Quantitative monitoring of acute experimental cutaneous pain and inflammatory vascular reactions

Effect of local analgesics, capsaicin and antihistamines

by Peter Bjerring

Denne afhandling er i forbindelse med nedenstående tidligere publicerede afhandlinger af Det lægevidenskabelige Fakultet antaget til forsvar for den medicinske doktorgrad.

Aarhus, den 28. maj 1991.

Søren Mogensen
dekan

This review and the following previously published papers have been accepted by the Medical Faculty of the University of Aarhus to be defended in public for the degree of Doctor of Medicine.

Aarhus, May 28th 1991.

Søren Mogensen
Dean

Forsvaret finder sted fredag den 28. juni 1990, kl. 13.00 i auditorium 424, Anatomisk Institut, Universitetsparken, Aarhus Universitet.

This thesis is based on the following publications, which will hereinafter be referred to according to the following enumeration:

(I)
Sensory and pain threshold characteristics to laser stimuli. Lars Arendt-Nielsen & Peter Bjerring. J Neurol Neurosurg Psych 1988; 51: 35–42.

(II)
Skin reflectance spectrophotometry. Peter Bjerring & Peter H. Andersen. Photodermatology 1987; 4: 167–171.

(III)
Laser-induced pain for evaluation of local analgesia: A comparison of topical application (EMLA) and local injection (Lidocaine). Lars Arendt-Nielsen & Peter Bjerring. Anesth Analg 1988; 67: 115–123.

(IV)
Depth and duration of skin analgesia to needle insertion after topical application of EMLA cream. Peter Bjerring & Lars Arendt-Nielsen. Br J Anaest 1990; 64: 173–177.

(V)
A quantitative comparison of the effect of local analgesics on argon laser induced cutaneous pain and on histamine induced wheal, flare and itch. Peter Bjerring & Lars Arendt-Nielsen. Acta Derm Venereol (Stockh) 1990; 70: 126–131.

(VI)
Vascular response of human skin after analgesia with EMLA cream. Peter Bjerring, Peter H. Andersen & Lars Arendt-Nielsen. Br J Anaest 1989; 63: 655–660.

(VII)
Methotrexate inhibits the human C5a-induced skin response in patients with psoriasis. Thomas Ternowitz, Peter Bjerring, Peter H. Andersen, Jens-M Schröder & Knud Kragballe. J Invest Dermatol 1987; 89: 192–196.

(VIII)
15-hydroxyeicosatetraenoic acid (15-HETE) specifically inhibits the LTB_4-induced skin response. Thomas Ternowitz, Peter H. Andersen, Peter Bjerring, Karsten Fogh, Jens-M Schröder & Knud Kragballe. Arch Dermatol Res 1989; 281: 401–405.

(IX)
Use of a new argon laser technique to evaluate changes in sensory and pain thresholds in human skin following topical capsaicin treatment. Peter Bjerring & Lars Arendt-Nielsen. Skin Pharmacol 1989; 2: 162–167.

(X)
Inhibition of histamine skin flare reaction following repeated topical applications of capsaicin. Peter Bjerring & Lars Arendt-Nielsen. Allergy 1990; 45: 121–125.

(XI)
Effect of antihistamines on argon laser induced cutaneous sensory and pain thresholds and on histamine-induced wheal and flare. Peter Bjerring. Skin Pharmacol 1989; 2: 210–216.

(XII)
Argon laser induced cutaneous sensory and pain thresholds in post-herpetic neuralgia. Quantitative modulation by topical capsaicin. Peter Bjerring, Lars Arendt-Nielsen & Ulla Søderberg. Acta Derm Venereol (Stockh) 1990; 70: 121–125.

Contents

LIST OF ABBREVIATIONS:

SP	: Substance P.
C5a	: C5 complement split product
NK	: Neurokinin
EMLA	: *E*utectic *m*ixture of *l*ocal *a*nalgesics

PREFACE

The underlying work, upon which this thesis is based, was carried out between 1985 and 1989 during my residency at the Department of Dermatology, Marselisborg Hospital.

I wish to express my sincere gratitude to my closest coworker lic. tech. Lars Arendt-Nielsen for this long period of conscientious team-work, and for many intense and inspiring discussions. Without his creative skills and enormous diligence this work would not have been possible.

I particularly wish to thank Peter H. Andersen, M.D. for his enthusiastic collaboration during the development of the reflectance spectroscopical technique. Also, I want to thank my coworkers, Knud Kragballe, M.D., Ph.D., Karsten Fogh, M.D., Ph.D., Thomas Ternowitz, M.D., Ph.D., Ulla Søderberg, M.D., and J-M Schröder, Ph.D, for their engagement and support.

I am deeply indebted to Professor Hugh Zachariae, M.D., Ph.D., Head of Department of Dermatology, Marselisborg Hospital, for his extensive personal support, for providing laboratory facilities and for his constructive criticism of the review article. Professor Annelise Rosenfalck, Head of Department of Medical Informatics, Aalborg University, and Palle Carlsson, M.D., Ph.D, Department of Anaesthesiology, Aarhus Amtssygehus, also provided excellent working conditions. Kurt Pfeiffer Petersen, M.Sc.(Pharm) is thanked for his advise and support. Finn Thøgersen is gratefully acknowledged for his highly skilled technical support. I am much obliged to Nicholas Coppard, Ph.D. for linguistic revision.

Financial support was received from The Danish Cancer Society, AstraGruppen A/S, The Danish Medical Research Council, The Danish Technical Research Council, The University of Aarhus, Edvard Carl Larsen's and Mrs. Larsen's Foundation.

CHAPTER 1

Introduction

1. Cutaneous pain and the relation to acute inflammation

In the beginning of this century, studies of the relationship between the peripheral part of the sensory nervous system and local inflammatory reactions were initiated by Sir Thomas Lewis with his now classical investigations of the cutaneous triple response (1). Experiments to elucidate vasodilator functions of the peripheral parts of the cutaneous sensory and pain systems were, however, performed half a century earlier (2), and were followed by other studies around the turn of the century (3,4). More recently, investigators have employed advancing technologies available at their particular time and developed neurophysiological and psychophysical methods for quantification of both clinical and experimental pain in humans (5–34,I,III,XI).

Around the middle of our century, general interest in the relationship between the nervous system and peripheral inflammatory reactions reached a minimum, and only a few researchers remained active in this field (35). However, the rediscovery of the phenomenon of neurogenic inflammation by Jancsó et al (36) renewed interest in this research area, and during the last decades, transmitters, modulators, hormones and specific receptors have been identified, which mediate information and control of different parts of the nervous system and the peripheral target tissues during pain and inflammation (37).

Although inflammation can occur in denervated tissue (38), recent investigations on neurogenic inflammatory mechanisms have led to the proposal of a major role for the peripheral sensory system in a variety of inflammatory disorders such as rheumatoid arthritis (39), fibrositis syndrome (40–42), and asthma (43). The recognition of early inflammatory components involved in a number of dermatological diseases including psoriasis (44) and cutaneous allergy (45–48) has also suggested a functional role for the cutaneous sensory nervous system.

Until now, the actual influence of the sensory nervous system in acute cutaneous inflammation has not been established due to methodological problems in quantifying both the activity of the nervous system (the cutaneous pain perception) and the neurogen-dependent inflammatory reactions of the skin. We found that improvements of existent methods were essential and, during the years 1985 to 1988, we developed techniques for the measurement of sensory- and pain perception and for dynamic monitoring of cutaneous vascular reactions. Also, clinical investigations were performed in order to compare the new tools with existing methods, and in order to evaluate these techniques in clinical dermatology. The present quantification of sensory and pain perception is based on psychophysical principles acknowledging Keele's (49) statement that "the verbal report of a person's experience is the primary source of information about pain, and is of more value than the measurement of objective accompaniments of pain".

The issues of this review are therefore monitoring of cutaneous sensory- and pain perception in relation to acute experimental inflammation (fig. 1).

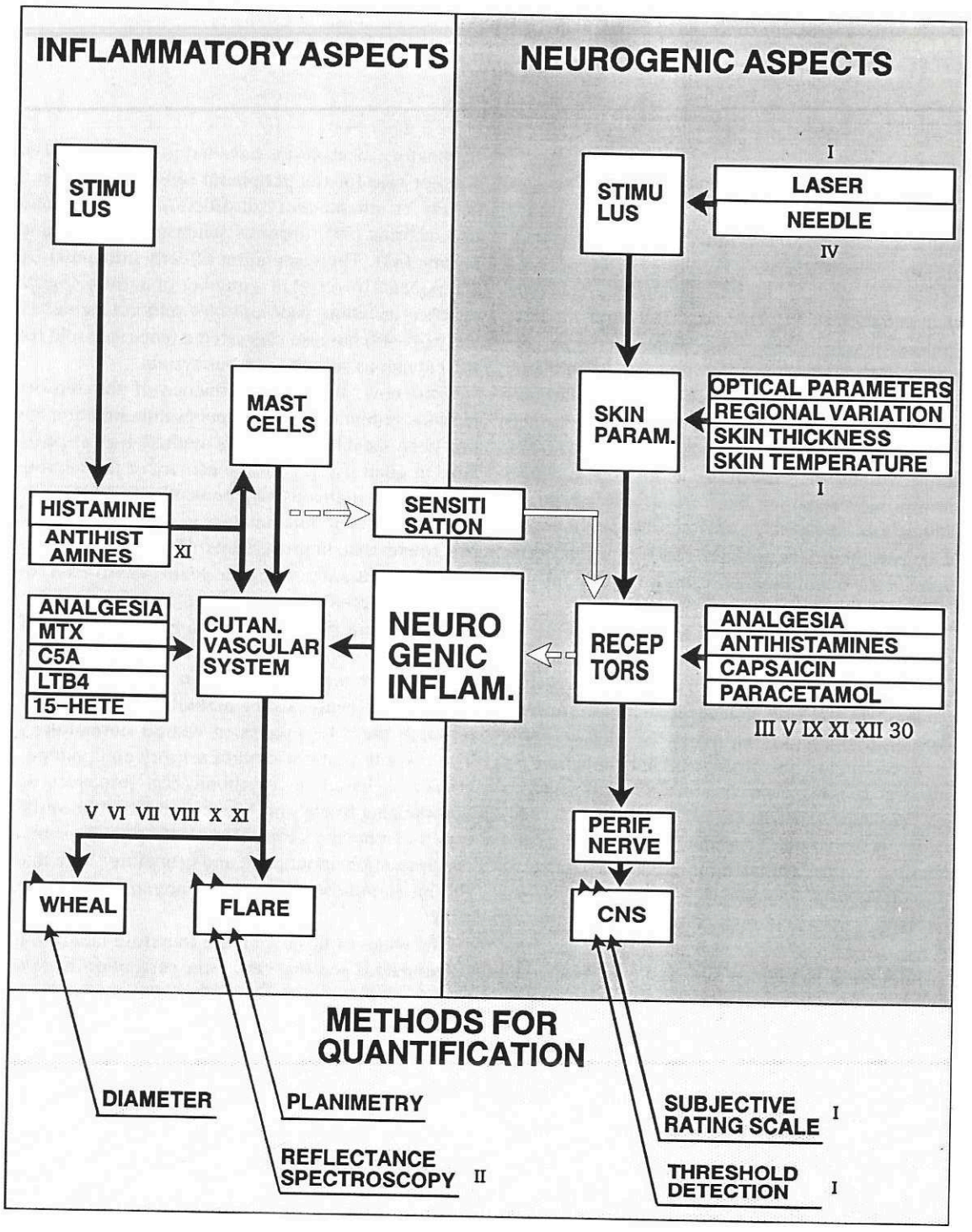

Fig. 1. A simplified schematic representation of different aspects of the relationship between cutaneous inflammation and the peripheral sensory nervous system. The Roman numbers refer to topics discussed in papers included in this thesis.

CHAPTER 2

The peripheral anatomical and neurophysiological basis of cutaneous pain perception

2.1. The cutaneous nociceptors – morphological aspects

Cutaneous pain is normally mediated by high threshold receptors, – the nociceptors, which respond selectively to potentially damaging high intensity stimuli (50). Also, stimulation of the afferent nerve fibres along the passage to the central nervous system may elicit pain (51). Morphologically, the receptor structures of sensory nerves of the skin may be either free nerve endings, mediating information of cold, warmth, itch or polymodal nociceptive events (pain), or corpuscular bodies e.g. Ruffini-, Pacinian-, and Meissner's corpuscles, Krause's end-bulbs and Merkel's touch spots (52–55). The nociceptors of the free nerve endings are mainly located subepidermally close to blood vessels and mast cells, but some nerve fibres penetrate the basal membrane and enter the epidermis (56,57). These fibres have mainly been observed to terminate in association with epidermal Merkel cells, and it is not known if they also mediate nociceptive responses.

2.2. The cutaneous nociceptors – physiological aspects

Different types of cutaneous nociceptors can be identified based on their response to various excitatory stimuli. They may be divided into three main groups, according to their assumed natural stimuli, e.g. mechanosensitive, thermosensitive and polymodal nociceptors (58–61). However, most nociceptors respond to several excitants and are thus polymodal nociceptors. The exact biochemical structure and the molecular reactions leading to the generation of action potentials in the nociceptor have not yet been elucidated. Nociceptors without any specialized function respond to noxious mechanical and heat stimuli. Recently, it has been suggested by LaMotte et al (62) that selective nociceptors may exist in the skin which are specifically responsive to itch and chemical stimuli.

Nociceptors signal not only when the noxious threshold has been exceeded, but they also supply information on the stimulus intensity by both the firing rate and the rate of increase in firing rate (63). A slow, spontaneous discharge of the nociceptor may occur. Only at higher discharge frequencies or by involvement of more nociceptors (spatial summation) pain is perceived (64,65). During thermal stimulation, the discharge frequency of the nociceptors is linearly related to the skin temperature (66,67). The fact that the threshold at which the nociceptors begin to fire does not correspond with the threshold for pain perception (64,68), inspired the suggestion that firing rates lower than those which elicit perception of pain may be used for transmission of other types of information, e.g. on homeostasis (69,70).

