin of patients suffering from so-called chronic fatigue syndrome compared with healthy controls, indicating hypersensitivity to pain stimulation in this group of patients (Vecchiet et al. 1996). IMES has also been used to induce pain in order to study cerebral processing of the nociceptive stimulation by positron emission tomography technique (Svensson et al. 1997a). In a recent study, IMES was used to evaluate the effect of ketamine on muscle pain induced by single vs. repeated electrical stimulation in patients suffering from fibromyalgia, and a significant increase to repeated IMES was found during ketamine infusion (Graven-Nielsen et al. 1999).

IMES has not previously been applied for the purpose of eliciting and studying referred muscle pain. However, electrical stimulation of the human gastrointestinal tract has been used for generating local and referred visceral pain (Arendt-Nielsen et al. 1997c; Drewes et al. 1997; Drewes et al. 1999a-b). IMES offers the advantage that several stimulus modalities can be applied exactly with respect to time and localization. Thus, it possesses the capability of eliciting and maintaining local and referred muscle pain in an “on and off” manner, allowing assessment of spatial and temporal aspects of various pain states. It is an easy method to use and has high local (94%) and referred (78%) pain response rates in healthy subjects (I).

However, IMES has some shortcomings compared with hypertonic saline. It excites all afferent and efferent nerve fibers in an unnatural way and bypasses the sensory nerve endings making observations of receptor transduction of limited value (Handwerker et al. 1993). Due to efferent-evoked activity IMES results in muscle twitches, which make pain assessment difficult (Handwerker et al. 1993).

Muscle pain in humans has been produced by intraneural electrical stimulation of nociceptive afferent fibers (Torebjörk et al. 1984; Simone et al. 1994; Marchettini et al. 1996). The technique allows recording from the same fibers as when various stimuli are applied to the receptive field. However, the method is technically difficult and requires some experience. Further studies looking at various aspects of local and referred muscle pain using this method are anticipated.

In the present experiments, IMES was used to generate local (I - IV) and referred (I - III) muscle pain. Needle electrodes (Dantec, 13R24, Denmark) with uninsulated tips (3 mm) were inserted 15 mm into the anterior tibial muscle. This was done 15 and 16 cm distal to the lower tip of the knee cap (apex patellae) (I - III) and in the middle part of the brachioradialis muscle (IV), always 10 mm apart along the muscle fiber direction. A computer-controlled constant current stimulator (Aalborg University, Denmark) was connected through a wire to the needle electrodes and used to induce electrical stimulation. Each stimulation consisted of five constant current rectangular pulses (1 ms) delivered at 200 Hz. It was possible to interrupt an electrical stimulation at any time, if the subject requested so. The experimental set-up was arranged in a way that the subjects had no clue as to the intensity of the stimulation, however, they were informed about the duration time of each stimulus. Threshold determinations and other procedures related to the set-up are described in the next chapter.

2.4 Summary

Various methods have been used to induce human muscle pain and pain-related phenomena for experimental purposes (Table 8.2). So far, injection of hypertonic saline has been the most reliable method to induce and study referred muscle pain. When using this model, however, it is difficult to time duration and the intensity of muscle pain. In order to look further into the mechanisms of local and referred muscle pain, a new model was developed. By using IMES, which has been used extensively to test muscle somatosensory sensibility and to induce muscle pain, it was possible to induce and control local and referred muscle pain in a high percentage of the subjects tested.

3. MANIFESTATION AND ASSESSMENT OF MUSCLE PAIN

3.1 Pain thresholds, pain intensity ratings, and pain area size

PT to IMES was determined in the studies (I - IV). Local PT and referred PT were defined as the lowest stimulus intensity required for the subject to describe a “just barely painful” sensation at the local and referred pain area. The subject signaled “pain” / “no pain” verbally. PT was determined by a staircase regime consisting of five ascending and four descending series of stimuli (Gracely 1994). The mean of five ascending thresholds was determined as the PT (Fig. 3.1).

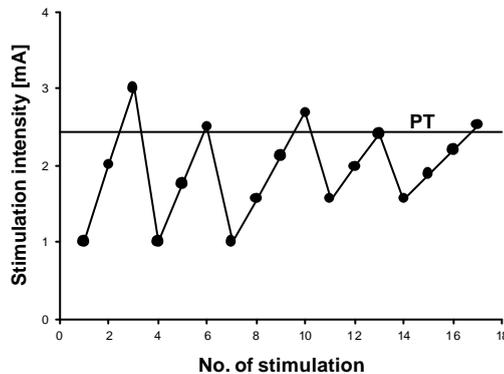


Figure 3.1. Stimulus intensity as a function of the number of stimuli.

The local muscle PTs were similar to those reported previously using electrical needle stimulation in healthy subjects (Brucini et al. 1981; Duranti et al. 1988; Vecchiet et al. 1988; Giamberardino et al. 1988; Vecchiet et al. 1990a; Vecchiet et al. 1993; Arendt-Nielsen et al. 1997b). In comparison, patients suffering from muscle pain have demonstrated significantly lower PT to IMES compared with controls suggesting a state of hyperalgesia in the investigated muscles (Vecchiet et al. 1988; Vecchiet et al. 1990a; Vecchiet et al. 1993; Sørensen et al. 1998).

In general, significantly higher stimulus intensity was required to elicit referred pain compared with local pain (I). This is in accordance with previous experimental and clinical studies (Inman et al. 1944; Torebjörk et al. 1984; Hong et al. 1996; Graven-Nielsen et al. 1997b-c).

In order to find the “optimal” stimulation paradigm (defined as: the stimulation at which most subjects perceived referred pain and at which the least stimulation intensity was required), nine different continuous stimulation paradigms were tested (1, 10, and 20 s stimuli combined with 2, 5, and 10 Hz) and no significant differences were found (I). Stimulation with 2 Hz often caused the subject’s foot to move repeatedly, thus masking the perception of referred pain at the anterior part of the ankle, while 5 and 10 Hz stimulation caused a maintained muscle contraction. Although not significant, referred pain tended to be generated in more subjects when stimulation frequencies were high (5 or 10 Hz), and when stimulation times were long (10 or 20 s). In comparison, another study of experimentally induced referred muscle pain (intraneural microstimulation) has shown that at low stimulation frequencies (1-3 Hz), high stimulation intensities (0.7 V) are required to generate referred pain compared with high frequencies (10 - 100 Hz) which generated referred pain with a significantly lower stimulus intensity (0.22 V) (Torebjörk et al. 1984). Based on the results obtained, it is difficult to point out an “optimal” stimulation frequency and duration. In the following experiments, however, stimulation at 10 Hz for at least 10 s was chosen as the paradigm for the generation of referred muscle pain.

The possibility of selectively stimulating various sub-populations of nerve fibers could have added new perspectives to the present study. A device, (Neurometer[®] Current Perception Threshold) claiming to have these properties, has been available for some years for the purpose of evaluating and quantifying sensory function by transcutaneous electrical nerve stimulation. By stimulating at 5 Hz, 250 Hz, and 2000 Hz, a selective stimulation of C-, A δ - and A β -fibers should be possible. Nevertheless, a recent technological review of the device concluded that the data presented so far were insufficient to prove the potential of this method (Technology review 1999).

A significantly positive correlation was found between the stimulus intensity and local and referred pain intensity ratings (I). Spatial summation is a well described feature in many experimental pain models of cutaneous pain (Andersen et al. 1994), deep pain (Simone et al. 1994; Marchettini et al. 1996; Graven-Nielsen et al. 1997c), and visceral pain (Arendt-Nielsen et al. 1997c; Drewes et al. 1997). The mechanism responsible for spatial summation observed is most likely an additional recruitment of

nociceptor units (Handwerker et al. 1993) resulting in an increased noxious input to the dorsal horn neurons and consequential an increased pain perception.

Referred pain appeared with various delays in the present studies (I - III), ranging from simultaneously with local pain to a delay of in average 43 s. A difference in stimulus intensities could account for the variances of referred pain onset (I). A more consistent delay of referred pain onset is characteristic for hypertonic saline experiments (Graven-Nielsen et al. 1997c). The reason for the difference in time delay between the two models could be due to different excitation mechanisms of the nociceptive afferents and/or due to central mechanisms (temporal summation or hyperexcitability). IMES causes a direct excitation of all afferents (Handwerker et al. 1993), whereas excitation of nociceptive afferents by hypertonic saline is postulated to a result of osmotic changes (Graven-Nielsen et al. 1997d).