Microneurographic studies using the methods of Torebjörk & Hallin (71) to detect the innervation area (72) and type of sensory stimuli which may excite single sensory nerves (73), have contributed to the elucidation of some components of the structure of peripheral nerve fibre organization. Lynn & Shakhanbeh (74) found that low-frequency discharge in C-fibres of the skin in the range of 0.1 to 1 Hz resulted in vasodilatation without any sensation of warmth or pain, and that a firing rate of more than 1 Hz was essential to generate a sensation of pain. This indicates that the afferent C-fibres may have at least a dual mode of operation. Firstly, at lower stimulus intensity, the fibres respond with low frequency (< 1 Hz) discharge which triggers local tissue reactions as vasodilatation, and, secondly, at higher stimulation strengths, the subject's perception of pain is evoked in addition to triggering of local tissue vaso-reactions. Lynn (75) found that low frequency activity in Aδ-fibres also generated minor local vasodilatation.

A possible third mode of action of the peripheral sensory nerves was proposed by Wall (76) who suggested that these nerves might convey non-electrical information by axonal transport of chemical substances from the peripheral micro-environment to the central nervous system, thereby signalling in-

formation on the chemical environment of the nerve endings (77). Though the theory may explain different observations of symmetrical inflammatory skin diseases (44,48,78), no experimental evidence for this theory has yet been presented.

2.3. Afferent fibres mediating pre-pain (warmth) and pain

Based on differences in conduction velocities, two populations of afferent nerve fibres, which serve the sensation of pain and warmth in the skin can be identified, i.e. small myelinated Aδ- fibres (79–82) and unmyelinated C-fibres (83). Fibres mediating pre-pain sensations were studied by LaMotte et al (84), and it was found that the conduction velocity of these fibres was 1.5 ± 0.5 m/s corresponding to that of the C-fibre population. Others have found similar or only slightly different conduction velocities from 1.2 m/s (85), to 0.5 m/s (86–88). Using microneurographic techniques, the subjective perceptions to noxious (painful) stimuli can be divided into 1), a sharp, pricking first pain, which is mediated by Aδ-fibres (12,89), and 2), a slow, dull, burning, and poorly localized pain, which is associated with activity in the C-fibre population (63,71,80,90,92–99).

Aδ-fibre mediated nociceptors are activated at the threshold for the sensation of an immediate, sharp pin-prick sensation, which correspond to peripheral nerve conduction velocities above 6 m/s. We investigated nerve conduction velocity in relation to the quality of pain and found that argon laser stimuli with intensities well above the pain threshold induced a well defined, immediate and sharp pinprick sensation followed by a slowly rising, long lasting and burning after-sensation (31). The burning after-sensation suggests recruitment of unmyelinated C-fibres. Also, sensations of this quality evoked by other stimulation methods have been shown to be caused by activity in high-threshold C-fibre innervated nociceptors (83,100–104).

2.4. The peripheral neuronal target for cutaneous laser stimulated pre-pain and pain perception

The exact peripheral neural events and the type of receptors activated by experimental argon laser stimulations are not known. This is related to lack of knowledge of the exact anatomy, biochemistry and function of the nociceptor. In addition to stimulation of receptors, excitation of the distal, intracutaneous part of the nerve fibre proximal to the receptor may occur and lead to pain perception. However, based on the results of investigations of the optical properties of the skin (105), the distribution of sensory nerve endings in the skin (57) and on the cutaneous penetration of brief (200 ms) high energy argon laser light, it may be assumed that argon laser induced pain stimulation evokes painful sensations due to stimulations of the cutaneous nociceptor and not the nerve fibres.

Previous investigators have identified electrical activity in peripheral C-fibres during acute cutaneous pain of different origin (83,92,106), and others have reported Aδ-fibre activity during such stimulations (51,79,94). Using CO_2 laser stimulations of short durations, Pertovaara et al (107) recorded activation of cutaneous Aδ-fibres, whereas Bromm & Treede (96) found that CO_2 laser impulses stimulated both Aδ-fibres and C-fibres of the skin. This apparent discrepancy may be explained by the fact that Bromm & Treede applied stronger stimuli, capable of exciting even high-threshold C-fibres and thus produced both sharp pin-prick- and burn type pain sensations. Under these conditions, pre-pain (warmth) sensations were seldom reported. The pin-prick sensation is registered much faster than the C-fibre mediated pre-pain sensations, and central mechanisms presumably inhibit pre-pain sensations at higher laser intensities (31,108). Also, stimulations by the CO_2 laser at pre-pain intensities only infrequently elicited a perception of warmth (64,89). In contrast, an argon laser induced pre-pain sensation of warmth can be elicited in most individuals (31).

The optimal method for evaluation of the function of different nerve fibre populations in the perception of cutaneous pre-pain and pain stimuli seems to be microneurography of peripheral nerve fibres. This is a sophisticated and time consuming technique (58,91), which unfortunately, has not yet been used to evaluate the argon laser technique as a stimulator for cutaneous pre-pain and pain perceptions.

In conclusion, argon laser light pulses which induce pre-pain sensations seem to stimulate warmth-sensitive low threshold C-fibre mediated receptors, whilst at higher energy levels, the perception of immediate, sharp, pricking pain would appear to be mediated by high-threshold, heat sensitive Aδ-fibres.

2.5. The anatomical and biophysical parameters of the skin

The regional variations of biophysical skin parameters may be of major importance in both clinical and experimental cutaneous threshold and suprathreshold sensory and pain determinations (109). Irradiation of the skin by visible or infrared light is associated with two complicated phenomena of scattering and absorption. Both theoretical and experimental techniques such as surface thermometry (110), experimental implantations of thermocouples (107, 111,112), and invasive fibre-optic measurements of the irradiance in different areas of the tissue (113) have been employed to determine the distribution of either intracutaneous or subcutaneous temperature, irradiance, or dynamics of heat distribution. The relevance of these studies may be limited however in the description of *in vivo* phenomena since mathematical models assume a series of prerequisite standardizations to reduce complexity which may not always apply due to local and regional variations of the micro-architecture of the tissues, whilst experimental, therapeutical or monitoring procedures often disrupt the integrity of the tissue. It has, for example, not been possible to record intracutaneous temperatures either during or immediately after short laser pulses under normal conditions, i.e. with preserved micro-architecture, normal dermal blood flow, normal content of haemoglobins, and normal heat dissipation from the surface.

Radiant heat stimulation may penetrate the skin layers differently according to anatomical region (114), metabolic (115) and hormonal status (116–118), time of day (119), regional blood perfusion, and racial (120,121) variations. From surface and intracutaneous measurements, a series of theoretical thermodynamic models for the skin have been constructed, based on several different tissue models. Buettner (122) employed a homogeneous tissue model, which predicted a temperature maximum at the level of 1 mm below the skin surface and a corresponding skin temperature minimum 1.8 mm below the surface at the level of the arteriolar-venous plexus. Several other thermodynamic skin models have been proposed (112, 123,124). Recently, Lahaye & van Gemert (125) calculated temperature profiles in the skin during laser induced blood vessel destruction for the treatment of haemangiomas and their results may also be applicable in studies of radiant heat induced pain. Due to a close anatomical proximity of the dense cutaneous nervous plexus and the superficial vessels of the skin, similar temperature profiles for vessels and nociceptors may be assumed. Hardy (126) determined the intracutaneous temperature threshold for the perception of pain to be 45.7°C. This seems to be generally accepted, even though other investigators have reported both higher and lower values (63,64,127,128).

CHAPTER 3

Methods for elicitation of experimental cutaneous pain

Cutaneous pain can be elicited experimentally by a series of different stimulation modalities. These may be based on either 1), fixed stimulation intensity and different stimulation duration or 2), fixed stimulation duration and variation of the stimulus intensity. The tests are normally repeated until a specific predetermined perceptional limit is reached or the perception is in accordance with a specific perceptional rating. Different methods and their limitations have been extensively reviewed previously (51,129–131). Generally, experimental pain stimulators can be classified according to the physical nature of the stimulus, – mechanical, electrical, chemical, or thermal.

3.1. Mechanical stimulation

Elicitation of pain by mechanical stimulation (15,123,132–134) is always accompanied by firm touching or stabbing of the skin. The subject may experience difficulty in describing a complex sensory stimulus of pain combined with sensations of touch and pressure. The peak pain intensity is often reached slowly and at various rates. Mechanical stimulation by shear, pressure or vibration may generate pain which in part originate from underlying structures, e.g. tendons, muscles, fascia, ligaments, and periost. This may obscure the subject's interpretation of the cutaneous nociceptive signal and make subjective ratings difficult.

Although mechanical stimulation seems to be best suited to the study of visceral and deep somatic pain from tendons, periost and muscles (135,136), pricking of the skin's surface or inserting needles into the skin, with subsequent determination of pain threshold or ratings of suprathreshold pain sensations on a visual analogue scale (VAS), may be adequate methods for determination of analgesia of the skin (137,138). Juhlin & Evers (139) reviewed the methods of pin-pricking for evaluation of the effect of topical analgesia (EMLA cream, an *e*utectic *m*ixture of *l*ocal *a*nalgesics) for minor skin surgery (140) and for venous cannulations in children. The pin-prick method may supply information on analgesia level in the outer layers of the skin, and may be a supplement to the non-tactile radiant heat of CO_2- and argon laser stimulation techniques. These latter techniques are assumed to generate a more selective form of pain (28,88,107,141,142). Other investigators have inserted thick needles or cannulas into the skin to investigate the effect of topical EMLA cream analgesia in the deeper layers of the skin and in the subcutaneous tissues. Determinations of this type have been used to estimate the utility of EMLA application for procedures such as i/v cannulation (143–148) or other forms of mechanical incision procedures, such as split skin grafting (149,150), or minor skin surgery (151–153).

To allow comparison with the argon laser for stimulation of sensory- and pain perceptions (III), we examined the depth-duration relationship after epicutaneously applied EMLA cream using a specially developed method of slow, micrometer-controlled, vertical insertion of a thick (18G, 1.2 mm) intravenous cannula. We registered the depth at which the subject first reported the slightest sensation of touching by the needle, and the depth at which the sensation was perceived as a distinct, sharp pain (IV). The depth at which the sensory and pain thresholds were reached increased linearly with the application time of EMLA cream (fig. 2). For application times shorter than 120 min, the depth of analgesia increased during the period after removal of the cream, which suggests different time constants for skin -penetration, -accumulation and -elimination of the drugs. The gradual onset of analgesia after EMLA cream application seems to make it suitable for investigations of the cutaneous nociceptors and the dynamics of skin analgesia. Results of this type of investigation may also be important in clinical practice. The manufacturer of EMLA cream recommends that it is applied under plastic film occlusion for one hour. Our studies of EMLA analgesia depths (IV) (and also argon laser stimulated pain threshold determinations (III)) suggest that the present recommendations for the use of these topical analgesics should be reconsidered in order to obtain maximal effect.

The needle insertion method may be associated

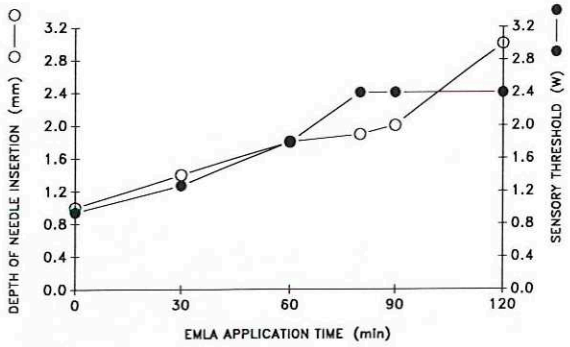

Fig. 2a. The effect of EMLA cream on the cutaneous sensory threshold measured by needle insertion (○-○) and by argon laser induced sensory threshold determination (●-●).

with a significant receptor sensitization, – a state of enhanced nociceptor responsiveness. The sensitization of the nociceptors is mediated by local algesic inflammatory mediators released in response to tissue injury (155–157). These mediators exert their effect on thin myelinated Aδ-fibres (158–161), and decrease perception thresholds (hyperalgesia and hyperpathia) (158,162). Generally, the use of experimental pain stimulation methods which induce local damage and receptor sensitization is declining due to lack of stability of baseline receptor excitability.

3.2. Electrical stimulation

Electrical stimulation is a technically convenient method for elicitation of experimental pain, and the use of electrical current is a method which has been widely employed for direct stimulation of nervous pathways (71,163–165) and for cutaneous sensory and pain threshold- and supra-threshold pain stimulation (6,166–168). A considerable experimental bias may be anticipated as the electrical stimulation of mixed cutaneous nerves primarily activate thick, myelinated nerve fibres, which convey afferent signals of touch and temperature. Only stronger stimuli activate thin pain-mediating fibres. The resulting afferent central input is a compound signal which comprises different sensory modalities. The subjective perception is therefore usually described as a peculiar, unfamiliar sensation (169,170).

3.3. Chemical stimulation

Induction of experimental pain by application of chemicals was introduced by Lewis (171) and has since been used extensively to elicit experimental cutaneous pain (92,172–176). For an extensive review of the early literature, see Keele & Armstrong (6). Application or injection of chemicals has also been used for elicitation of oral mucosal pain (177) as well as for the study of pain in muscles and ligaments (171). Chemical stimulation of cutaneous pain is always followed by some degree of injury to the skin resulting in sensitization and hyperalgesia (178). This excludes repeated measurements on the same or adjacent skin areas. Although considered of reduced utility today (177) a commonly used technique was to apply chemical substances on the exposed base of cantharidin (174) or suction (179) induced skin blisters. It was found difficult to obtain reproducible results because the blister formation requires either removal of the epidermis or at least detachment of epidermis from the dermis, and formation of cantharidin- or suction-induced skin blisters (179) may thus induce inflammation and pain *per se*. Neurogen-dependent inflammation and sensitization is also generated as nerve fibres which cross the basal membrane will be ruptured during formation of the blister. After chemical stimulation, the maximal pain sensation does not always reach its peak immediately. This could be due to an indirect action of the algesic substances, presumably by generation or activation of endogenous secondary algesic tissue- or plasma- derived pain producing substances. Also, temporal and spatial summations in the central nervous system of afferent impulses from chemogenic sensitive C-fibres might in part account for the observed latency from a chemical pain stimulation to the perception of pain (5,62,94).