In the present experiment, a significant correlation between the size of local and referred pain areas and local and referred sensation/pain intensity ratings was observed (I). Similar observations have been seen in studies where sequential infusions of hypertonic saline into a muscle resulted in increasing number of subjects experiencing referred pain and increasing areas of referred pain (Graven-Nielsen et al. 1997b), and where intraneural, electrical stimulation of muscle afferents at constant frequency and intensity evoked an expansion of the projected pain area over time (Marchettini et al. 1996). Temporal summation in experimental visceral pain studies has demonstrated an increase in the size of the referred pain areas (Ness et al. 1990; Arendt-Nielsen et al. 1997c; Drewes et al. 1997; Drewes et al. 1999b). An increased nociceptive input to the dorsal horn neurons, which generates an expansion of receptive fields of the dorsal horn neurons (Hoheisel et al. 1989; Hu et al. 1992), may account for the expansion of referred areas observed during repeated stimulation (I).

In summary, observations from the present studies indicate that referred muscle pain is dependent on both spatial and temporal summation and support the general idea that an amount of local pain input during a time interval is required to elicit referred pain (Bonica 1990).

3.2 Appearance, quality, and distribution of local and referred muscle pain

When IMES was used, it was possible to elicit local pain and referred pain in 94% and 78% of the 18 subjects, and it was possible to elicit local pain with 93% of all stimuli and referred pain with 64% of 162 stimuli (I). One subject reported referred pain before the local muscle sensation turned into pain during some of the stimulation paradigms, and this explained why it was only possible to generate local muscle pain in 93% of stimulation (I). Experimentally referred muscle pain can be generated by injection of hypertonic saline in 40–85% of the subjects (Graven-Nielsen et al. 1997a-c). In contrast, pressure stimulation is less effective in generating referred muscle pain if the pressure site is without taut bands or trigger points (24% of the subjects experienced referred pain) (Hong et al. 1996). A possible explanation for this observation could be that pressure stimulation induces a weaker excitation of nociceptors and that the duration of pressure stimulation is shorter compared with electrical stimulation or hypertonic saline injection. In support of this, a recent study comparing pressure stimulation to needle injection showed that referred pain is elicited significantly more often by needle stimulation (Hong et al. 1997).

The quality of local and referred muscle pain induced by electrical stimulation was described by a Danish version of the McGill Pain Questionnaire (Drewes et al. 1993). The local pain quality was described as “drawing” (70% of the subjects), “boring” (50%), “penetrating” (40%), and “taut” (40%). The referred pain quality was described as “sharp” (60% of the subjects), “taut” (60%), and “tight” (40%) (I). These descriptions are in line with previous experimental studies with saline-induced pain (Feinstein et al. 1954; Svensson et al. 1996; Graven-Nielsen et al. 1997a) and a clinical study comparing experimental muscle pain and the pain experienced by patients suffering from myofascial pain patients (Stohler et al. 1994).

The distribution of local and referred pain was recorded in all experiments. The subjects indicated the distribution of the local and referred pain either on an anatomical drawing (I) (Fig. 3.3) or directly on the skin (II - IV). Local pain was located around the stimulation needles, and referred pain was located on the anterior part of the ankle (Fig. 3.3A). Previous recordings of referred pain elicited by stimulation of the proximal part of the TA have shown a similar localization:

injection of bradykinin/serotonin (Fig. 3.3B) (Babenko et al. 1999a), injection of hypertonic saline (Fig. 3.3C) (Kellgren 1938; Graven-Nielsen et al. 1997a-c), or pressure stimulation at a trigger point (Travell et al. 1983). Inman and Saunders investigated the distribution of referred pain in relation to local pain (Inman et al. 1944). Based on their observations, it was suggested that referred pain followed more frequently the distribution of sclerotomes (muscle, fascia, and bone) than the classical dermatomes (Foerster 1933). The distribution of referred pain observed in the present studies (I - III) seemed to fit better with the distribution of sclerotomes compared with dermatomes. A recent study, however, has questioned the rigid mapping of nerve roots and their sensory distribution (Slipman et al. 1998). A needle tip stimulated the human cervical nerve roots (C4 to C8) prior to diagnostic fluoroscopically guided nerve root block. The sensory sensation was recorded on an anatomical map. Although the distribution of sensory sensation resembled the classic dermatomal maps for cervical nerve roots, symptoms were frequently provoked outside of the distribution of classic dermatomal maps. In summary, the distribution of referred pain observed in various studies has only made minor contributions to the investigation of the mechanism responsible for referred pain.

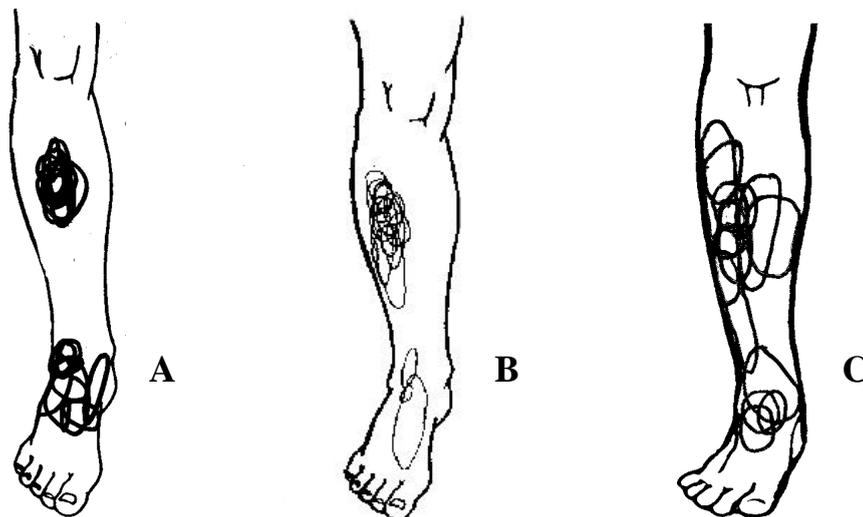


Figure 3.3. Anatomical drawing of the right lower leg used to mark the subjects' local pain and referred pain and the pain areas (local pain proximal and referred pain distal) generated from 60 s stimulation at 10 Hz in 10 subjects [A] (I), from injection of bradykinin (10 nmol) and serotonin (20 nmol) in 10 subjects (Babenko et al. 1999a) [B], and from injection of 0.5 ml 5% saline in 10 subjects (Graven-Nielsen et al. 1997a) [C].

3.3 Inter- and intrasession variability in pain thresholds

When IMES was used, reproducible local PT (I and IV) and referred PT (II - III) could be obtained between sessions (Fig. 3.4). However, a small, but significant difference was found for local PT, and referred PTs were tested within the same session (I). The first local and referred PTs were smaller than the subsequent values in the primary experiment. A similar increase in intrasession local PT has been described when IMES was used (Brucini et al. 1981). In the following experiments, however, no significant differences were found in local PT (IV) or referred PT (III). When assessed repeatedly, a difference in time intervals between PT determinations (15 min in (I) and 5 min in (III and IV)) could explain the difference of PT determinations in intersession variability. An increase or decrease in perceived pain over time is a well-known phenomenon. The observed suppressed perception of pain at the PT could be either peripheral or centrally mediated. As mentioned earlier, electrical stimulation activates nociceptive afferents directly, and any modulation at receptor level is by-passed (Handwerker et al. 1993), making a peripheral mechanism less likely. Alternatively, the suppression may be due to central processes, e.g. habituation or training effect (Ernst et al. 1986; Gracely 1989).

In addition to reproduce local and referred PT, another valuable feature of the IMES pain model is the capability of generating reproducible local and referred pain areas which remain constant within (I) and between sessions (II).

3.4 Summary

IMES was applied to the right tibial anterior muscle, and pain occurred around the stimulation needles and was referred to the anterior part of the right ankle. Significantly higher stimulus intensity was required to elicit referred pain compared with local pain, and in most cases a time delay of seconds was observed from the elicitation of local pain and appearance of referred pain. A significant correlation between stimulus intensity and local and referred pain intensity, and between stimulus intensity and the size of local and referred pain areas was also found. These observations are consistent with other experimental and clinical pain studies and seem to indicate that spatial and temporal summation are both important factors in the

referred pain phenomena. Local and referred pain thresholds and size of local and referred pain areas were reproducible within and between experiments. Taken together, IMES is a useful model of referred muscle pain. Further studies of the mechanisms of referred muscle pain using IMES will be described in the following chapters.