3.4. Thermal stimulation with contact elements

Experimental pain may also be evoked by skin contact with high or low temperature probes

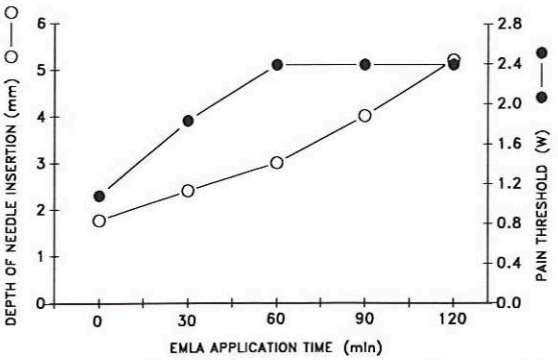

Fig. 2b. The effect of EMLA cream on the cutaneous pain threshold measured by needle insertion (○-○) and by argon laser induced pain threshold determination (●-●).

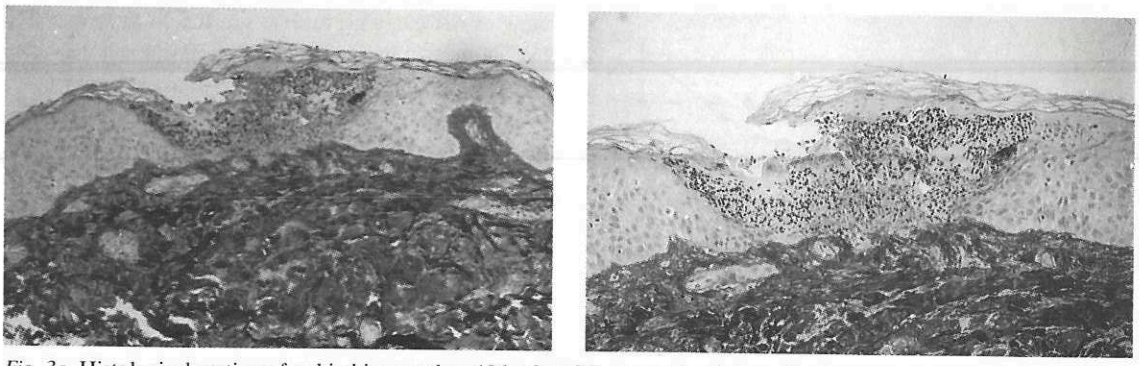

Fig. 3a. Histological section of a skin biopsy taken 18 h after CO_2 laser stimulation. The laser parameters were: Power 3.5 W, duration 0.2 s, spot size 3 mm. The high absorption of the CO_2 laser irradiation in the outermost skin layers induces increased skin temperature and superficial damage. Low power view: original magnification × 100, high power view: original magnification × 400.

(131,180,181). Experimental pain stimulators have been developed in which contact probes, (or thermodes), can change the skin surface temperature to preset values in fractions of a second. However, the heat transfer is accomplished by direct contact between thermodes and the skin and therefore not only nociceptors, but also receptors for other sensory modalities will be stimulated (89).

3.5. Non-touching thermal stimulation

Techniques allowing thermal stimulation of the skin without contact have been developed by Hardy et al (5,182,183). Their initial studies resulted in construction of the first reliable optical instrument, the Hardy-Wolff-Goodell radiant heat dolorimeter. The mode of action was based on pain stimulation by visible and infrared radiation generated by an electric bulb, from which the light was transmitted through a lens system to the skin. Subsequently, numerous investigators employed this apparatus or modifications of it (79,111,122), and significant knowledge of normal and pathological pain mechanisms has since been accumulated. The basic properties of the method have been reviewed by Procacci (131). A major disadvantage of this method is that it requires blackening of the skin by e.g. carbon black or India ink to obtain a high cutaneous absorption of the light energy. A series of methodological problems have also been encountered using the infrared radiant stimulator for pain threshold determinations. Different investigators experienced difficulties in relating experimental laboratory findings to clinical observations when long stimulation times were applied. Also, inconsistencies between results obtained in different laboratories, even when similar methods and equipment were used, were encountered (8,181,184). Finally, the constant time/ variable intensity method for pain stimulation can induce skin damage, and if such damage occurs, nociceptors in both the test area and the area surrounding the stimulated tissue will be sensitized (84,158,159,185–187). Methodological problems such as these forced Goetzl et al (188) to conclude that the infrared radiant heat stimulation method lacks reproducibility.

Recent experiments with a visible light laser as pre-pain and pain stimulator (I) indicate that visible light, which in part is conveyed by radiation in the skin, is a reliable heat energy source for cutaneous stimulation. However, as part of the laser energy is reflected off the skin surface, it is essential that the skin reflectance can be monitored (I,II,189).

3.5.1. CO_2 laser stimulation

Brief pulses from a CO_2 laser have recently been introduced as a new source of noxious thermal energy for experimental pain stimulation of the skin. This laser emits a spectrally narrow band of radiation in the far infrared region (10600 nm). CO_2 laser radiation has been extensively used for cutaneous pain stimulation (I,12,22,64,68,87,89,103, 107,191–193), and this method allows selective thermal stimulations at both noxious and non-noxious levels to be applied to the skin without significant stimulation of other sensory modalities. One major problem which has been observed by several investigators however, is that the CO_2 laser induces superficial tissue damage, especially when the stimulus is very short (0.1 ms to 0.1 s) and the laser energy is high. Energy from the CO_2 laser beam is, for

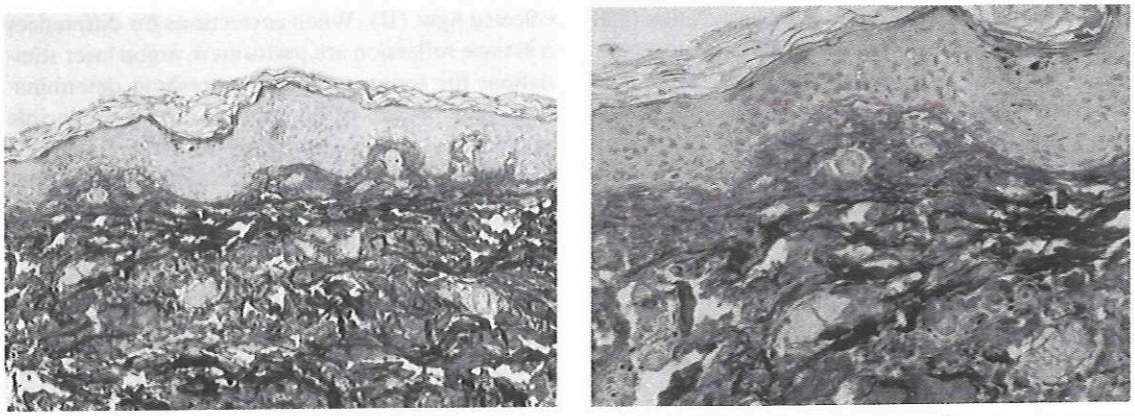

Fig. 3b. Histological section of a skin biopsy taken 18 h after argon laser stimulation. The laser parameters were: Power 3.5 W, duration 0.2 s, spot size 3 mm. No damage could be observed. Low power view: original magnification × 100, high power view: original magnification × 400.

practical purposes, totally absorbed in water molecules in the stratum corneum (194). This may result in rapid temperature rise, often to tissue-destructive levels. Carbonization of the skin surface may occur at energy levels just above the pain threshold at levels normally used for experimental elicitation of pain perception in lightly pigmented skin (1,12,22,195), (fig. 3a, 3b and 4). The CO_2 laser energy is transmitted from the stratum corneum to the lower layers of epidermis and to the papillary dermis, where most nociceptors are situated, exclusively by tissue conduction.

3.5.2. Argon laser stimulation

The argon laser was introduced into medicine in the mid-sixties. It was initially used for coagulation of diabetic retinopathy and later used in dermatology for treatment of haemangiomas. Willer et al (196) introduced this laser to cutaneous neurophysiology for elicitation of flexor reflexes. In contrast to the

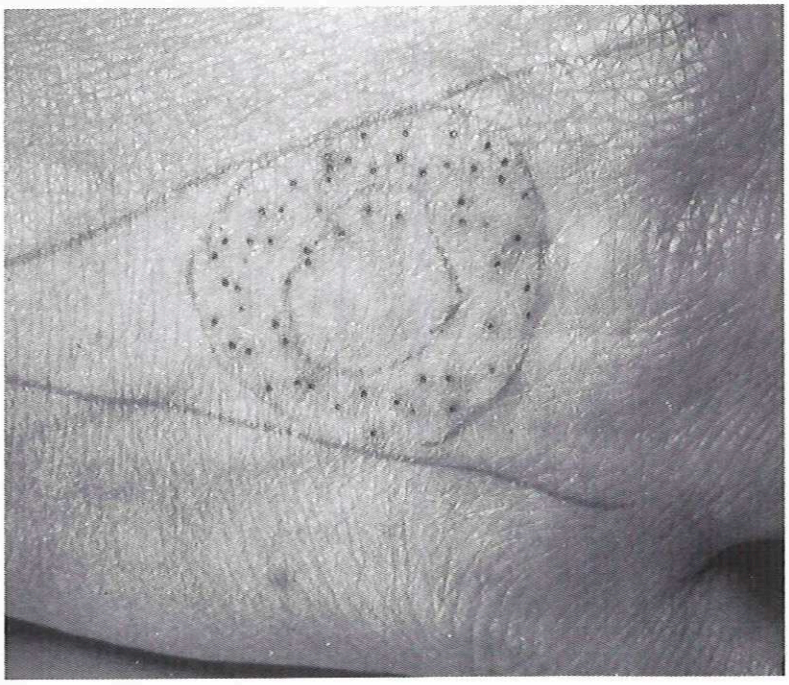

Fig. 4. Dorsum of the hand 18 h after CO_2 laser (outer ring) and argon laser (central area) irradiation. The laser parameters were: Power 3.5 W, duration 0.2 s, spot size 3 mm. No damage could be observed after the argon laser irradiation.

CO_2 laser the argon laser emits visible radiation (488 and 515 nm), which partially penetrate the upper layers of the skin. The argon laser possesses a range of technical characteristics, e.g. high output power (5–10 W) which can be gated into single pulses by simple external electronics, and a flexible fibre optic light guide system. Based on these properties we find that the argon laser is particularly well suited for experimental pain stimulation (I). Part of the incident light from the argon laser is reflected by the surface of the skin (105,197) and only 50 – 90 % (depending on skin colour) of the laser energy is absorbed and utilized for stimulation. Therefore, the ratio between reflected and absorbed argon laser energy is of major importance for standardization of the argon laser technique for pain stimulation. We developed an apparatus for the recording and characterization of optical parameters of the skin in order to determine the ratio between the absorbed and reflected light (II). When corrections for differences in surface reflection are performed, argon laser stimulations for sensory and pain threshold determinations have proven to be accurate and reproducible both in basic cutaneous neurophysiology (I,IX,X, 29,31,200), and in clinically related investigations (III,XI,XII,30,142,202,203).

The high surface temperatures which are registered after CO_2 laser stimulation are not observed after argon laser stimulation due to partial cutaneous penetration of the argon laser wavelengths. Argon laser stimulations are generally regarded as non-destructive. We investigated this topic by comparing pain stimulations at the same high energy level using either argon- or CO_2 laser (I,29). The CO_2 laser produced significant skin damage whereas the argon laser left the skin undamaged at similar energy levels (fig. 3a, 3b and 4).

CHAPTER 4

Quantification of pre-pain and pain perception

Pain is a qualitative, subjective phenomenon which does not easily lend itself to quantitation. However, objective measurements can be made along the peripheral part of the nociceptive pathway with a high degree of accuracy (71,91,164). Investigation of the relationship between the discharge in human nociceptive C-fibres and the subject's rating of his pain sensation has shown that subjective ratings were better estimates of the stimulus size than discharge rates of individual nerve fibres (63). Recently, several investigators have correlated subjective reports of pain with event-related electrical brain potentials as an objective measurement of responsiveness to suprathreshold noxious thermal stimulations (I,12,28, 31,88,95,192,195,204). The objectively measured values can be used in addition to the subject's own report. It cannot be more accurate than the report itself, as it is a subjective decision whether a certain stimulation is painful or not (63). Finally, studies of subjective responses to noxious stimuli are complicated by the subject's previous pain experience (68).

4.1. Quantitative subjective experimental pain measurement techniques

Following short experimental thermal stimulation, three well defined subjective perceptions can be identified, a lower level of sensation, below which nothing can be felt (the sensory threshold), a lower level of sharp, pin-pricking pain perception (the pain threshold), and an upper level of pain tolerance (the tolerance level) (131). Determinations of the tolerance level may lead to tissue damage, and therefore, may be a matter for ethical consideration (205). Consequently, we have not performed laser stimulations at energy intensities capable of reaching this subjective pain level. Between these fixed levels, defined by the sensory threshold, the pain threshold and the tolerance level, ratings can be performed using different types of descriptive terms (I). Perceptions below the pain threshold, including miscellaneous subjective sensations termed pre-pain sensations, have been described after electrical stimulation, CO_2 laser (mainly touching sensations) (I,22,-29,64,87, 195,206), and argon laser stimulation (I,31). The most reproducible pain perception seems to be the threshold for the slightest sharp painful sensation which can be perceived (the pain threshold).

4.1.1. Sensory and pain thresholds

Both sensory (warmth) and heat pain thresholds may be determined with high accuracy (20) in the non-trained subject after only a few test trials, provided the type of stimulus is familiar to the subject. Results obtained by different stimulation methods support the finding that C-fibres may mediate the perception of warmth just above the sensory threshold and that thin, myelinated, Aδ-fibres are involved in the perception of pricking pain at the pain threshold after brief argon laser stimulations of the skin.

The subjective discrimination between 1) no sensation, 2) pre-pain and 3) pain can be determined very accurately if the skin is stimulated by both a series of increasing energy levels (from below the threshold) and by a series of decreasing energy levels (from above the threshold) (I).

The reliability of threshold determinations seems to be strongly dependent on the stimulus used. In a comparison of different pain stimulators (20) electrical stimulation had the best reproducibility. Under normalized laboratory conditions using healthy volunteers, electrically induced pain thresholds had a coefficient of variation of less than 5 % during immediate test- re-test sessions, and less than 15 % for inter-session tests. However, electrical stimulation is a non-physiological and "non-familiar" sensation to which the subjects have to become accustomed to. Other types of stimulation may be regarded as more natural. Infrared CO_2 laser stimuli are perceived as light touching to sharp pinprick, eventually followed by a long-lasting, burning pain (I,12,22,29,206). Argon laser stimuli are perceived a faint sensation of warmth to very strong pin-pricks followed by a long-lasting, burning pain (I, 31,196).

If the argon laser intensity and physiological and optical parameters of the skin can be thoroughly controlled (I), both sensory and pain thresholds are sensitive and reproducible parameters. In our expe-

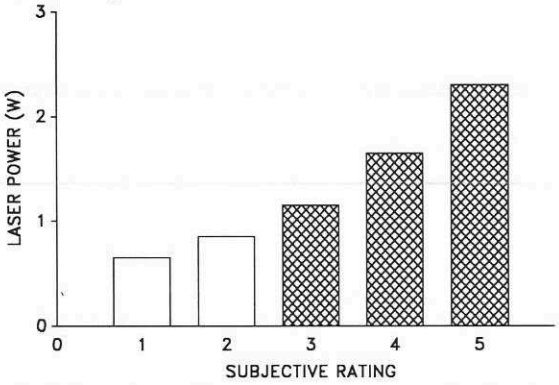

Fig. 5. The relationship between laser power and subjective pre-pain and pain ratings in fair caucasian skin. 1= faint warmth, touching or late pin-prick (threshold of sensation), 2 = distinct warmth or faint, late pin-prick (pre-pain), 3 = distinct, immediate pin-prick (pain threshold), 4 = sharp, distinct pin-prick (moderate pain), 5 = an intense pin-prick sensation (strong pain).

rience, the coefficients of variation is 9.3 % for the sensory (warmth) threshold and 4.3 % for the pricking pain threshold (I). Furthermore, it should be emphasized, that these figures are the summarized variance i.e. the sum of variances of stimulation, skin optics and thermodynamics, nociceptor -sensitivity and -density, and finally the central processing and verbal reporting. It is difficult to evaluate the exact contribution of each component participating in this complex pathway. Only the stimulation parameter, the argon laser energy can readily be monitored. We calculated a coefficient of variance of the laser energy to be as low as 1.8 % for a series of 16 stimulations.

Many investigations in pain research are based on signal detection- or the sensory decision- theories. The basis for separating the subject's response into a sensory and a decision process has been questioned (8,23). According to the sensory decision theory, any variation in an observed threshold level is a result of modulations of sensory transduction mechanism or related to the type and strength of the stimulus, whereas the decision process is inherently invariable. However, we find that variability in threshold levels may arise from either the sensory transduction or the decisive process, and that these entities cannot be separated easily. Investigations combining microneurography of peripheral sensory nerves and threshold determination after argon laser stimulation should be initiated for the evaluation of the relative contribution of peripheral sensory transduction in this process.

It can be concluded that the present use of a stimulation method which selectively elicits warmth or pin-prick sensations, seems to be advantageous compared to other methods.

4.1.2. Pain ratings and descriptions

Numerical estimates of subjective pain are used in both experimental and clinical pain measurements. The most commonly used methods of subjective pain assessments include numerical and verbal category scales, and visual analogue or colour scales, in which subjective estimates may be assumed to reflect pain intensity (14,18,19,21,50,63,207–214). These rating scales may focus on different specific aspects of pain, and are often designed for a special test situation. Rating scales may also be used for subjective estimates of experimental pain (12,31, 196), (fig. 5).

The quality of subjective pain level estimation is limited by the skill of the observer and the type of experimental pain stimulation used (18). Sternbach (205) pointed out that subjective pain level estimations might be distorted by psychological and social factors (see also section 4.3.).

Registration of a series of subjective pain responses was suggested by Beecher (184), to be a valuable tool in pharmacological measurement. Stevens (215) explored the relationship between stimulation intensity and the subjective perception of different stimuli reported by the subject. He found that the subjective estimation of different sensory modalities obeyed a general "psychophysical power law" i.e., $\varphi = k \times \Phi^n$, where φ is the subjective intensity of sensation, Φ is the stimulus intensity, k is a constant, and ^{n} is the power exponent. Power exponents have been determined for many different sensory modalities. Physical stimuli as brightness of light, length of an object judged visually, and painful cold and heat have power exponents of approximately 1, indicating that a linear relation exists between stimulus intensity and subjective perception. In contrast, electrical skin stimulation has a power exponent of 3.5. A linear correlation between stimulation intensity and subjective classification of pain perceptions has also been established for argon laser induced heat pain (31).

4.2. Objective quantitation of pain perception

Objective methods for quantitation of experimental pain perception are based on the registration of (electro)physiological or behavioral patterns. We have previously employed recording of electrical brain responses after peripheral cutaneous argon laser stimulations (28–31,88,200). Recordings of this kind have also been performed during electrical stimulation (166,216,217), mechanical stimulation (218), CO_2 laser stimulation (192), and focused ultrasound stimulation (219). This issue is, however, beyond the scope of the present review.

4.3. Factors which may influence the validity of sensory and pain threshold determinations

Determination of sensory and pain threshold depends on the registration of a binary response (presence or absence of a specific sensational quality). Binary responses have been widely used in measurement of experimental pain. These methods are based on the volunteer's ability to report whether a subjective sensation is present or not. Often, a series of short stimuli of increasing or decreasing intensities is applied. Under normal experimental conditions, the subject's ability to communicate information of sensory or pain perception after stimulation is only impaired if the pain stimulus very strong and the perception is near the tolerance threshold. Chapman et al (23) reviewed this topic and showed that infrared heat pain stimulation in combination with threshold determinations could not demonstrate analgesic effects of clinically proven drugs quantitatively, and Beecher (220) also failed to find a relationship between the action of analgesic agents and the alteration of experimental pain threshold in humans.

Psychological factors may, under special conditions, modulate the pain perceived and the reliability of pain threshold has therefore been disputed. It was found to be susceptible to the influence of psychological manipulations by guided suggestions (experimenter bias) or autosuggestions (placebo response) or both (221). We have also investigated the effect of guided suggestions during hypnosis, and found that both hyperalgesia and analgesia could be induced in susceptible individuals (200). In such situations, objective quantifications, e.g. evoked brain potentials (28), may be used as an objective correlate to the subjective perception.

Different physiological and psychological factors which can affect the variability of pain threshold, e.g. cooperation and awareness of the volunteers, must also be taken into consideration. This means that the subjects must be trained in the observation and reporting of experimental pain. This is especially important for the quantification of nonphysiological pain sensations after electrical stimulation. A sinusoidal variation of the pain threshold with a cycle duration of approximately one month, – the circatrigintan rhythm, has previously been described (131,222). In fertile women this threshold varies according to the phase of the menstrual cycle (25 days), and in young men, the cycle time was determined to be 24 days. Older men and postmenopausal women had a cycle time of 30 – 32 days. These cyclic variations are very important factors, which should be taken into consideration when sequential determinations of pain thresholds during long-term comparative studies are performed. We have also found a variation in both thermal sensitivity and pain perception during the day, as part of an circadian rhythm. This has previously been described for pricking pain in men, but not in females (222,223). Part of the circadian variation can be inhibited by naloxone (224).

Both spatial and temporal summation in the central nervous system may influence sensory and pain determination (5,90,94,225). In order to avoid these phenomena, and also to avoid receptor sensitization due to release of inflammatory mediators, we decided to refrain from stimulating the same skin area more than once during a test session.

We found that a 3 mm stimulation area and a duration of 200 ms constitutes the best combination of stimulation parameters (I). This spot size provides a relatively large area for stimulation and it overlaps several receptor fields (7,71,91,226). This size of area does not require very high energy density for pain stimulation, whereas smaller areas might lead to skin burns due to generation of very high intracutaneous temperatures (27), (fig. 3a, 3b, and 4).

The effect of different stimulation times was also investigated. Ideally, the energy pulse should be kept extremely short, but this would require a very high energy density and a substantial risk of burning the skin surface during transmission of the stimulation energy to the deeper skin layers. In the present studies, the laser intensity was always kept below 3.2 W using the 3 mm laser spot size and 200 ms stimulation duration. Laser energy levels of 0.5 to 1.0 W and 1 to 1.5 W were usually sufficient to reach

the sensory and pain thresholds respectively, in normal, lightly pigmented skin on the C7 dermatome (dorsum of the hand). We also compared the argon laser (Cooper Medical, USA) and the CO_2 laser (Sharplan, Israel) as pain stimulators (I). The CO_2 laser often induced superficial skin burns at stimulation intensities just above the pain threshold. In contrast, no clinical or histological damage was observed after argon laser stimulation at energy levels which induced the same perception of pain.

4.4. Conclusions

The determination of heat induced sensory and pain thresholds by a series of short argon laser impulses of both increasing and decreasing intensity may be an accurate and reproducible method for the determination of the functional state of the peripheral part of the cutaneous sensory system. Standardized conditions should however be maintained for the central afferent and the perceptional part of the nociceptive system. Thresholds may be assumed to reflect the liminal perception for activity generated in low threshold thermal C-fibre innervated receptors (sensory threshold), high threshold Aδ-fibre mediated nociceptors (the first pricking pain) and high threshold C-fibre mediated nociceptors (the burning after-sensation). It may also be concluded that the argon laser method for pain stimulation may be superior to previously used focused bulb- and CO_2 laser based methods for pain stimulation.

CHAPTER 5

The optical properties of the skin – compensation for partial surface reflection of argon laser irradiation

Natural variations of skin colour and transparency due to melanin pigmentation, skin thickness and vascular status (227) were not routinely monitored in previous experimental pain stimulating systems. In the Hardy-Wolff-Goodell apparatus, a focused electric bulb provided radiant heat in a continuous, broadband spectrum ranging from the visible spectrum (400–700 nm) into the infrared. The peak wavelength of the emitted spectrum depends on the glowing filament temperature. To eliminate any wavelength-dependent tissue absorption, the stimulated skin surface was blackened to obtain a high absorption of the energy (fig. 6). Blackening of the skin eliminates the possibility to monitor vascular reactions during a stimulation session. Conversely, the CO_2 laser emits radiation in the far infrared region and since this region of the spectrum is strongly absorbed by water molecules no blackening is required to obtain a high absorption in the outermost few nanometres of the stratum corneum.

The argon laser emits visible radiation at two wavelengths, 488 nm (blue) and 515 nm (green), with a power distribution between these wavelengths of approximately 1:3 in the power range used. A shift towards a higher proportion of the green (515 nm) component occurs for increasing power, but the two wavelengths penetrate the upper skin layers with nearly the same attenuation (105).

The absorbed fraction of the pain-stimulating light energy which is absorbed by the skin can be determined accurately. Only an insignificant part of the absorbed energy penetrates the full skin thickness (105), and only a small fraction of the irradiation passes beyond the upper vascular plexus. Therefore, the absorbed fraction of an incident beam of radiant energy can be calculated from scanning reflectance spectrograms. The absorbed energy equals the incident energy minus the reflected fraction. Reflectance spectroscopy of the test areas is necessary in order to ensure that the optical conditions of the skin remain constant during and between different test sessions of pain stimulations (I,II). Argon laser stimuli at energy levels sufficient to elicit sensations of either warmth or pricking pain, do not induce receptor sensitization judged by threshold variation after repeated stimulations. It has been suggested that release or generation of sensitizing proinflammatory substances (228) might account for the immediate, sharp pin-prick pain after argon laser stimulations. However, the short time constants (229) involved in generation of action potentials after laser stimulation indicate that either the cutaneous nociceptors or the distal parts of afferent nociceptive Aδ-fibres are the primary target for the argon laser stimulation. This is in accordance with recordings of event-related brain potentials, which can be detected only 0.3 sec after the start of a 0.2 sec laser stimulation at the C7 dermatome (28,31). This short latency corresponds to the time required for transmission of the signal through peripheral and central nervous pathways. Also, we observed no alteration in sensory or pain threshold or latency after suppression of inflammatory mediators (230) by topical application of an non-steroidal antiinflammatory substance (indomethacin cream 5%, Amuno, MSD, FRG) (unpublished results).

5.1. The optical properties of human skin

The optical properties of the skin are very complex and are still not fully described. The skin is a highly heterogenous tissue and mathematical models such as Beer's law, which may be used to determine the attenuation of the laser energy level in a homogeneous tissue, cannot be applied directly. Due to this complexity, all models are based on a series of approximations, which may be valid only under certain conditions (111,112,231–235). A simple but still useful optical model is the multi-layer model. Here, the skin is conceived as a series of homogenous either absorbing, scattering, or reflecting layers (236,237). A beam of visible light aimed at the skin surface will sequentially penetrate an outer, strongly scattering stratum corneum, an absorbing layer comprising melanin in the epidermis and haemoglobins in the

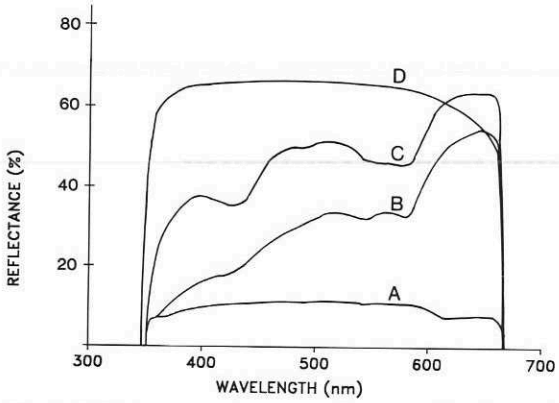

Fig. 6. Reflectance spectroscopy of human skin. Spectral range: 350 nm – 670 nm. A = skin blackened by colloidal carbon. B = moderately pigmented skin. C = fair caucasian skin. D = reflectance from collagen fibres in vitro.

upper dermis, and a collagenous reflecting layer in the deeper parts of dermis. Part of the light travelling through the skin layers passes the upper two layers twice. The stratum corneum, which provides diffuse forward scattering, contributes less than 8% to the total skin reflectance (105,238). The absorbance of epidermal melanin is strongly wavelength-dependent with high absorption in the ultraviolet part of the spectrum (105,239). The two types of haemoglobins, oxygenized and deoxygenized (reduced) haemoglobin have distinctly different absorption spectra, which can be detected separately by spectroscopy. The dermal collagen present in all layers of the dermis mostly reflect visible light in a wavelength-independent manner (fig. 6, curve D). Theoretical considerations of the interaction between argon laser irradiation and skin have been performed in order to improve the techniques for treatment of haemangiomas (231). Since the irradiation target for laser therapy, the superficial blood vessels, are located in close proximity to the target for experimental pain stimulations, the nervous plexus in the skin (240), these theories may also apply for argon laser induced sensory and pain stimulations.