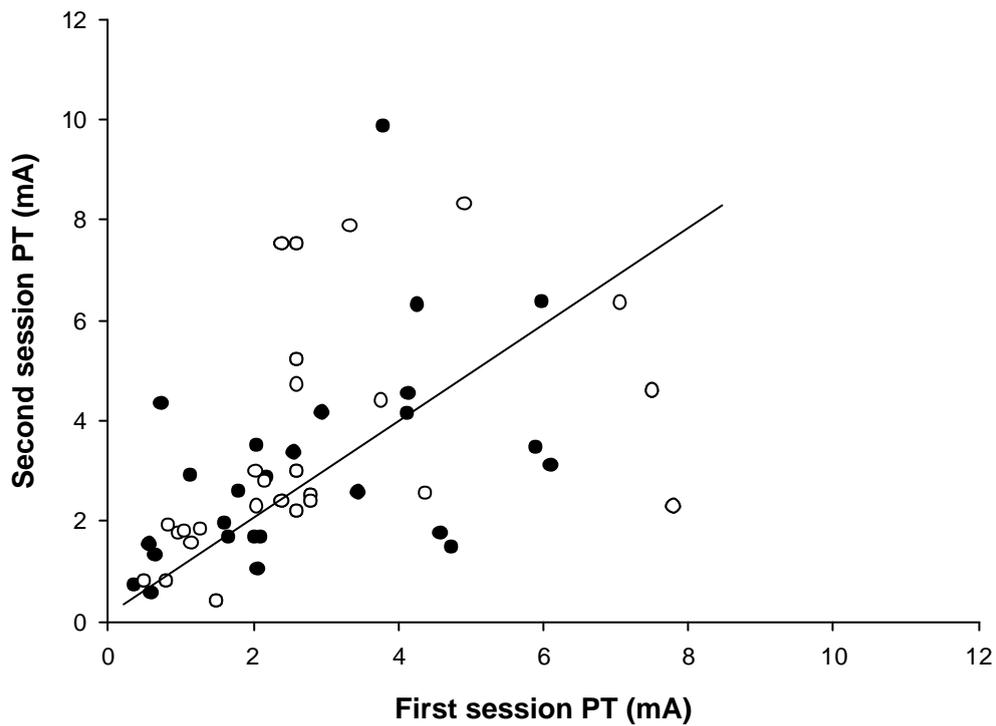


Figure 3.4. Plot of local (●) and referred (○) PT determinations from the first session as a function of the PT determination from the second session (I-IV).

4. MODULATION OF LOCAL AND REFERRED MUSCLE PAIN

In this chapter, a description of the three anesthetic techniques used in the project to modulate local and referred muscle pain will be presented. The rationale of using these techniques and the results obtained in the experiments are discussed in correlation to previous observations and experiments.

4.1 Nerve blocking techniques

Frequently, various drugs are tested for their analgesic and anesthetic properties in experimental settings and pain-related diseases. However, the opposite condition enables us to examine the neurophysiology of various experimental and clinical pain states. Using three well-known anesthetic techniques, which will be described briefly, attempts were made to investigate mechanisms responsible for local and referred muscle pain.

The anesthetic effect of an eutectic mixture of lidocaine and prilocaine (EMLA[®] cream) has been documented experimentally and clinically (Evers et al. 1985; Bjerring et al. 1990; Juhlin et al. 1990). It has been used in experiments where the skin component in pressure pain perception was minimized (Kosek et al. 1995; Graven-Nielsen et al. 1998b; Kosek et al. 1999). The skin and subcutis are anesthetized to a depth of 5 mm, 30 min. after the cream is applied for 90 min (Bjerring et al. 1990).

Injection of local anesthetics into tissue and around nerves has been used extensively in pain research. The classical work by LaMotte et al. (1991) demonstrated that a spreading hyperalgesic area induced by capsaicin injection does not cross a strip of skin which is anesthetized by local anesthetic. Vecchiet et al. showed that hyperalgesia in skin, subcutis, and muscle elicited by the injection of hypertonic saline into muscle tissue is eliminated by injecting 1 ml of 2% carbocaine into the same spot as the hypertonic saline (1988). In another experimental study, pain intensity ratings and areas were significantly smaller when the investigated tissue (periosteum, tibial anterior muscle and subcutis) was anesthetized with carbocaine before hypertonic saline was injected (Graven-Nielsen et al. 1997a). Divergent results

of anesthetizing referred pain areas have been demonstrated (for review: Table 8.3), and possible mechanisms are discussed later in this chapter. Blocking bundle of nerves such as the brachial plexus has also been used to show the effect of pain referred to a phantom limb in angina pectoris patients (Cohen et al. 1943) and pain referred to the upper limb in a healthy subject who had hypertonic saline injected to an interspinous ligament (Feinstein et al. 1954).

Selective nerve compression block is a useful technique in human pain research. It is generally applied by two methods: 1) A pure nerve compression block can be achieved by applying force (a rubber sting with weights on each side) to a superficial nerve (e.g., the radial nerve near the wrist or the ulnar nerve at the elbow) (Kellgren et al. 1948; Torebjörk et al. 1973), and: 2) a combined compression-ischemia nerve block induced by inflating a tourniquet around a limb at a level above the systolic blood pressure (Sinclair et al. 1950; Sinclair et al. 1960; Moddel et al. 1977, II and III). It has been demonstrated that compression blocks produce large diameter fiber blockade before small diameter fiber (Bishop et al. 1933; Gasser 1935; Clark et al. 1935; Landau et al. 1953). Microneurographic recordings have confirmed that loss of tactile sensation and loss of cold sensation are associated with myelinated fiber block. Whereas loss of dull pain sensation and loss of warmth sensation are associated with unmyelinated fiber block (Torebjörk et al. 1973; Mackenzie et al. 1975; Hallin et al. 1982). When differential nerve block is used, hyperalgesia (induced by capsaicin) to brush-evoked pain is shown to be mediated by A β -fibers, while hyperalgesia to punctate-stimuli is mediated by C-fibers (Kilo et al. 1994). Furthermore, Tinel's sign (tingling sensation in the distal end of a limb when percussion is made over the site of a divided nerve) can be mediated by myelinated and unmyelinated afferents (Yarnitsky et al. 1991).

If a complete nerve block is wanted in addition to or after a differential nerve block, a modified IVRA block can be used. A pressure of > 400 mmHg is demonstrated not to be sufficient to block unmyelinated nerve fibers (Dahlin et al. 1989). In order to ensure a total nerve block, lidocaine was injected into a vein on the dorsal side of the foot (III) or hand (IV) immediately (III) or 35 min (IV) after the tourniquet had been inflated. The intravenous regional block was introduced by Bier in the beginning of

this century (1908), and it is often used for short, open, surgical procedures to ensure a total nerve fiber block (Wedel 1993). IVRA has been used in experimental settings, where the analgesic effect of lidocaine, prilocaine, morphine, and saline is tested (Arendt-Nielsen et al. 1990). In conclusion, lidocaine and prilocaine induced a quick onset of anesthesia compared with morphine and saline. However, no difference in the analgesic effect was found between morphine and saline, indicating that ischemia by itself is, to a degree, sufficient to generate anesthesia.

4.2 Modulation of local muscle pain

Many studies have been performed to identify nociceptive afferents since Gasser suggested that pain was mediated by thin afferents (Gasser et al. 1929; Gasser 1935). Muscle nociceptors have been identified in animals and are known to respond to high intensities of mechanical, thermal, and chemical stimulation (Mense 1993; Mense 1996). A study has reported that group III and IV fibers mediate muscle pain in humans (Simone et al. 1994). However, only a fraction of group III and IV afferents (33% and 43%) is known to mediate nociception (Mense et al. 1985).

In order to characterize the present muscle pain model, an experiment was setup to study which nerve fibers are mediating experimental human muscle pain, induced by IMES (IV). Previously, impulse activity has been recorded from cat group III muscle afferents (thin myelinated) in response to high electrical stimulus intensities (in average 24 times the threshold for activation of group I afferents (thick myelinated)) (Paintal 1960). However, very little information pertaining to electrical stimulation of muscle afferents in humans is available. Electrical activity from human muscle afferents has been recorded from experimental nociceptive stimuli (pressure and capsaicin (1 ml of 0.01%)) by microneurography (Simone et al. 1994; Marchettini et al. 1996), and an activation group III and IV afferents was reported. Based on measurements of nerve conduction velocity, an analysis of cerebrally evoked potentials to intramuscular electrical pain induction showed that a mixed population of nerve fibers was activated during stimulation (Svensson et al. 1997b).

A differential nerve block was applied to the upper arm proximal to the muscle pain area in the brachioradial muscle. The nerve block was induced by a combination of

compression-ischemia nerve block combined with IVRA 35 min after the inflation of the tourniquet. Muscle pain induced by IMES was gradually reduced in correlation with sensory modalities associated with myelinated nerve fibers and abolished completely at the time of the induction of IVRA (Table 4.1 and IV). This observation suggested that IMES induced muscle pain was mediated by myelinated nerve fibers. Although, a stimulus intensity of 150% of the muscle pain threshold was used to induce muscle pain, the lack of involvement of unmyelinated nerve fibers in the mediation of electrical muscle pain could perhaps be due to insufficient stimulus intensity.

time [min]	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Pinprick (A δ)	-	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-
Touch (AB)	-	-	-	-	-	-	-	-	0	0	0	0	0	-	-	-
Proprioception (AB/A δ)	-	-	-	-	0	0	0	0	0	0	0	0	0	-	-	-
Heat detection (C)	-	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-
Pressure pain (A δ /C)	-	-	-	-	-	-	-	-	0	0	0	0	0	0	-	-
Electrical muscle pain	-	-	-	-	-	-	-	0	0	0	0	0	0	-	-	-

Table 4.1. The changes of sensibility to the modalities tested during the progressive nerve block where “-“ indicates “no significant change in sensibility”; “↓” shows “a significantly decreased sensibility”; and “0” indicates “total inhibition” (IV).