5.2. Argon laser stimulation and skin optics

Two primary modes of energy transfer are involved in the argon laser stimulation of cutaneous nociceptors. The laser energy reaches the level of the nociceptors partly as radiation and partly by heat conduction. The wavelength dependent attenuation of radiation transmitted through the skin (105) causes most of the incident light to be transformed into heat along the pathway to the level of the most dense network of nociceptors around the vessels in the superficial dermis. However, a significant part of the incident irradiation in the blue and green (488 nm and 515 nm) spectrum is reflected from the surface of the skin. The magnitude of this fraction depends mainly on the amount of skin pigments (oxygenized and reduced haemoglobins and melanin) (fig. 6) (I,II,241). The fraction of the stimulating laser energy which is retained by the skin can then be calculated. This fraction constitutes the true pain stimulation energy, and therefore reflectance data should always be reported when absolute values of laser energies applied on the skin surface are reported. With a few exceptions, dosimetric data reported in the literature originate from readings of laser power meters, assuming a 100 % absorption in the skin. The same type of considerations apply for other types of optical stimulation of the skin e.g. low level laser treatment (242–244).

CHAPTER 6

Acute experimental non-immunological skin inflammation – neurogenic aspects

6.1. Aδ- and C-fibre primary afferents and the pathophysiology of acute cutaneous inflammation

Upon stimulation, peripheral nociceptors signal the occurrence of a peripheral noxious stimulus to the central nervous system and release potent inflammatory neuromediators into the local peripheral innervation field. Action potentials travel not only toward the spinal cord but also spread antidromically to nearby peripheral branches of the same nerve fibre, where impulses spread towards the peripheral endings of the nerve fibre branches. The neurogenic spread of inflammation can be encountered as secondary hyperalgesia surrounding a traumatized skin areas (185,186). Hyperalgesia is produced by the release of secondary inflammatory mediators which decrease the threshold for firing of the nociceptors and induce a state of ongoing activity. Sensitization of primary afferent nociceptors decreases the thresholds (36,161,174,245–247). The peripheral sensory neurons may thus have a dual (both afferent and efferent) function (76,248–250). Release of neurotransmitters occurs at both the central and peripheral nerve endings (251), but approximately 90% of the neurotransmitters produced in the nerve cell body are transported to the peripheral endings and released into the tissue upon stimulation (42). In animals, this neurogenic inflammation can be elicited by antidromical electrical stimulation of dorsal roots or peripheral sensory nerves (252). It leads to both vasodilation and plasma protein extravasation near the nerve fibre endings (4,36,253–258). In human skin, the local reaction after antidromic electrical stimulation is less pronounced as only the flare reaction can be elicited (75,259).

6.2. Sensitization of nociceptive receptors

Receptor sensitization has only been shown to occur in Aδ-fibre mediated nociceptors (160,260). In contrast, vasodilation (flare) is mediated in particular by nociceptive C-fibres (173,261) and only to a small degree by the Aδ-fibres (74,262). Sensitization can be induced either by neurogenic inflammation, e.g. antidromical electrical stimulation or local application of inflammatory chemicals (263,264) e.g. mustard oil (allyl-isothiocyanate), or by non-neurogenic inflammatory mediators as carrageenin, dextran or formalin (36). The effect of neurogenic influence on inflammation can be demonstrated by e.g. surgical sensory denervation, after neonatal systemic capsaicin treatment or after long term topical capsaicin treatment. Capsaicin is a selective neurotoxin which is well known as the pungent principle in hot peppers and chilies. The exact mode of action is not known. After local, peripheral application it induces release of neurotransmitters from both peripheral and central endings (36,265–270). Cutting of the nerve fibres and capsaicin application both abolish the neurogenic part of an inflammatory reaction (271).

6.3. The axon reflex flare

The concept of axon reflex flare and neurogenic inflammation has undergone a series of evolutionary processes since the beginning of this century (fig. 7). In 1927, Lewis (1) described the axon reflex flare as an erythema mediated through activity in a single neuron short arc axon reflex, in which both ortho- and antidromical propagation of the axon potential to other branches of the same nerve fibre occurred (36,272–274). Accordingly, the flare should be confined to the most distal arborizations of neurons projecting into the inflamed area, and the spread of flare should be inhibited by local analgesics. In a later model (275), the spread of flare is described as a cascade of local neuro-mediator release between neighbouring fibre endings arranged in parallel. Helme and McKernan (276) found that capsaicin-induced flare on the forehead in humans did not spread across the midline to the area innervated by the contralateral trigeminal nerve. This favours the concept of neurally mediated spread of inflamma-

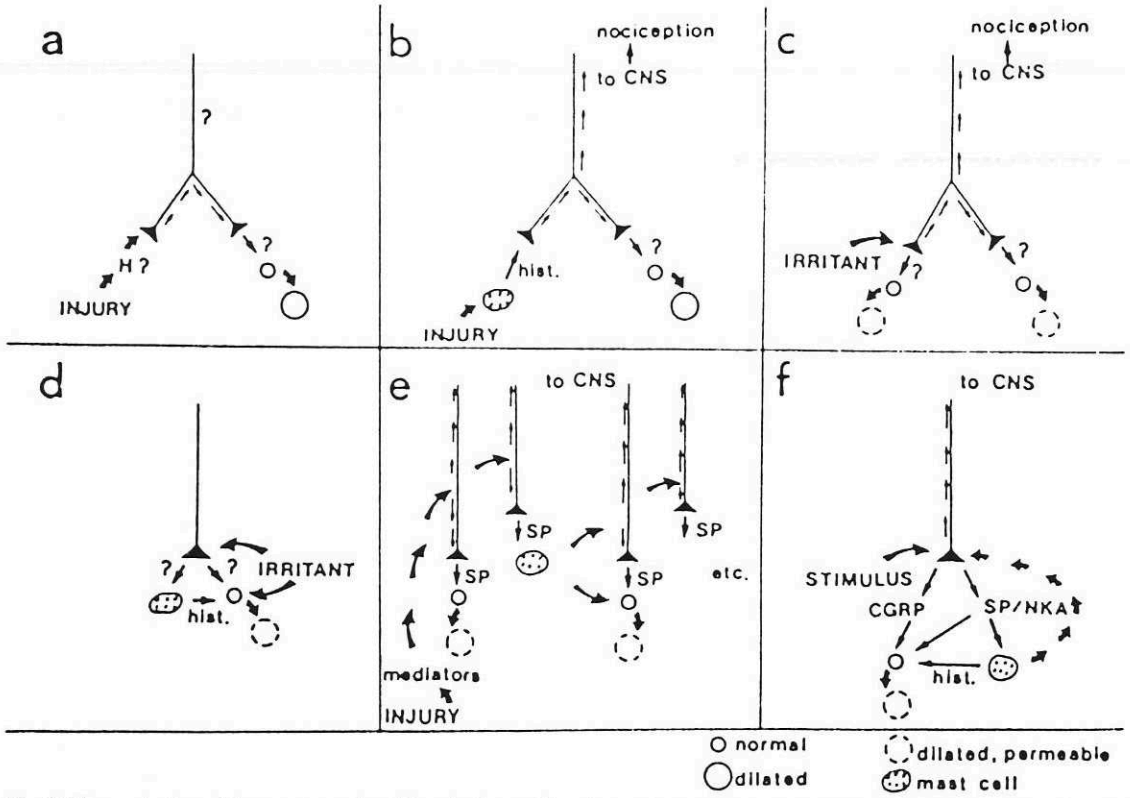

Fig. 7. The evolution of the concept of antidromical vasodilatation and neurogenic inflammation. The axon reflex flare (a) (1). The involvement of mast cells (b) (291). Neurogenic inflammation (c) (36,343). Neurogenic oedema (d) (478). The axon response – a cascade theory (e) (258,275). The response of the primary afferent terminal – a current concept (f). (After Chahl 1988 (256). (Reprinted with the permission of the author and the copyright holder, Pergamon Press, Oxford).

tion (277). According to the "cascade theory" the flare reaction will be limited to an area delineated by the spatial distribution of blood vessels, which are innervated by branches of the fibres terminating near the trauma or histamine skin prick. Our experiments with EMLA analgesia of the skin question this theory. A flare reaction generated by a histamine skin prick applied in the centre of a small area of EMLA treated skin (3 cm diameter) is inhibited in the EMLA treated area, but reappear outside the EMLA treated area (V). This finding is in accordance with the "neurally mediated spread" theory. The size of the skin area which is supplied by the collateral axon network of fibres terminating near the trauma or histamine skin prick will limit the area of neurally spreading of the flare reaction. C-fibre innervation fields have been determined by microneurography to be in the order of 1 cm^2 (64,92), which is much smaller than the flare responses normally encountered in humans. This is in favour of the "cascade theory". A plausible explanation of the different experimental findings is that both mechanisms may be operating simultaneously.

6.4. Mast cells and peripheral sensory nerves

The functional role of the sensory nervous system in acute inflammation has been addressed by several recent investigators. The suggestion has been put forward that cutaneous mast cells and the peripheral sensory nervous system may communicate. Mast cells are highly active in the initiation of both immediate hypersensitivity reactions, neurogenic inflammation (279), (fig. 7), and non-immunological inflammatory reactions (280–284). Mast cells undergo hyperplasia in chronic inflammatory conditions, which may also indicate a functional role in these situations (285,286).

Cutaneous mast cells, besides being located perivascularly, have been found in close proximity to peripheral nerve terminals (287–289), and may even be selectively innervated by afferent fibres. Celander & Folkow (290) suggested the existence of a

local neuro-effector unit involving mast cells and the peripheral sensory nervous system. The same sensory nerve fibre is assumed to deliver branches in upper layers of the skin, where noxious stimuli are likely to occur, and branches which terminate around blood vessels and mast cells. Short arc axon reflexes may mediate antidromic action potentials, generated in the superficial branches following a noxious stimulus, to the deep, perivascular terminals. Here, local release of neurotransmitters influence mast cell- and vessel- functions. This theory is supported by 1) studies of Kiernan (291), who suggested that cutaneous mast cells might even be selectively innervated, and 2) by light microscopic observations of direct contact between nerve fibres and mast cells (287). Also, ultrastructural studies of animal skin show that thin unmyelinated nerve fibres and mast cells may be invested by the same perineurial cell suggesting a functional unit (287,292).

6.5. Histamine and neurogenic inflammation

Histamine is an algesic agent and is assumed to be a major mediator of cutaneous pain (171,293). Histamine stimulation of peripheral nerves generates action potentials in thin myelinated Aδ-fibres and unmyelinated C-fibres and releases neurotransmitters locally (36,294,295). The neurotransmitters contribute to the formation of wheal and flare either by secondary release of endogenous histamine (272), or directly by a histamine-like effect on endothelial cells and vessel wall smooth muscles (296–299). Substance P (SP)(300,301) has been shown to stimulate the synthesis and release of mediators e.g. histamine (302) prostaglandins (303,304) and leukotrienes (305–308). Injection of SP into the skin produces physiological changes similar to the initial events of acute inflammation: vasodilation and increased vascular permeability (36,253,309,310), mast cell degranulation (295), accumulation and adhesion of leukocytes at venule walls, and stimulation of phagocytosis by polymorphonuclear leukocytes (311). The increased vascular permeability may also lead to algesic kinin formation in the skin (312).

6.6. Elicitation of acute non-immunological inflammation by histamine skin pricks

Histamine is the classical mediator of inflammation and is generally used to elicit acute experimental non-immunological inflammation of the skin (313). It is used clinically as positive control test for immediate type reaction tests, either by intradermal injections, superficial scarification through a drop of histamine solution or superficial pricking of the skin with a lancet covered by lyophilized histamine (Phazet, Pharmacia, Sweden) (314). However, circadian variations of the reaction size occur. These should be recognized and corrections should be made if investigations are carried out at different times of the day (315). Histamine increases the micro-vascular permeability as described in detail by Lewis (1). Classical studies by Majno et al (316), and by Hultström & Svensjö (317) show that following exposure to histamine, vessel leakage occurs from post-capillary venules. In low concentrations, histamine sensitizes nearby nociceptors, and in higher concentrations nociceptive and itch mediating receptors are activated sufficiently to generate a sensation of pain and itch (318). Histamine may initiate cutaneous inflammatory reactions, but it cannot sustain inflammation (319). Consequently, histamine induced acute inflammatory reactions will subside in minutes, if secondary mediators are not generated or released in sufficient amounts to perpetuate the reaction. In the skin, endogenous histamine is mainly confined to the mast cells (320–322), where it is synthesized and stored, bound to proteoglycans in secretory granules (323) and released during mast cell degranulation or degeneration (324).

The histamine effects are mediated through characteristic receptors. In the skin, plasma extravasation and itch is mediated by the H_1 receptor, whereas flare, and probably also itch, is mediated through the H_2 receptor. Recently, an inhibitory action of histamine on lymphocyte-mediated inflammatory reactions has been attributed to the H_2 receptor on leukocytes (325–327). This receptor may also mediate inhibition of mast cells, basophils and polymorphonuclear leukocytes (328,329). Finally, an H_3 receptor, previously described in central pain regulation systems, may be involved in neurogenic vasoreactions (330–334).

6.7. Peripheral mediators of acute non-immunological inflammation

The axon reflex flare involves release of both preformed pro-inflammatory mediators and/or local production of various mediators of inflammation, including kinins, prostaglandins, leukotrienes and interleukins (310,334–336). These may be synthesized from plasma precursors or released from inflammatory reactive cells. The physiological reaction is clinically recognized by the cardinal components

of inflammation: rubor (redness), calor (heat), tumour (swelling), dolour (pain) and *functio laesae* (diminished or loss of function).

6.8. The relationship between the peripheral autonomic nervous system and experimentally induced pain and inflammation

In addition to thin myelinated and unmyelinated sensory afferent nerve fibres, the peripheral limb of the sympathetic nervous system may contribute to the neurogenic component of inflammation (337). Linde et al (338) demonstrated increased vascular permeability following sympathetic nerve stimulation and Engel (339,340), showed that lumbosacral sympathectomy substantially reduced baseline plasma extravasation. Chemical sympathectomy inhibits plasma extravasation evoked by intradermal injection of compound 48/80, a potent histamine depleting agent (341). Inflammation induced by intradermal injection of non-neurogenic inflammatory mediators, e.g. carrageenin, dextran or formalin has also been shown to be significantly reduced in sympathectomized rats (342–343).

Neurogenic inflammation is not dependent on the sympathetic or parasympathetic nervous systems (4,75,291). However, activation of nociceptive afferents may increase the activity of postganglionic sympathetic efferents as well as sympathetic efferent activity and may induce antidromic activity of the sensory afferents with subsequent local release of neurotransmitters. Capsaicin treatment, which does not affect autonomic fibres (344), may modulate the initial local inflammatory events in localized skin trauma elicited by firm stroking on the skin surface with a blunt object. This procedure will initiate a sequence of events termed "the triple response" by Lewis (1) which consists of a primary local erythema due to dilatation of superficial vessels of the skin, a peripheral flare elicited by a local axon reflex, and a late wheal reaction, indicating increased permeability of local vessels (256).

6.9. Inhibition of nociception and inflammation by local analgesics

The effect of different application times on the action of the local analgesics lignocaine and prilocaine (EMLA cream) has been thoroughly investigated using many different pain stimulating procedures (for a review, see 139). In studies employing pinpricks (139), argon laser stimuli (III), or deep needle insertions (IV) as pain stimulators, it was found that the pain inhibitory effect of EMLA cream increased with application time. The analgesic effect reached a maximum approx. 2 h after application, and a linear relationship between duration of application and analgesic effect could be demonstrated (III). These results indicate, that both superficial pain induced by pin-pricks, the intracutaneous pain threshold quantified by the argon laser stimulations, and the deep cutaneous and subcutaneous pain elicited by introduction of a thick intravenous cannula into the skin, can be modulated quantitatively by EMLA. The analgesic effect correlated to the inhibition of flare area after histamine skin pricks (V). The wheal reaction was not affected by local analgesia. This is in accordance with findings of Pipkorn and Andersson (345) who tested the acute immunological inflammatory reactions in EMLA treated areas. The present findings suggest that the excitability of cutaneous nociceptors, evaluated by argon laser stimulated thresholds determinations, may be a sensitive indicator of the cutaneous vascular reactivity to acute inflammatory reactions induced by histamine skin pricks. However, the inverse relationship may not always be found, i.e. the magnitude of the vascular reaction after a histamine skin prick does not necessarily reflect the excitability of the peripheral sensory nervous system. This is supported by the investigations of systemically administered antihistamines (XI,346). All antihistamines inhibit the inflammatory response after histamine skin pricks, but some do not exert any direct inhibition of nociception. The cutaneous vascular reactions induced by EMLA cream were investigated by Juhlin & Rollman (347), Bjerring et al (VI) and Juhlin & Evers (139). Using different application times, these groups found a biphasic, vascular effect of EMLA cream. After longer application times, an initial pallor was followed by increasing erythema, indicating a sequence of different events during analgesia (348). This biphasic reaction was accelerated in patients with atopic dermatitis (139,349,350) presumably due to enhanced skin penetration of EMLA in these patients. However, the exact mode of action of local analgesics on the cutaneous vascular system still remains to be elucidated.

6.10. Effect of topical application of capsaicin

Local treatment of the skin with a solution of capsaicin (trans-8-methyl- N-vanillyl-6-noneamide) or resiniferatoxin inhibits sensory neuronal excitation. Both substances are neurotoxins with selective ef-

fects on neuropeptide-containing small-diameter sensory afferent fibres (352–363). The neurogen mediated part of an acute inflammation can be inhibited by these substances. The antiinflammatory effects are dependent on an intact peripheral sensory nervous system (36). Capsaicin depletes SP (364) and somatostatin (365) and presumably other neurotransmitters (366,367) from sensory afferents. During the initial depletion or release of neurotransmitters, action potentials are generated. If the depletion occurs rapidly, the process will be perceived as an intense, stinging and burning pain (368–372). After the initial phase, a state of desensitization leading to total analgesia is produced (XII,369). The neurogen-dependent part of local inflammation induced by histamine skin pricks (X) and by antidromic electrical stimulation (248,373) may also be inhibited by capsaicin treatment. In rats, both systemic and topical capsaicin treatment can cause widespread degeneration of nerve fibres but in humans the sensory nerve fibres are normally only temporarily inhibited after local capsaicin treatment (364). This has been inferred from estimates of the number of opiate receptors which was found to be unaffected by capsaicin treatment (374). Studies performed in rats have indicated that capsaicin treatment affects chemonociceptive C-fibres selectively, with little or no loss of function of the Aδ- and Aβ-fibre mediated sensations (36,375,376). In humans, we found a strong inhibition also of Aδ-fibre mediated pricking pain after repeated capsaicin applications (IX). The neurotoxic effects of capsaicin seem to be associated to its pungent properties, and non-burning congeners fail to induce inflammation after topical application (377). Recently, ruthenium red was described as a specific inhibitor of capsaicin (378). This compound may be of interest in future studies of the functions of capsaicin.

In humans, repeated applications of capsaicin on the skin does not produce any significant inhibition of that part of the inflammatory reaction which is not influenced by the sensory nervous system. Plasma extravasation, monitored for example by the wheal formation after histamine skin pricks, could not be modified quantitatively by capsaicin (X). The observed inhibitions of erythema (flare) and nociception have different kinetics. Data from two experiments (IX,X) show that the flare is inhibited at a much faster rate than argon laser induced pin-prick pain (fig. 8). This may be due to different effect on the fibre populations involved in the generation of

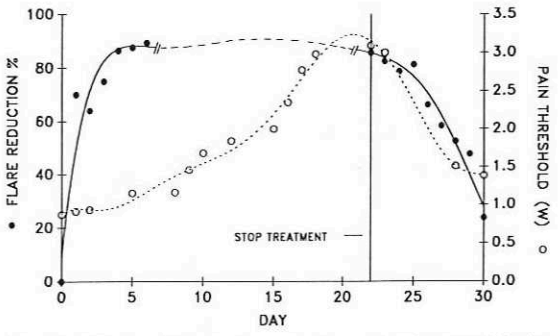

Fig. 8. Effects of topical application of 1% capsaicin in ethanol on inhibition of flare reactions (●) and on argon laser induced pain threshold (○). The inhibition on the flare reaction develops faster than inhibition of the pricking pain sensation.

his tamine induced flare and in pain perception after short argon laser pulses.

The selective action of capsaicin on Aδ- and C-fibre mediated sensory functions, renders capsaicin a valuable tool for investigation of the sensory nervous system. Total anaesthesia cannot be obtained, however, as fibres mediating e.g. pressure and vibration are not affected by capsaicin. Interestingly, LaMotte et al (62) have reported an effect of capsaicin on the itch mediating subpopulation of cutaneous C-fibres, which suggests a possible role of capsaicin in the symptomatic treatment of itching conditions.

6.11. Analgesic effect of antihistamines

H_1 antihistamines were previously used for local analgesia in dentistry (379–381), and in various animal studies, a series of antihistamines of both the H_1 and H_2 class were shown to possess analgesic effects (382). However, also conflicting reports of the analgesic effect of antihistamines have been published (383–385). These discrepancies may presumably be due to different test systems, dosage schedules, or routes of administration. Histamine H_1 receptors have been identified on primary afferent nerves (295), and inhibition of histamine binding to nerve terminals may reduce the excitability of pain mediating nerve fibres to histamine. This effect has been observed for the itch sensation (386). Part of the antiinflammatory effects of those antihistamines which also possess analgesic properties, may be attributed to a direct inhibition of cutaneous nociceptors with consequent inhibition of the neurogenic component of inflammation. As yet no clinical studies have focused on this effect of antihistamines.

Argon laser-stimulated sensory and pain thresh-

olds were used for determination of the antinociceptive effects of antihistamines in humans and these were correlated to their antihistaminic effect on standardized histamine skin pricks (XI). However, a correlation between antihistaminic efficacy and analgesic effect could not be established. Only diphenhydramine, an H_1 antihistamine with both peripheral and CNS effects, induced moderate analgesia, as measured by an increase in the argon laser-stimulated pain threshold. The effect was not assumed to be due to a central sedation, as sedatives e.g. diazepam, meprobamate, and barbiturates do not elevate the pain threshold (383). Still, the mechanisms involved in analgesia produced by systemic administration of antihistamines are unknown.

6.12. The neurogenic influence on inflammation and the immune system

Inflammation-induced activation of sensory Aδ- and C- fibres releases SP and other neurotransmitters either directly or via histamine release from mast cells (272,295,387,388). To date, three SP receptors have been identified; neurokinin (NK)-1 (SP preferring), NK-2 (NK-A preferring) and NK-3 (NK-B preferring) (389–392). Due to the multiple actions of SP, it has not been clarified as yet which receptors reported are involved in neurogenic inflammation. However, Piotrowski et al (393) showed that the SP receptor on mast cells differs from that involved in the production of a wheal response in human skin. SP receptors have been demonstrated on mast cells, polymorphonuclear leukocytes and macrophages (391–395). The physiological relevance of SP is indicated by the fact that *in vitro* stimulation with SP in physiological concentrations (30 ng/ml), may induce interleukin-1 secretion from the macrophage cell line P388D1 (396). This effect can be inhibited by NK-1 receptor antagonists. Proliferation of human T lymphocytes may be stimulated *in vitro* by SP at 10^{-10} M (397), and phagocytosis may be enhanced by SP (398). Capsaicin, which induces release of SP from nerve endings, inhibits the rate of immunoglobulin secretion in the rat (399), and it also inhibits the stimulation of human B-lymphocyte differentiation by SP and NK-A (395,400).

Thus, evidence is accumulating that the peripheral sensory nervous system may be a potential initiator and modulator of skin inflammation by peripheral secretion of neuropeptides (401,402).

6.13. Vascular effects of intradermal injections of C5a and leukotriene B4

The inflammatory effect of the 74 amino acid complement fragment C5a is independent of local mast cell degranulation. This type of inflammatory mediator is not significantly inhibited by antihistamines or by inhibitors of kinin formation (403,404). In the skin both the C5a inflammatory response and that of the arachidonic acid-derived leukotriene B4 (LTB4) have been shown to be delayed in onset, presumably because LTB4 and C5a trigger reactions between polymorphonuclear leukocytes and vascular endothelial cells resulting in increased vascular permeability (405–407). We investigated, if also these types of inflammation could be monitored objectively by reflectance spectroscopy. Yancey et al (408) found, that C5a produced maximal wheal reactions 20 min after administration and maximal erythema (flare) after 10 min. This time for the flare maximum was in accordance with our findings of maximal erythema index recorded by reflectance spectroscopy (VII,VIII). The actual mechanisms behind the C5a-induced neurogenic part of acute inflammatory reactions (flare) and the differences in kinetics of the flare -area and -intensity are not known (see chapter 6.3). The time course of the development of a flare reaction after C5a injection is comparable to that induced by histamine. This suggests either direct effects on the nerve terminals or involvement of mast cell products. The latter suggestion is supported by observations of mast cell degranulation in C5a test sites by both light- and electron microscopy of biopsies (411).

CHAPTER 7

Methods for static and dynamic measurements of the cutaneous vascular responses during acute experimental non-immunological inflammation

The physiological reactions of wheal (plasma extravasation) and flare (erythema) can be quantified according to various principles. In most investigations, only the wheal is monitored. The wheal can easily be measured having relatively well defined borders, and it is relatively insensitive towards manipulation and touching of the test area.

The flare reaction may, in contrast, be difficult to quantify. It is often only faintly discernible from normal skin and the size reaches a maximum after a few minutes and then decreases. The flare area is sensitive to light mechanical traumas to the skin, and may easily be increased by gentle handling of the skin surface by callipers, rulers, or pens, used, for example, for measurement of diameters or for tracing the border on a transparent plastic film for planimetry. In order to eliminate these methodological problems, we have developed a method for non-invasive, non-tactile quantitation of skin flare and for the monitoring of the time intensity relationship during the early phases of experimental acute skin inflammation (II, VI, VII, VIII). Various other methods are currently used for this purpose, and in the following sections, a survey of some non-invasive techniques for measuring the wheal and flare reactions is provided.

7.1. Plasma extravasation (wheal)

The central wheal reaction induced by a histamine skin prick is assumed to be a direct histamine effect on the endothelial cells of the vessel wall, and cannot be reduced by infiltration of local analgesics, topical application of EMLA cream or topical application of capsaicin (V,X). Similar results were reported by Jancsó et al (343) and by Pipkorn & Andersson (345).

Plasma extravasation to the intercellular space produces oedema (413) accompanied by a more or less pronounced migration of cells. It is generally agreed that the wheal can be quantified non-invasively by measurement of the diameter, or preferably, by measurement of two orthogonal diameters. Planimetry of wheal areas can be performed, but due to the often small areas involved, these measurements require a high degree of technical skills to be accurate. For special purposes, determination of oedema volume may supply further information.

7.1.1. Quantification of wheal by skin thickness

Increased skin thickness, induced by inflammatory oedema, has been quantified by soft tissue X-rays (414), skin fold measurements (415) and by measurements on histological specimens (416). The radiographic method is hampered by the requirement for very expensive equipment and is best suited to measurements on large skin surfaces. The skin fold method includes at least some subcutaneous tissue in the skin thickness determination, and is not suited for tightly bound skin. Skin thickness determinations on histological sections of the skin are often inaccurate due to artifacts produced during dehydration, cutting and fixation of the tissues. Repeated measurements on the same area are not possible (417). Recently, high frequency ultrasonographic a-scans have been applied to the measurement of skin thickness (418). This technique was later improved and is now very accurate (419). Ultrasound measurements have been used for quantification of histamine wheal thickness (420) and for quantification of skin thickness increments in patch testing (421). The ultrasound b-scan method has also been employed in measurements of skin thickness (422,423).