4.3 Modulation of referred muscle pain

During the last century, several theories on the origin of referred pain have been suggested (see next chapter). In order to illuminate possible mechanisms of referred pain, a number of case reports and experiments have been published on the effect of anesthetizing the referred pain area. In most cases viscerally referred pain has been investigated, and contradictory results have been demonstrated (for review: Table 8.3). Weiss and Davies published the first large study (1928). They found that patients suffering from various diseases (e.g. angina pectoris, pleuritis, stomach ulcer, chronic cholecystitis, salpingitis, and kidney stones) experienced pain at structures (most often the skin) located at a distance from the affected organ(s), which could be partially and in some cases completely abolished by infiltrating the area with a local anesthetic. Similar findings were demonstrated in other studies, where

experimental pain (pressure stimulation of the diaphragm (Morley 1937); distention of duodenum by a balloon (Jones 1943); injection of hypertonic saline (Klingon et al. 1958; Hockaday et al. 1967)) produced pain referred to the skin at distant sites, which were abolished with the injection of local anesthetics. In most cases the referred pain returned once the anesthetic effect had diminished.

On the contrary, a number of clinical cases and studies have not been able to demonstrate any effect of anesthetizing the referred pain area (Table 8.3). Wollard et al. found minor or no change of referred pain intensity in an anesthetized skin area (1932). Furthermore, Kellgren did not see any decrease in referred pain intensity, when he anesthetized areas in the hand to which saline induced muscle pain was referred (1938).

Several explanations have been offered to the divergent results obtained when an area of referred pain is anesthetized, namely: 1) The variation in the number of structures (skin, subcutis, fascia, muscle, tendons, ligaments, and bone) anesthetized. This is probably a major source of error because referred pain areas, and especially visceral referred pain, tend to be located on the truncus where complete anesthesia of a referred pain area is difficult. 2) The duration and level of pain at the primary site. 3) The site of the primary pain (skin, viscera, and deep structures). 4) Whether sensory changes (hypersensitivity) take place at the referred pain site. Referral of visceral pain have divided referred pain into: a) referred pain without hyperalgesia (segmental pain) where no effect of anesthetizing the referred pain area is observed, and: b) referred pain with hyperalgesia (true parietal pain) where anesthetizing the referred pain area results in a cease or a considerably diminishing effect (Procacci et al. 1986; Ness 1995; Giamberardino et al. 1996).

In order to determine if it is possible to anesthetize referred pain, two experiments were preformed (II and III). Due to the obvious problems of blinding a study involving compression-ischemia nerve blocks and local anesthetics, it was decided first to use a non-invasive technique to anesthetize the skin area of referred pain (II). The main purpose of the experiment was to quantify a potential reduction of referred pain induced by IMES and subsequently quantify somatosensory changes in the area

in the placebo session. In the next experiment, the effect of a differential and complete nerve block of afferents from the referred pain area was tested (III).

In a placebo-controlled experiment (II), an eutectic mixture of lidocaine and prilocaine was applied to the skin lying over the referred pain area for 90 min to quantify the skin component of referred muscle pain. A cream was chosen instead of injection as this method minimized the spread of the drug to subcutaneous tissues. Local pain and referred pain were induced and maintained for 10 min, and a 22.7% reduction of the referred pain intensity was observed in the local anesthetic group compared with the placebo group (Fig. 4.1). A similar result has been reported in one case where another technique of skin anesthetization was used. Ethyl-chloride sprayed onto the saline-induced referred pain area resulted in “greatly reduced referred aching” (Whitty et al. 1958). Observations, where the superficial part of referred pain area has been anesthetized, seem to suggest that referred pain is, to some degree, dependent on an input from cutaneous afferents (for review: Table 8.3).

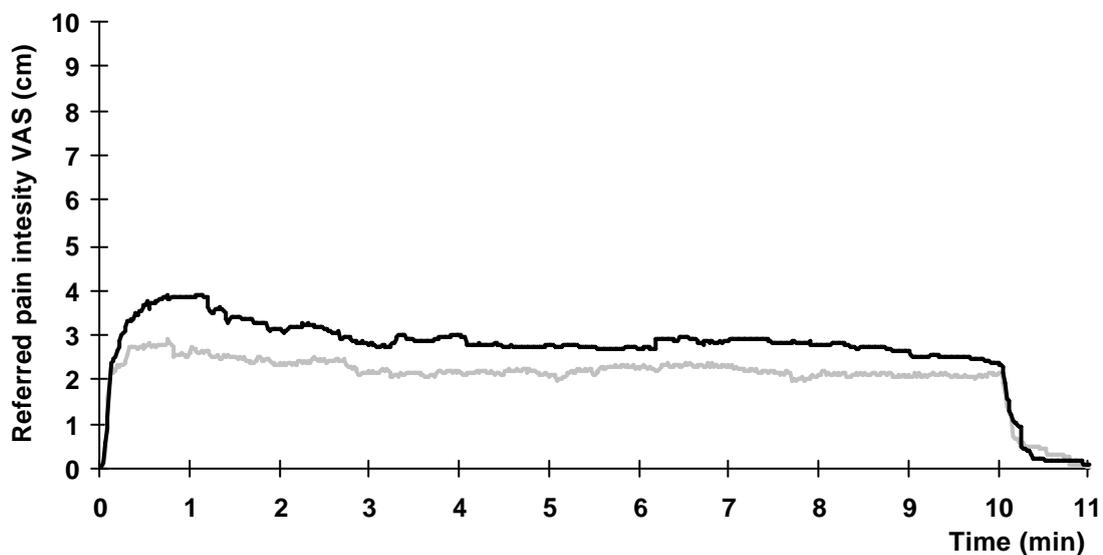


Figure 4.1. Referred pain intensity as a function of time during 10 min IMES (gray line: anesthetic cream; black line: placebo cream) (II).

Although, a reduction of referred pain was found, somatosensory changes to pinprick perception and pressure pain perception in the referred pain area were not observed during the placebo session (II). This does not correlate with the idea of segmental pain, as proposed by others (Procacci et al. 1986; Ness 1995; Giamberardino et al. 1996), and could be a sign of fundamental differences in the mechanisms of visceral and somatic pain (see discussion in chapter 5). However, methodological differences may be responsible for the divergent results. Previous studies of somatosensory changes in the referred pain area of deep pain origin have revealed contradictory results (increased (Kellgren 1938; Feinstein et al. 1954), unchanged (Steinbrocker et al. 1953), and decreased (Graven-Nielsen et al. 1997c; Graven-Nielsen et al. 1998b) sensibility to pressure).

To completely block all afferents from the referred pain area, the next experiment was undertaken. Using a combination of two techniques, differential nerve blocking with an inflated tourniquet around a limb and IVRA, an experiment looking at the referred pain intensity during 60 min of progressive nerve blocking was performed (III).

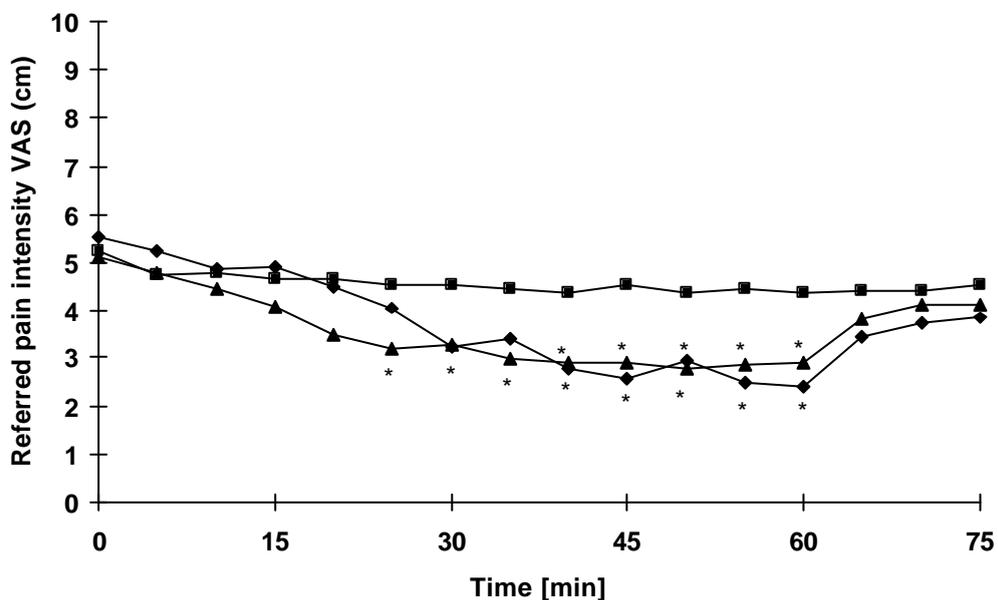


Figure 4.2.

Referred pain intensity as a function of time. Asterisks indicates a significant decrease in referred pain intensity in the compression block experiment (◆) and the compression block combined with IVRA experiment (▲) compared with the control experiment (■) (III).

Referred pain was elicited for 10 s with 5-min intervals at 150 % of the referred PT throughout the experiments. Even though referred pain intensity was reduced by 40.2% while myelinated nerve fiber function was abolished and an additional reduction was not observed, the limb containing the referred pain area was completely anesthetized (myelinated and unmyelinated nerve fiber block) (Fig. 4.2) (III). This substantial observation suggests that referred pain has a peripheral component associated with intact myelinated nerve fiber function. Several cases, in which the afferents supplying a limb have been completely blocked, have reported similar findings. Two patients with anginal pain, which in part was referred to a left amputated arm, experienced moderate/complete relief of their referred pain, when the left brachial plexus was anesthetized (Jones 1943). Injection of hypertonic saline into the interspinous ligament (C₆/C₇) caused a dull, deep aching pain on the ulnar side of the forearm in a healthy subject. Blocking the brachial plexus on the same side caused Horner's syndrome and a complete paralytic and anesthetic upper arm. However, another injection of hypertonic saline resulted in the same type of pain with the exact same location, "only slightly less intense" compared with the previous injection (Feinstein et al. 1954).

There seems to be evidence to state that referred pain is, to a degree dependent, on an intact peripheral nervous system. In the next chapter, this evidence will be discussed in relation to the different theories proposed on the mechanisms of referred pain.

4.4 Summary

Various anesthetic techniques were used to investigate the influence of peripheral pathways in experimental muscle pain and referred pain. Muscle pain generated by IMES seems to be mediated by myelinated nerve fibers. So far, contradictory observations of anesthetizing the area of referred pain have been reported, and a number of explanations have proposed. A source of error is the anesthetic technique applied since injection of local anesthetic may not anesthetize all structures involved in the referred area, and, in addition, the stimulus methods and intensity may vary between experiments. IMES was used in two different experiments in which the referred pain area was anesthetized. In the first experiment, the referred pain area was

superficially anesthetized, resulting in a 22.7% reduction of the referred pain intensity (II). Complete anesthesia of the referred pin area reduced the referred pain intensity by 40.2% in the second experiment (III). The inability to abolish referred pain by anesthetizing it clearly indicates that central mechanisms play a role in the elicitation and maintenance of referred pain. Also, the peripheral component of referred pain seems to be mediated by myelinated nerve fibers.

5. DISCUSSION

5.1 Neurophysiological mechanisms for referred pain

Clinically and experimentally referred pain is characterized by the occurrence after injury of deep tissues such as muscles and joints or viscera. Usually it develops in the same or adjacent anatomical segment (Weiss et al. 1928; Inman et al. 1944; Torebjörk et al. 1984; Giamberardino et al. 1994; Graven-Nielsen et al. 1997a; Simons et al. 1998; I - III). The referred pain area is often described as “tender”, and an examination reveals hyperalgesia (Head 1893; Procacci et al. 1986; Vecchiet et al. 1992; Giamberardino et al. 1994). The distribution of referred pain increases with intensity and duration of the pain from the injured tissue (Arendt-Nielsen et al. 1997c; Drewes et al. 1997; Graven-Nielsen et al. 1997c; I).

The mechanisms responsible for pain referral to other structures are not known in detail, nevertheless, several theories have been suggested and will be briefly summarized.

The convergent-projection theory: Based on the ideas of Sturge (1883) and Ross (1888), Ruch (1961 and 1979) proposed that afferent fibers of different origin converge onto common spinal neurons (Fig. 5.1A). The core of this suggestion is that the nociceptive activity from the spinal cord is misapprehended as originating from other structures. This could explain the segmental nature of referred pain and the increased referred pain intensity recorded when local pain was intensified (Arendt-Nielsen et al. 1997c; Drewes et al. 1997; Graven-Nielsen et al. 1997c; I). However, it does not explain the delay in the development of referred pain following local pain as observed by others (Inman et al. 1944; Graven-Nielsen et al. 1997a-b; I). Also, referred pain has not been demonstrated to be a bi-directional phenomenon (e.g., muscle pain in the anterior tibial muscle produces pain in the ventral part of the ankle, but the opposite condition has not been demonstrated). Finally, the threshold for local and referred pain is different (Inman et al. 1944; Sinclair et al. 1948; Torebjörk et al. 1984; Hong et al. 1996; I).

The convergence-facilitation theory: MacKenzie (1893) was also inspired by the ideas of Sturge (1883) and Ross (1888). He believed that viscera were totally insensitive

and that non-nociceptive afferent input to the spinal cord created an “irritable” focus in the spinal cord (Fig 5.1B). This focus would make other somatic inputs appear in an abnormal fashion and in some cases even be perceived as referred pain. The theory was not recognized, mainly due to the fact that it did not accept true visceral pain. In recent years, however, MacKenzie’s simple idea of an irritable focus has reclaimed awareness under another term - central sensitization. The somatosensory sensibility changes reported in referred pain areas (Steinbrocker et al. 1953; Hockaday et al. 1967; Arendt-Nielsen et al. 1997b; Graven-Nielsen et al. 1997c) could in part be explained by similar mechanisms in the dorsal horn neurons, and the delay in appearance of referred pain demonstrated in various studies (Graven-Nielsen et al. 1997c; I) could also be explained since the creation of central sensitization may take time.

The axon-reflex theory: Bifurcation of afferents from two different tissues has been suggested as an explanation to referred pain (Fig. 5.1C) (Sinclair et al. 1948). Although, bifurcation of nociceptive afferents from different tissues (muscle and skin: (Mense et al. 1981) and intervertebral discs and skin: (Takahashi et al. 1998)) exists, it is generally agreed that these types of neurons are rare (McMahon 1994). A time delay in the appearance of referred pain, different local PT and referred PT, and somatosensory sensibility changes in referred pain area cannot be explained by this theory.

The thalamic-convergence theory: Theobald suggested that referred pain appeared as a summation of input from the injured area and the referred pain area within neurons in the brain, and not in the spinal cord (Fig. 5.1E) (1949). A recent study of referred pain in monkeys applying computer simulations has demonstrated several pathways, which converge on different cortical and sub-cortical neurons (Apkarian et al. 1995). Numerous experimental and clinical studies (for review: Table 8.3) including II and III have documented an effect of anesthetizing the area of referred pain, and therefore referred pain may likely not be explained solely by a central mechanism.

The above-mentioned theories lack some of the referred pain characteristics previously described in this chapter. Recently, Mense suggested an interesting theory,

especially from a “referred muscle pain” point of view, which is known as *the central-hyperexcitability theory* (Fig. 5.1D) (Mense 1994).

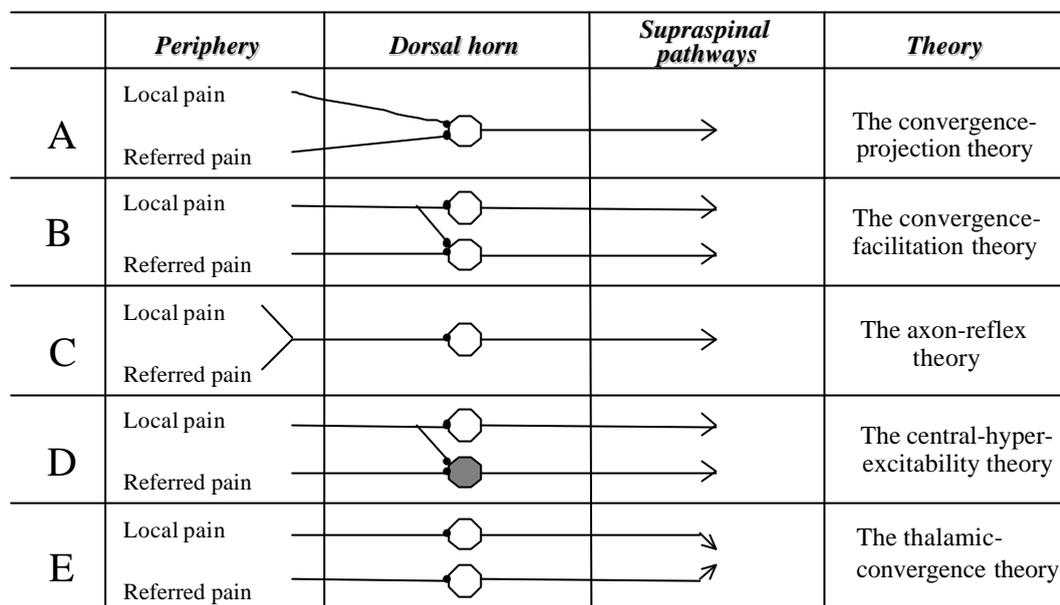


Figure 5.1. The different possible mechanisms of referred pain. Dorsal horn neurons are shown as open circles, and the shaded circles indicate connectivity changes in the dorsal horn. Part of the figure is modified from (Selzer et al. 1969).