7.1.2. Quantification of wheal reactions by skin elasticity

Several methods for the determination of skin elasticity *in vivo* have been introduced (424–426). Alterations in the mechanical properties of the skin have been shown to be a significant finding in inflammation. The oedema induces a relative increase in the non-elastic mass of the skin, and the tensile distensibility and hysteresis is increased in the wheal

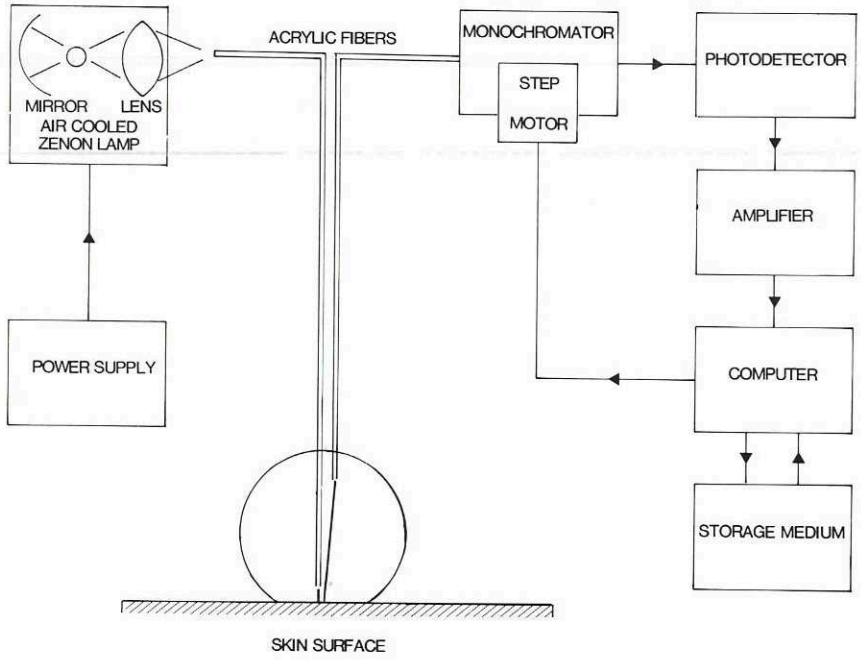

Fig. 9. Diagram of the computer controlled reflectance spectrophotometer. (Reprinted with the permission of Munksgaard International Publishers Ltd.).

area (420). In humans, the wheal reaction is only slightly affected by sensory nervous activity, which indicates that skin elasticity measurements may not be of particular value for quantification of the neurogenic part of inflammation in humans.

7.2. Monitoring of the histamine induced flare reaction

The physiological flare reaction may be monitored non-invasively by monitoring the vasodilatation which produces both increased blood content in superficial cutaneous vessels, clinically encountered as an erythema (427), and increased blood flow in the superficial vascular plexus resulting in increased skin temperature. Rating of erythemas for the quantification of inflammatory vascular reactions was used in early studies by Lewis (428) and Winder et al (429), who used coloured tiles or cards for visual comparison of skin colours. More modern techniques for the monitoring of flare reactions may principally be divided into 1) tracing of the flare circumference for planimetric area calculations, 2) determination of the relative increase of blood content in the superficial vessels by reflectance spectroscopy, and 3) monitoring the increase in skin perfusion, either directly by blood flowmetry, or indirectly by the elevation of skin temperature. We developed a method for dynamic reflectance spectroscopical monitoring of vascular reactions in the skin (VI). We have also used reflectance spectroscopic monitoring combined with flare area determinations (VII,VIII) and finally, flare area determinations were used alone (V,X,XI,430). In principle, reflectance spectroscopy should be used when repeated, time-course measurement are essential and flare area determinations when single measurements would suffice. We found a good correlation between flare area and erythema index kinetics. (VIII)

7.2.1. Subjective visual quantification of flare area by planimetry

Flare area has traditionally been quantified by tracing the circumference with a pen on a transparent plastic film for subsequent manual or computerized planimetry (V,XI,X,XI,430). In studies which require less accuracy, calculations based on the mean of two orthogonal diameters can be used as a measure of flare area, but due to the variability in shape of the reactions, the method cannot be recommended for accurate determinations. The planimetry method is, however, not suited for time-course measurements, as even light manipulations of the sensitized skin by the tracing pen may trigger further increase of the flare area.

7.2.2. Objective quantification of flare by reflectance spectroscopy

Visual readings of the flare reactions may be sufficient in clinical research, but for quantitative time-course investigations the more accurate, but also more tedious reflectance spectroscopic method should be used (II,431–433). The spectral reflectance of the skin may conveniently be expressed as computer calculated pigment indices, suitable for statistical analysis (236,238). The scanning reflectance spectrophotometer may, however, also be used for accurate determinations of concentrations of the main chromophores (oxidized haemoglobin, reduced haemoglobin and melanin) in the skin (239,434).

The principle of scanning reflectance spectroscopy is to irradiate the skin surface with a spectrally well-defined quasi-continuous light source (320 nm to 1200 nm). The light which is reflected back from the skin surface is collected by an integrating sphere and guided to a monochromator for quantification (fig. 9).

A major advantage of the reflectance spectroscopic method is that repeated measurements do not interfere with the natural course of the flare reaction, and repeated measurements can be performed at intervals of less than 30 sec in order to monitor the dynamics of flare reactions. Finally, computer-controlled scanning reflectance spectrophotometers are highly accurate, but the price of the instruments restricts their use to a few experimental set-ups (II,VII,114,236,435).

7.2.3. Accuracy of reflectance spectroscopy for skin measurements

We have tested the accuracy of reflectance spectroscopic measurements on human skin in vivo by the variance of repeated measurements on the same skin surface (3.1%, n=10), and by comparison of measurements on UV-induced erythemas using reflectance spectroscopy and laser Doppler flowmetry (II,436,437). We found a high degree of correlation (r=0.96) between the reflectance spectroscopically determined EI and the laser Doppler blood flow measurements (114).

7.3. Objective quantification of flare reactions by cutaneous blood flow

The erythema associated with acute inflammatory reactions in the skin may also be monitored by measurement of the concomitant increase in cutaneous blood flow. Photoelectric pulse plethysmographic measurements (438,439) and radioactive ^{99}mTC-pertechnetate- or ^{133}Xenon wash-out methods have been extensively used (440–442). Photopletysmographic methods seem to be too insensitive for detection of the minute increases in superficial skin blood flow in a flare reaction and the isotope wash-out techniques are time consuming. Non-invasive laser Doppler blood flowmetry was introduced in 1977 (443), and was later improved by Tenland (436). Although objective quantifications of blood flow may be performed continuously only relative variations of blood flow are measured. Good correlation has been shown with direct capillary microscopy (444) and with the ^{133}Xenon wash-out techniques (445), and this method is now widely used in cutaneous vascular research (74,104,262,446,447). We found, however, that skin reflectance spectroscopy was a more sensitive technique for detecting minimal erythemas than laser Doppler blood flowmetry (114).

7.4. Objective, indirect quantification of flare reactions by measurement of skin temperature

Increased skin temperature following increased skin blood flow in the flare area of an inflammatory reaction has been detected by thermometric measurements, either by surface contact probes or by thermography (110,124,126,441,448–453), or by intracutaneous implantations of thermocouples (126,234). Increased skin temperature may be used as an indirect measure of increased blood flow during inflammation, and it has been assumed to reflect the degree of vasodilation in inflammation. Increase in skin surface temperature does not necessarily indicate an increase in blood flow, but can be influenced by many different factors, e.g. the stratum corneum water content and evaporation, heat loss by radiation, the ambient temperature, and air -flow and -humidity. However, since the instrument is inexpensive, it is still widely used.

CHAPTER 9

Summary in Danish

Emnet for oversigten og de 12 originalarbejder er kvantitativ monitorering af den perifere del af hudens nociceptive nervesystem. På baggrund af eksperimentel hæmning af hudens nociceptorer er der foretaget undersøgelser af nociceptorernes funktion sat i relation til hæmning af det akutte inflammatoriske respons efter histaminpriktest i huden. Den inflammatoriske reaktion er kvantiteret ved hjælp af et computerstyret reflektansspektroskop, som med stor nøjagtighed bestemmer hudens erythem som udtryk for graden af inflammatorisk vasodilatation. Mange metoder til kvantitering af smertehæmning i huden har gennem tiden været afprøvet, men manglen på en entydig smertestimulations-metode, der udelukkende stimulerer smerte og ikke samtidigt andre sansekvaliteter, har begrænset værdien af disse. Med udviklingen af en metode til kortvarig varme- og smertestimulation af huden med en argon laser er der åbnet mulighed for at stimulere varme- og smertefølsomme sansenerver uden samtidig at beskadige det stimulerede væv. Teknikken er anvendt dels til basale undersøgelser af perifere aspekter af hudens sansenervesystem og dels til undersøgelse af effekten af forskellige metoder til at hæmme smerteopfattelse fra huden. Metoden er baseret på stimulation af huden med kortvarige argonlaser impulser af stigende intensitet. Det registreres, hvilken laserenergi som netop svarer til mindste sanseopfattelse (sensorisk tærskel), og hvilken der netop svarer til den mindste skarpe nålestikssmerte (smertetærsklen). Ved lasereffekter over smertetærsklen kan den subjektive smerteopfattelse udtrykkes via en deskriptiv skala for smerteintensitet.

Ud over at informere centralnervesystemet om potentielle vævsskadelige påvirkninger spiller det sensoriske (nociceptive) nervesystem en rolle i modulation af inflammatoriske reaktioner. Elektrisk, mekanisk, termisk eller kemisk stimulation af perifere sansenerver udløser inflammatoriske reaktioner i nervernes innervationsområder via frigørelse af proinflammatoriske neuropeptider fra nerveender. Denne proces kaldes neurogen inflammation. Ved andre non-neurogene former for inflammation påvirkes de sensoriske nerver af lokale inflammatoriske mediatorer, og bidrager til inflammationen ved lokal sekretion af neurotransmittere. I originalarbejderne er effekten af forskellige typer af smertehæmmende stoffers virkning undersøgt dynamisk med hensyn til den tidsmæssige relation mellem applikation og smertehæmmende virkning i huden og med hensyn til deres effekt på eksperimentel, akut inflammation. Der er foretaget undersøgelser af gradueret analgesi, induceret enten ved applikation af lokalanalgetika, capsaicin (chilipeber ekstrakt) samt peroralt indgivne antihistaminer. Capsaicin, et selektivt neurotoxin der udtømmer sansenervernes indhold af neuropeptider, er fundet effektivt til hæmning af postherpetiske neuralgismerter. Den hæmmende effekt af analgetika og capsaicin på inflammation findes relateret til graden af analgesi af huden. Der fandtes derimod ikke relation mellem antihistamineffekt og analgetisk effekt efter systemisk administration af antihistaminer.

De udviklede metoder synes at kunne finde anvendelse dels i den basale humane neurofysiologi og dels ved undersøgelser af dynamiske effektforløb af analgetika og antiinflammatoriske farmaka.

References

1. Lewis T. The blood vessels of the human skin and their responses. London: Shaw and Sons, 1927: 46–66.
2. Stricker S. Untersuchung über Gefasserwurzeln des Ischiadicus. Sitz Kaiserl Acad Wiss (Wien) 1876.
3. Frey von M. Beiträge zur Physiologie des Schmerzsinnes. Ber ü d Verhandlungen d k Sachs Ges d Wiss z Leipzig 1894; 46: 185–196, 288–296.
4. Bayliss WM. On the origin from the spinal cord of the vasodilator fibres of the hind limb, and on the nature of these fibres. J Physiol 1901; 26: 173–209.
5. Hardy JD, Wolff HG, Goodell H. Studies on pain. A new method for measuring pain threshold observations on the spatial summation of pain. J Clin Invest 1940; 19: 649–657.
6. Notermans SLH. Measurement of the pain threshold determined by electrical stimulation and its clinical application. Part I. Method and factors possibly influencing the pain threshold. Neurology 1966; 16: 1071–1086.
7. Torebjörk HE, Hallin RG. Activity in C fibres correlated to perception in man. In Hirsch C, Zotterman Y, eds.: Cervical pain. Oxford: Pergamon Press, 1973: 171–177.
8. Chapman CR. Measurement of pain: Problems and issues. In JJ Bonica, Albe-Fessard D, eds.: Advances in pain research and therapy, New York: Raven Press, 1975; 1: 345–353.
9. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1975; 1: 277–299.
10. Dubuisson D, Melzack R. Classifications of clinical pain descriptions by multiple group discriminant analysis. Exp Neurol 1976; 51: 480–487.
11. Stowell A. Cerebral slow waves related to the perception of pain in man. Brain Res Bull 1977; 2: 23–30.
12. Carmon A, Mor J, Goldberg J. Application of laser to psychophysiological study of pain in man. In Bonica JJ, Albe-Fessard D, eds.: Advances in pain research and therapy. New York: Raven Press 1976; 1: 375–380.
13. Clark WC. Measurement of pain in humans: Signal detection theory and pain. Neurosci Res Progr Bull 1978; 16: 14–27.
14. Wolff BB. Behavioral measurement of human pain. In Sternbach RA, ed.: The psychology of pain, New York: Raven Press, 1978: 129–168.
15. Procacci P. Methods for the study of pain threshold in man. In Bonica JJ et al., eds.: Advances in pain research and therapy. New York: Raven Press, 1979; 3: 781–790.
16. Rohdewald P, Derendorf H, Elger CE, Knoll O. Quantifizierung der dampfung in der schmerzempfindung mit hilfe evozierter potentiale nach gabe schwacher analgetika. Z EEG-EMG 1980; 11: 199–204.
17. Campbell JA, Lahuerta J. Physical methods used in pain measurements: a review. J Royal Soc Med 1983; 76: 409–506.
18. Carlsson AM. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. Pain 1983; 16: 87–101.
19. Grossi E, Borghi C, Cerchiari EL, Della Puppa T, Francucci B. Analogue chromatic continuous scale (ACCS): a new method for pain assessment. Clin Exp Rheumatol 1983; 1: 337–340.
20. Wolff BB. Laboratory methods of pain measurement. In Melzack R, ed.: Pain measurement and assessment. New York: Raven Press, 1983: 7–13.
21. Gaston-Johansson F. Pain assessment: differences in quality and intensity of the words pain, ache and hurt. Pain 1984; 20: 69–76.
22. Biehl R, Treede R-D, Bromm B. Pain ratings of short radiant heat pulses. In Bromm B, ed.: Pain measurement in man. Neurophysiological correlates of pain. Amsterdam: Elsevier Science Publishers BV, 1984: 397–408.
23. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: An overview. Pain 1985; 22: 1–31.
24. Fishman B, Pasternak S, Wallenstein SL, Houde RW, Holland JC, Foley KM. The Memorial Pain Assessment Card. A valid instrument for the evaluation of cancer pain. Cancer 1987; 60: 1151–1158.
25. Gracely RH, Lota L, Walter DJ, Dubner R. A multiple random staircase method of psychophysical pain assessment. Pain 1988; 32: 55–63.
26. Arendt-Nielsen L, Bjerring P. Laser induced pain for evaluation of local analgesia: A comparison of topical application (EMLA) and local injection (Lidocaine). Anesth Analg 1988; 67: 115–123. (III).
27. Arendt-Nielsen L, Bjerring P. Sensory and pain threshold characteristics to laser stimuli. J Neurol Neurosurg Psych 1988; 51: 35–42. (I).
28. Arendt-Nielsen L, Bjerring P. Cortical response characteristics to noxious argon laser stimulation. Clin Evoked Pot 1988; 5: 11–19.
29. Arendt-Nielsen L, Bjerring P. Reaction times to painless and painful CO2 and argon laser stimulation. Eur J Appl Physiol 1988; 58: 266–273.
30. Arendt-Nielsen L, Bjerring P. Evaluation of acetaminophen (Setamol) analgesia by argon laser induced pain related cortical responses. In Wallinga W, Boom HKB, deVries J, eds.: Electrophysical kinesiology, Proceedings ISEK. Amsterdam: Elsevier, 1988: 195–198.
31. Bjerring P, Arendt-Nielsen L. Argon laser induced single cortical responses: a new method to quantify pre-pain and pain perceptions. J Neurol Neurosurg Psych 1988; 51: 43–49.
32. Bjerring P. Effect of antihistamines on argon laser induced cutaneous sensory and pain threshold and on histamine induced wheal and flare. Skin Pharmacol 1989; 2: 210–216. (XI).