Recordings from a dorsal horn neuron in animals have revealed that noxious stimuli to a receptive field in a muscle generated within minutes receptive fields at a distance from the original receptive field (Hoheisel et al. 1990; Hoheisel et al. 1993). The appearance of two new receptive fields could indicate that latent convergent afferents on the dorsal horn neuron are opened by noxious stimuli appearing from muscle tissue (Mense 1994), and this facilitation of latent convergence connections could appear as referred pain. Recent observations from the same group have demonstrated that substance-P released from the terminal ends of primary afferents plays a role in the connectivity in the dorsal horn (Hoheisel et al. 1995). Furthermore, an expansion of receptive fields proximal to the normal receptive field was found in a study where experimental myositis is induced, and afterwards application of antagonists to three different neurokinin receptors are effective in preventing the induced hyperexcitability (Hoheisel et al. 1997). The idea of this theory falls in line with several of the

characteristics of referred pain (dependency on stimulus and a delay in appearance of referred pain compared with local pain), however, the proximal appearance of receptive fields, thought of as referred pain, is in contrast to the reports from a majority of the experimentally referred pain studies including healthy subjects (Kellgren 1938; Inman et al. 1944; Arendt-Nielsen et al. 1997a-c; Graven-Nielsen et al. 1997a-c; I - III). Clinical studies looking at the spread of experimentally induced referred pain in patients suffering from whiplash syndrome and fibromyalgia have demonstrated proximal as well as distal referral of pain (Johansen et al. 1999; Graven-Nielsen et al. 1999). A possible explanation to the divergence in these observations could be that an already ongoing pain is necessary to induce a state of hyperexcitability in the spinal cord resulting in proximal and distal referral compared with the one-sided distal referral in healthy subjects.

The findings in the experiments, in which the referred pain area was exposed to various degrees of anesthesia (skin anesthesia resulted in referred pain reduction by 22.7% (II) and a complete nerve block in 40.8% reduction of referred pain intensity (III)), indicated that afferents from the referred pain area were involved in the processing of referred pain. Although human skin nociceptors are known not to have any resting activity (Campbell et al. 1996), a reduction of activity from other skin receptors (e.g., thermal receptors and possibly low-threshold mechano-receptors) could explain the reduced referred pain in the skin anesthetic study (II). A clear indication that spinal and/or supraspinal mechanisms were involved in the appearance of referred pain, was observed in the complete nerve block study (III), where an additional reduction of referred pain was observed, nevertheless, referred pain was still perceived.

Based on the pain model developed in this study and the observations derived, it seems evident that referred muscle pain depends on peripheral and central mechanisms. Although the hyperexcitability theory (Mense 1993; Hoheisel et al. 1993; Mense 1994) is based on animal studies where receptive fields appeared within minutes and not within seconds in humans as referred pain areas (I - III), the idea of latent connections between dorsal horn neurons is convincing. An intact connection

between the dorsal horn neurons and the potential receptive field/referred pain area seems to be mandatory, if they are to appear with full magnitude. In order to explain the referred pain, which could not be anesthetized, supraspinal mechanisms that could mimic the mechanisms seen in the dorsal horn region cannot be excluded. If the processing of local and the referred pain is not done in the same supraspinal pathways and centers, neuro-imaging techniques (positron emission tomography and functional magnetic resonant imaging) will possibly be able to visualize the nociceptive processing of referred pain in humans

5.2 Summary

Referred pain has been known and described for more than a century and has been used extensively as a diagnostic tool in the clinic. Several theories regarding appearance of referred pain have been suggested, and basically they state that nociceptive dorsal horn neurons receive convergent inputs from various tissues, thus higher centers cannot identify the actual input source. The data obtained in the present experiments (I - III) and previous clinical and experimental experiments are evaluated in comparison with the features of each theory. The rigidities of previous theories have been replaced by newer theories where plasticity of neurons in the spinal cords dorsal horn plays a central role. Noxious stimuli to a receptive field in a muscle are suggested to generate, within minutes, receptive fields at a distance from the original receptive field due to openings of latent connections between dorsal horn neurons. Many features of referred pain (intensity, time duration, and distribution) can be explained by this theory. However, data obtained from experiment II and III also suggest that the referred pain intensity induced by IMES and partly blocked by anesthetizing the afferents from the referred pain area is dependent on input from the area. Therefore, a combination of peripheral and central mechanisms seems to be required for referred pain to appear.

6. CONCLUSION

Pain and relief of pain have been and are still one of the greatest challenges in medicine. Muscle pain is a major factor in many disorders such as injuries, degenerative diseases, and cancer. The mechanisms underlying muscle pain are not fully understood. A particular problem in muscle pain is the relationship between local and referred muscle pain. Experimental pain models are useful in basic pain research, because they allow a standardized activation of the nociceptive system and measurements of evoked responses. When healthy subjects are used, confounding factors (e.g., habitual pain, psycho-social aspects of pain, emotions, etc.) can be minimized, thus improving the validity of the study.

An electrical muscle pain model was constructed and applied on healthy subjects. The model was found suitable for inducing local and referred muscle pain. It was demonstrated that local pain was elicited around the stimulation needles (proximal part of the tibial anterior muscle) and referred pain appeared at a distal site (the ventral part of the ankle) (I). Referred pain required significantly higher stimulus intensity compared with local pain (I), and referred pain appeared later than local pain (I - II). The sizes of local and referred pain areas were correlated to pain intensity (I), and local and referred pain thresholds were reproducible within and between sessions (I - IV). Experimentally (electrical stimulation and infusion of hypertonic saline) induced muscle pain seems to be mediated by myelinated and unmyelinated afferents (IV) and the peripheral component of referred muscle pain by myelinated afferents (III). Furthermore, cutaneous anesthesia of the referred pain area resulted in a reduction of referred pain intensity of 22% (II), while a complete nerve block of afferents from the referred pain area resulted in a 40% reduction (III). In summary, observations from the presented experiments (I - IV) suggest that elicitation of referred muscle pain is depending on and correlated to local muscle pain. Peripheral input from the referred pain area is involved, but is not a necessary condition for referred pain to appear (II - III).

The present studies as well as others suggest that central hyperexcitability is involved in the generation of referred pain, but further investigations on mechanisms of

referred pain are needed. Spinal cord and higher centers are likely structures to study. In humans, non-invasive approaches such as neuro-imaging (e.g., positron emission tomography and functional magnetic resonant imaging) may be helpful. Furthermore, pharmacological modulation of experimentally induced referred pain may contribute with additional information.

The data presented in this thesis have made further contributions to understanding the mechanisms of muscle and referred pain that can be helpful in diagnosis, control, and treatment of muscle pain. Moreover, the intramuscular, electrical pain model may be helpful in future clinical studies when aspects of muscle pain are investigated.

DANISH SUMMARY / DANSK SAMMENFATNING

Diagnosticering og behandling af smerter har været, er og vil fremover være en af de største udfordringer i klinikken. Muskelsmerter er en vigtig komponent i mange sygdomme og specielt i muskel- og ledsygdomme af degenerativ karakter. De patofysiologiske mekanismer, som ligger tilgrund for muskelsmerter, er for en stor dels vedkommende helt ukendte eller kun delvist belyst og overvejende hos dyr. Et forhold, som er meget interessant i muskelsmerte sammenhæng, er relationen mellem (lokal) muskelsmerte og smerter, som opstår på baggrund af disse i andre nærliggende strukturer (meddelt smerte). Med eksperimentelle smertemodeller er det muligt at opnå vigtig informationer om basale smertemekanismer, fordi de tillader en standardisering af smertestimuli. Brug af raske forsøgspersoner tillader desuden observationer uden slørende faktorer, som f.eks. psykosociale aspekter og habituelle smerter.

I det aktuelle Ph.D. studie blev en elektrisk muskelsmerte-model konstrueret og appliceret på raske forsøgspersoner. Formålet med studiet blev defineret som: 1) at opstille en smertemodel, hvor man kunne "tænde og slukke" lokal og meddelt muskelsmerte (I - IV); 2) at karakterisere lokal og meddelt muskelsmerte med hensyn til smerteintensitet, -areal, og -beliggenhed (I) samt teste variabiliteten af disse faktorer mellem forsøgene (I - IV); 3) at modulere lokal og meddelt muskelsmerte med velkendte anæstesiteknikker for at undersøge de enkelte smerterers neurofysiologiske egenskaber (II - IV).

I forsøgene observeres, at lokal muskelsmerte fremkaldes i området omkring stimulationsnålene (proksimale halvdel af den anteriore tibialis muskel), og meddelt smerte opstår adskilt og distalt herfor (anteriore del af anklen/forfoden) (I - IV). Der kræves en signifikant højere stimulusintensitet for at fremkalde meddelt smerte i forhold til lokal smerte (I), og meddelt smerte opstår senere end lokal smerte (I og II). Arealet af både lokal og meddelt smerte er korreleret til stimulus intensiteten (I). Lokale og meddelte smertetærskler og arealet af meddelt smerte er reproducerbart mellem sessioner (I - IV). Myeliniserede og ikke-myeliniserende nervefibre medierer eksperimentelt (intra-muskulær elektrisk stimulation og infusion af hypertont saltvand) fremkaldt lokal muskelsmerte (IV), mens meddelt smerte medieres af

myeliniserede nervefibre (III). Bedøvelse af huden over det meddelte smerteområde resulterer i en 22% reduktion af meddelt smerteintensitet (II), mens en komplet bedøvelse af de afferente nerver fra området med meddelt smerte resulterer i en 40% reduktion af meddelt smerte (III). På baggrund af disse resultater kan man konkludere, at meddelt smerte opstår som følge af lokal muskelsmerte, og der synes at være både en spatial og temporal afhængighed. De afferente nerve fibre fra det meddelte smerteområde er involveret i meddelt smerte, men er ikke nødvendige for, at meddelt smerte opstår (II – III).

Central hypereksitabilitet kan være en mulig mekanisme i fremkaldelsen af meddelt smerte, og yderligere undersøgelser af dette er påkrævet. Centre lokaliseret i den forlængede rygmarv eller på et højere cerebralt niveau er specielt interessante, da de, som vist i dette Ph.D. studie, må være involveret i fremkaldelsen af meddelt smerte. Non-invasive undersøgelser i form af billeddannelse (f.eks. positron emission tomografi (PET) og funktionel magnetisk resonans billeddannelse (fMR)) kan vise sig at være anvendelige, når man vil lokalisere de områder i central nervesystemet, som er involveret i bearbejdningen og opfattelsen af lokal og meddelt smerte. Ligeledes kan farmakologiske undersøgelser, hvor modulation af eksperimentelt fremkaldt meddelt smerte forsøges, bidrage med oplysninger om mekanismer, som ligger tilgrund for denne form for smerter.

Den elektriske muskel-smertemodel præsenteret i denne Ph.D. afhandling har belyst forskellige aspekter af lokal og meddelt muskelsmerte og bidraget med ny viden om disse. Den anvendte smertemodel vil fremover kunne finde anvendelse i både eksperimentelle og kliniske studier af muskel- og meddelt smerter, og dette kan i længden være med til at finde en mere rationel behandling af muskelsmerter.

8. TABLES

Table 8.1 Chronological review of experiments involving intramuscular electrical muscle stimulation				
Reference	No. of subjects	Aim of study	Investigated muscle	Findings
Meadows 1970	1	Assessment of intramuscular sensibility	M. quadriceps vastus medialis	Variation of PT to electrical stimulation was found when the needle was moved
Brucini et al. 1981	8 patients 8 controls	Muscle PT in patients with osteoarthritis in the knee	M. quadriceps vastus medialis	↓ muscle PT in patients
Duranti et al. 1983	11 healthy	Relationship between muscle PT and blink response threshold	M. quadriceps vastus medialis	Correspondence between muscle PT and blink response threshold
Bellini et al. 1984	18 healthy	Effect of vibratory stimulation on muscle PT and blink responses	M. quadriceps vastus medialis	Vibration ⇒ ↑ muscle PT / blink response threshold
Pantaleo et al. 1986	28 healthy	Effect of vibratory stimulation on muscle PT and blink responses	M. quadriceps vastus medialis	Vibration ⇒ ↑ muscle PT / blink response threshold
Duranti et al. 1988	15 healthy	Effect of TENS and IMES on muscle PT and blink responses	M. quadriceps vastus medialis	TENS / IMES ⇒ ↑ muscle PT
Vecchiet et al. 1988	15 healthy	Sensory-/PT in skin, subcutis, and muscle after induction of muscle pain	M. brachioradialis	↓ PT in skin, subcutis, and muscle during muscle pain
Vecchiet et al. 1989	8 patients 6 healthy	Muscle/subcutaneous PT at referred pain areas in patients suffering from renal/urethral colics	M. obliques externus	↓ PT in subcutis and muscle in the referred pain area in patients
Vecchiet et al. 1990a	36 patients	Muscle/subcutaneous PT at trigger point and referred pain area in myofascial pain patients	M. supraspinatus, m. longissimus and m. gluteus minimus	↓ Muscle/subcutaneous PT at trigger point and referred pain areas compared with contralateral side
Vecchiet et al. 1990b	9 patients	Muscle/subcutaneous PT at referred pain areas in patients suffering from renal/urethral colics	M. obliques externus	↓ PT in subcutis and muscle at the referred pain area
Kawakita et al. 1991	12 healthy	Methodological study of IMES in tender points	Tender points in various muscles	PT is repeatable within the same session

Table 8.1 continued				
Reference	No. of subjects	Aim of study	Investigated muscle	Findings
Vecchiet et al. 1992	9 patients 6 healthy	Muscle/subcutaneous/skin PT at referred pain areas in patients previously suffering from renal/uretral colic	“muscular tissue of the lumbar region (metamere L1)”	↓ PT in muscle, subcutis and skin at the referred pain area in patients
Vecchiet et al. 1993	6 healthy	Sensory-/PT in skin, subcutis, and muscle after induction of muscle pain	M. quadriceps vastus lateralis	↓ PT in skin, subcutis, and muscle
Vecchiet et al. 1994	35 patients 30 healthy	PT in skin, subcutis, and muscle in fibromyalgia (FM) and myofascial pain syndrome (MPS) patients compared with healthy subjects	M. deltoideus, m. trapezius	↓ PT in muscle, subcutis and skin in MPS and FM patients compared with controls
Giamberardino et al. 1994	15 patients 12 healthy	Evaluation of time as a factor in visceral referred pain in patients suffering from renal/urethral colic	“muscular tissue of the lumbar region (metamere L1)”	Time is only partly linked to the continuing presence of referred hyperalgesia after the removal of renal/uretral stones
Ishimaru et al. 1995	16 healthy	Analgesic effects of TENS and electroacupuncture (EA) on deep tissues in human subjects	“midpoint of the right anterior femoral region”	TENS ⇒ unchanged muscle PT EA ⇒ ↑ muscle PT
Vecchiet et al. 1996	21 patients 30 healthy	PT in chronic fatigue syndrome (CFS) patients	M. deltoideus, m. trapezius, m. quadriceps	↓ PT in muscle, subcutis and skin in CFS patients compared with controls
Arendt-Nielsen et al. 1997b	13 healthy	Assessment of temporal summation in local and referred muscle pain areas	M. tibialis ant.	↓ PT to repeated nociceptive stimuli in referred muscle pain area
Svensson et al. 1997a	11 healthy	Cerebral processing of muscle pain	M. brachioradialis	Increased cerebral blood flow was observed in the contralateral anterior cingulate cortex, primary sensorimotor cortex and lenticular nucleus during muscle pain
Svensson et al. 1997b	11 healthy	Comparison of central processing of nociceptive and non-nociceptive input from muscle and skin	M. brachioradialis	A difference is found in the electrophysiological processing of input from muscle and skin

Table 8.1 continued				
Reference	No. of subjects	Aim of study	Investigated muscle	Findings
Svensson et al. 1997c	10 healthy	Comparison of central processing of nociceptive input from muscle and skin	M. brachioradialis	A common mode of central processing of nociceptive input from muscle and skin is found
I	18 healthy	Method study local and referred pain induction by intramuscular electrical stimulation	M. tibialis ant.	Referred pain is dependent on temporal and spatial summation
II	14 healthy	Examine the effect of skin anesthetizing the referred pain area	M. tibialis ant.	Referred pain is dependent on sensory input from the periphery
Sörensen et al. 1998	12 patients 12 controls	Assessment of hyperalgesia in fibromyalgia (FM) patients	M. tibialis ant.	A state of deep hypersensitivity was found in FM patients
Slot Fenger-Grøn et al. 1998	30 healthy	Assessment of muscle pain induced by pressure and electrical stimulation	M. tibialis ant.	A correlation between muscle pain from pressure and electrical stimulation was found
Graven-Nielsen et al. 1998b	14 healthy	Study sensibility of deep tissue at various times during muscle pain within homo- and heterotopic areas	M. tibialis ant.	An inhibitory mechanism during muscle pain was shown to be effective for the deep tissue sensibility
Graven-Nielsen et al. 1998a	22 healthy	Quantification of deep and superficial sensibility in saline induced muscle pain	M. tibialis ant.	No muscle or subcutaneous hyperalgesia to electrical or pressure stimulation was found
III	12 healthy	Study on referred pain mechanisms by differential/complete nerve block from the referred pain area	M. tibialis ant.	Peripheral (myelinated nerve fibers) and central mechanisms are involved in the elicitation of referred pain
Graven-Nielsen et al. 1999	14 FM patients	Effect of ketamine on experimental referred pain and temporal summation	M. tibialis ant.	Ketamine results in diminished referred pain and temporal summation of electrical stimulation
IV	13 / 10 healthy	Study of experimental muscle pain mechanisms by differential/complete nerve block	M. brachioradialis	Experimental muscle pain is mediated by group III and IV fibers

Reference	No. of subjects	Aim of study	Applied stimulus	Investigated muscle	Findings
Kellgren 1938	3-14 healthy	Investigation of characters and distribution of pain produced by injection of hypertonic saline (HS)	Hypertonic saline	Various muscles	Local pain is referred to other regions and follows a certain pattern and is associated with tenderness
Steinbrocker et al. 1953	18 healthy	To repeat the above mentioned experiment	Hypertonic saline	Various muscles	Local pain is associated with various sensibility changes, may be referred to other regions and does not follow any patterns
Feinstein et al. 1954	78 healthy	To examine patterns, and sensory and autonomic changes in experimental muscle pain	Hypertonic saline	Various muscles	Local pain is associated with various sensibility changes, may be referred to other regions and seems to be following patterns
Klingon et al. 1958	1 healthy	To examine sensibility changes from deep pain	Hypertonic saline	Various muscles	Cutaneous hyperalgesia occurs at a predictable site determined by known segmental innervation of muscles
Torebjörk et al. 1984	10 healthy	To examine factors of importance in the induction of referred pain	Intraneural electrical stimulation	Muscles innervated by the median nerve	Referred pain is influenced by temporal and spatial summation and correlated to the stimulation frequency
Vecchiet et al. 1993	6 healthy	Sensory-/PT in skin, subcutis, and muscle after induction of muscle pain	Hypertonic saline	M. quadriceps vastus lateralis	↓ PT in skin, subcutis, and muscle at the referred pain site

Table 8.2 continued					
Reference	No. of subjects	Aim of study	Applied stimulus	Investigated muscle	Findings
Arendt-Nielsen et al. 1997b	13 healthy	Assessment of temporal summation in local and referred muscle pain areas	Hypertonic saline	M. tibialis ant.	Temporal summation from nociceptive input exists in local and referred muscle pain areas
Graven-Nielsen et al. 1997a	10 healthy	Examination of variability and sensory aspects of experimental muscle pain	Hypertonic saline	M. tibialis ant	reproducible intra-individual scores of pain-intensity, quality, distribution and cutaneous changes were obtained
Graven-Nielsen et al. 1997b	11 healthy	Examination of temporal and spatial aspects in experimental muscle pain	Hypertonic saline	M. tibialis ant	Experimental muscle pain is influenced by temporal and spatial summation
Graven-Nielsen et al. 1997c	11 healthy	Examine whether referred pain can be maintained by continuous stimulation and whether sensibility changes occur	Hypertonic saline	M. tibialis ant	Ongoing muscle pain can cause sensory changes in the referred pain area
I	18 healthy	Method study local and referred pain induction by intramuscular electrical stimulation	I.m. electrical stimulation	M. tibialis ant.	Referred pain is dependent on temporal and spatial summation
II	14 healthy	Examine the effect of skin anesthetizing the referred pain area	I.m. electrical stimulation	M. tibialis ant.	Referred pain is dependent on sensory input from the periphery
Graven-Nielsen et al. 1998a	14 healthy	Study sensibility of deep tissue at various times during muscle pain within homo- and heterotopic areas	Hypertonic saline	M. tibialis ant.	An inhibitory mechanism during muscle pain was shown to be effective for the deep tissue sensibility

Table 8.2 continued

Reference	No. of subjects	Aim of study	Applied stimulus	Investigated muscle	Findings
III	12 healthy	Study on referred pain mechanisms by differential/complete nerve block from the referred pain area	I.m. electrical stimulation	M. tibialis ant.	Peripheral (myelinated nerve fibers) and central mechanisms are involved in the elicitation of referred pain
Graven-Nielsen et al. 1999	14 FM patients	Effect of ketamine on experimental referred pain and temporal summation	Hypertonic saline	M. tibialis ant.	Ketamine results in diminished referred pain and temporal summation of electrical stimulation

Table 8.3 Review of the effect of anesthetizing experimentally and clinically referred pain					
<i>Partially or completely anesthetized referred pain</i>					
Reference	No. of subjects	Point of stimulation	Applied stimulus	Referred pain localization	Effect of anesthetic applied to the referred pain area
Weiss et al. 1928	25 patients unknown number of controls	Patients with referred pain suffering from various diseases (angina pectoris, pleuritis, stomach ulcer, chronic cholecystitis, salpingitis, kidney stones) Healthy controls: Distension of the lower esophagus with a balloon caused pain referred to an area between the shoulders, epigastrium and other portions of the anterior abdomen			Skin infiltration with Novocain abolished or modified the referred pain in all subjects
Morley 1937	1	1) Anterior right dome of the diaphragm 2) Posterior part of the right dome of the diaphragm	Pressure applied by a finger	Sharp pain in 1) the front of the center of the right clavicle and 2) suprascapular region	Skin infiltration with Novocain abolished or modified the referred pain
Jones 1943	? healthy	Distension of the first portion of the duodenum	Distension of a balloon	Mid-epigastric area	Abolition of referred pain
Cohen et al. 1943	2 patients	Myocardial infarction	Myocardial ischemia	“phantom” left arm	Abolished/significantly modified by anesthetizing left brachial plexus
(Theobald 1949	>3 healthy patients	The cervix uteri	Increasing faradic current	Area inferior to the umbilicus	Abolished at low currents, however, no or only partial abolishment was observed at higher currents
Feinstein et al. 1954	1 healthy	Interspinous ligament C ₆ /C ₇	0.5ml 6% saline	Dull, deep aching pain on the ulnar side of the left forearm	Left plexus brachialis block induced a complete anesthetic left arm but no change in referred pain intensity
Doran et al. 1954	unknown number of TB patients	The phrenic nerve	Electrical stimulation	An area around the shoulder tip	A more powerful stimulation was required to produce pain in the anesthetized area

Table 8.3 continued		<i>Partially or completely anesthetized referred pain</i>			
Reference	No. of subjects	Point of stimulation	Applied stimulus	Referred pain localization	Effect of anesthetic applied to the referred pain area
Klingon et al. 1958	1 healthy	Various muscles in neck/shoulder/arm	0.5 – 2.0 cc 5% saline	Various areas in neck/shoulder/arm	Deep pain was abolished by anesthetizing “metamerically” skin
Whitty et al. 1958	1 healthy	Interspinous ligament C ₇ /Th ₁	0.3ml 6% saline	C ₇ segment	Skin anesthetic (ethylchloride spray) greatly reduced referred pain and hyperalgesia
Hockaday et al. 1967	28 healthy	Interspinous spaces from C ₁ /C ₂ to L ₅ /S ₁	0.1 – 0.3 ml 6% saline	At a distance at various places	Anesthetizing skin/muscle caused a reduction/elimination of referred pain/hyperalgesia, however, it returned as soon as the anesthetic effect wore off.
II	14 healthy	Right tibial anterior muscle	I.m. electrical stimulation	Ventral part of the right ankle/forefoot	Skin anesthetizing the referred pain area resulted in a 22% reduction of referred pain
III	12 healthy	Right tibial anterior muscle	I.m. electrical stimulation	Ventral part of the right ankle/forefoot	Differential and complete nerve block of the referred pain area resulted in a 40% reduction of referred pain
<i>Unaltered referred pain</i>					
Woollard et al. 1932	9 subjects	The phrenic nerve	Light pinch	A small area above the shoulder/acromioclavicular joint	No change in referred pain intensity when stimulating the proximal end of the phrenic nerve
Kellgren 1938	1 healthy	1) Extensor digitorum muscle and 2) flexor digitorum profundus	0.2 cc6% saline	1) Area over the back of the ipsilateral hand and 2) ulnar side of the ipsilateral hand	No change in referred pain, however, tenderness in the areas disappeared

Table 8.3 continued		<i>Unaltered referred pain</i>			
Reference	No. of subjects	Point of stimulation	Applied stimulus	Referred pain localization	Effect of anesthetic applied to the referred pain area
Lewis 1942	1 patient	Angina pectoris	“Local anesthetic”	Area over the sternum	No change in referred pain
Theobald 1949	see above				
Hockaday et al. 1967	see above				

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