33. Arendt-Nielsen. Quantification of laser induced pain perception. Ph.D. Thesis, University of Aalborg, 1987.
34. Saito K, Markowitz S, Moskowitz MA. Ergot alkaloids block neurogenic extravasation in dura mater: Proposed action in vascular headaches. Ann Neurol 1988; 24:732–737.
35. Black S, Humphrey JH, Niven JS. Inhibition of mantoux reaction by direct suggestion under hypnosis. Br Med J 1963; 6: 1649–1652.
36. Jancsó N, Jancsó-Gábor A, Szolcsányi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol Chemother 1967; 31: 138–151.
37. Henry JL, Couture R, Cuello AC, Pelletier G, Quirion R, Regoli D.(Eds). Substance P and neurokinins. Proceedings of "Substance P and neurokinins-Montreal '86" A Satellite Symposium of the XXX International Congress of The International Union of Physiological Sciences. New York: Springer Verlag 1987.
38. Chapman LF, Goodell H. The participation of the nervous system in the inflammatory reaction. Ann NY Acad Sci 1964; 116: 990–1017.
39. Helme RD, Littlejohn GO, Weinstein C. Neurogenic flare responses in chronic rheumatic pain syndromes. Clin Exp Neurol 1987; 23: 91–94.
40. Levine JD, Moskowitz MA, Basbaum AI. The contribution of neurogenic inflammation in experimental arthritis. Immunol 1985; 135: 843–847.
41. Littlejohn GO, Weinstein C, Helme RD. Increased neurogenic inflammation in fibrositis syndrome. J Rheumatol 1987; 14: 1022–1025.
42. Levine JD, Terrence JC, Basbaum AI. The peripheral nervous system and the inflammatory process. In Proceedings of the Vth World Congress on Pain. Amsterdam: Elsevier Science Publishers, 1988: 33–40.
43. Lundberg JM, Martling C-R, Saria A, Folkers K, Rosell S. Cigarette smoke-induced airway oedema due to activation of capsaicin-sensitive vagal afferents and substance P release. Neuroscience 1983; 10: 1361–1368.
44. Farber EM, Nickoloff BJ, Recht B, Fraki JE. Stress, symmetry, and psoriasis: Possible role of neuropeptides. J Am Acad Dermatol 1986; 14: 305–311.
45. Braverman IM. The role of blood vessels and lymphatics in cutaneous inflammatory processes: An overview. Br J Dermatol 1983; 109, suppl 25: 89–98.
46. Lundblad L, Lundberg JM, Änggård A, Zetterström O. Involvement of capsaicin-sensitive nerves in the cutaneous allergy reaction in man. Acta Physiol Scand 1985; 124, suppl 542: 135.
47. Bernstein JE. Capsaicin in dermatologic disease. Seminars in Dermatol 1988; 7: 304–309.
48. Troilius A, Möller H. Unilateral eruption of endogenous eczema after hemiparesis. Acta Derm Venereol (Stockh) 1989; 69: 256–258.
49. Keele CA, Armstrong D (eds). Substances producing pain and itch. London: Edward Arnold Ltd., 1964.
50. Robinson CJ, Torebjörk HE, LaMotte RH. Psychophysical detection and pain ratings of incremental thermal stimuli: A comparison with nociceptor responses in humans. Brain Research 1983; 274: 87–106.
51. Zimmermann M. Neurophysiology of nociception. In Porter R, ed.: International review of physiology, neurophysiology II. Baltimore: University Park Press 1976; 10: 179–221.
52. Chouchkov C. Cutaneous receptors. Int Adv Anat Embryol Cell Biol 1978; 54: 3–62.
53. Iggo A. Cutaneous and subcutaneous sensory organs. Br Med Bull 1977; 33: 97–102.
54. Hensel H. Classes of receptor units predominantly related to thermal stimuli. In de Reuck AVS, Knight J, eds.: Ciba Foundation Symposium. Touch, heat and pain. London: Churchill LTD, 1966: 275–292.
55. Konietzny F, Hensel H. The neural basis of the sensory quality of warmth. In Kenshalo DR, eds.: Sensory Functions of the skin of humans. New York: Plenum Press, 1979: 241–259.
56. Cauna N. The free penicillate nerve endings of the human hairy skin. J Anat 1973; 115: 277–289.
57. Cauna N. Morphological basis of sensation in hairy skin. Progr Brain Res 1976; 43: 35–45.
58. Vallbo ÅB, Hagbarth KE, Torebjörk HE, Wallin BG. Somatosensory proprioceptive and sympathetic activity in human peripheral nerves. Physiolog Rev 1979; 59: 919–957.
59. Raja SN, Meyer RA, Campbell JN. Peripheral mechanisms of somatic pain. Anesthesiology 1988; 68: 571–590.
60. Sinclar D. Mechanisms of Cutaneous Sensation. New York: Oxford University Press, 1981.
61. Hensel H, Iggo A, Witt I. A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. J Physiol (London) 1960; 153: 113–126
62. LaMotte RH, Simone DA, Baumann TK, Shain CN, Alreja M. Hypothesis for novel classes of chemoreceptors mediating chemogenic pain and itch. In Dubner R, Gebhart GF, Bond, MR, eds.: Proceedings of the Vth World Congress on Pain. Amsterdam: Elsevier Science Publishers BV, 1988: 529–535.
63. Gybels J, Handwerker HO, Van Hees J. A comparison between the discharges of human nociceptive nerve fibres and the subject's ratings of his sensations. J Physiol (Lond.) 1979; 292: 193–206.
64. Bromm B, Jahnke MT, Treede RD. Responses of human cutaneous afferens to CO2 laser stimuli causing pain. Exp Brain Res 1984; 55: 158–166.
65. Gad J, Goldscheider A. Über die Summation von Hautreizen. Z Klin Med 1892; 20: 339–373.
66. Van Hees J. Human C-fiber input during painful and nonpainful skin stimulation with radiant heat. In Bonica JJ, Albe-Fessard D, eds.: Advances in pain research and therapy. New York: Raven Press, 1976; 1: 35–40.
67. Zotterman Y. ed.: Sensory functions of the skin in primates. Oxford: Pergamon Press, 1976.
68. Kenton B, Coger R, Crue B, Pinsky J, Friedman Y, Carmon A. Peripheral fiber correlates to noxious thermal stimulation in humans. Neurosc Lett 1980; 17: 301–306.
69. Khayutin VM, Baraz LA, Lukoshkova EV, Sonina RS, Chernilovskaya PE. Chemosensitive spinal affe-

rents: Thresholds of specific and nociceptive reflexes as compared with thresholds of excitation for receptors and axons. In Iggo A, Ilyinski OB, eds.: Progress in brain research. Amsterdam: Elsevier, 1976: 43; 293–306.
70. Zimmermann M. Peripheral and central nervous mechanisms of nociception, pain, and pain therapy: Facts and hypotheses. In Bonica JJ et al., eds.: Advances in pain research and therapy. New York: Raven Press 1979; 3: 3–32.
71. Torebjörk HE, Hallin RG. Identification of afferent C units in intact human skin nerves. Brain Research 1974; 67: 387–403.
72. Baranowski R, Lynn B, Pini A. The effects of locally applied capsaicin on conduction in cutaneous nerves in four mammalian species. Br J Pharmac 1986; 89: 267–276.
73. Lynn B, Carpenter SE. Primary afferent units from the hairy skin of the rat hind limb. Brain Research 1982; 238: 29–43.
74. Lynn B, Shakhanbeh J. Neurogenic inflammation in the skin of the rabbit. Agents Actions 1988; 25: 228–230.
75. Lynn B. Neurogenic inflammation. Skin Pharmacol 1988; 1: 217–224.
76. Wall PD. Alterations in the central nervous system after deafferentation: Connectivity control. In Bonica JJ, Lindblom U, Iggo A, eds.: Advances in pain research and therapy. New York: Raven Press, 1983; 5: 677–689.
77. Chahl LA. Pain induced by inflammatory mediators. Mechanisms of pain and algesic compounds. In Beers RF, Basset EG, eds. New York: Raven Press, 1979: 273–284.
78. Thomsen K. Unilateral skin conditions after hemiparesis. Acta Derm Venereol (Stockh) 1989; 69: 544.
79. Beck PW, Handwerker HO, Zimmermann M. Nervous outflow from cat's foot during noxious radiant heat stimulation. Brain Res 1974; 67: 373–386.
80. Adriaensen H, Gybels J, Handwerker HO, Van Hees J. Response properties of thin myelinated (Aδ) fibres in human skin nerves. J Neurophysiol 1983; 49: 111–122.
81. Adriaensen H, Gybels J, Handwerker HO, Van Hees J. Receptive properties of human Aδfibers. In Bonica JJ et al., eds.: Advances in pain research and therapy. New York: Raven Press, 1983; 5: 95–98.
82. Sumino R, Dubner R, Starkman S. Responses of small myelinated warm fibers to noxious heat stimuli applied to the monkey face. Brain Research 1973; 62: 260–263.
83. Van Hees J, Gybels JM. Pain related to single afferent C fibers from human skin. Brain Research 1972; 48: 397–400.
84. LaMotte RH, Thalhammer JG, Torebjörk HE, Robinson CJ. Peripheral neural mechanisms of cutaneus hyperalgesia following mild injury by heat. J Neurosci 1982; 2: 765–781.
85. Darian-Smith I, Johnson KO, LaMotte C, Shigenaga Y, Kenins P, Champness P. Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli. J Neurophysiol 1979; 42: 1297–1310.
86. Konietzny F, Hensel H. Warm fiber activity in human skin nerves. Pflügers Arch. 1975; 359: 265–267.
87. Carmon A, Friedman Y, Coger R, Kenton B. Single trial analysis of evoked potentials to noxious thermal stimulation in man. Pain 1980; 8: 21–32.
88. Arendt-Nielsen L, Bjerring P. Selective averaging of argon laser induced pre-pain and pain related cortical responses. J Neurosci Methods 1988; 24: 117–123.
89. Carmon A, Mor J, Goldberg J. Evoked cerebral responses to noxious thermal stimuli in humans. Exp Brain Res 1976; 25: 103–107.
90. Price DD. Characteristics of second pain and flexion reflexes indicative of prolonged central summation. Exp Neurol 1972; 37: 371–387.
91. Torebjörk HE, Hallin RG. Responses in human A and C fibres to repeated electrical intradermal stimulation. J Neurol Neurosurg Psych 1974; 37: 653–664.
92. Torebjörk HE. Afferent C units responding to mechanical, thermal, and chemical stimuli in human non-glabrous skin. Acta Physiol Scand 1974; 92: 374–390.
93. Beitel RE, Dubner R, Harris R, Sumino R. Role of thermoreceptive afferents in behavioural reaction times to warming temperature shifts applied to the monkey's face. Brain Research 1977; 138: 329–346.
94. Price DD, Hu JW, Dubner R, Gracely RH. Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain 1977; 3: 57–68.
95. LaMotte RH, Campbell JN. Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgments of thermal pain. J Neurophysiol 1978; 41: 509–528.
96. Torebjörk HE, Ochoa JL. Specific sensations evoked by activity in single identified sensory units in man. Acta Physiol Scand 1980; 110: 445–447.
97. Hallin RG, Torebjörk HE, Wiesenfeld Z. Nociceptors and warm receptors innervated by C fibres in human skin. J Neurol Neurosurg Psych 1981; 44: 313–319.
98. LaMotte RH. Information processing in cutaneous nociceptors in relation to sensations of pain. Fed Proc 1983; 42: 2548–2552.
99. Torebjörk HE, LaMotte RH, Robinson CJ. Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: simultaneous recordings in humans of sensory judgments of pain and evoked responses in nociceptors with C-fibers. J Neurophysiol 1984; 51: 325–339.
100. Bessou P, Perl ER. Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. J Neurophysiol 1969; 32: 1025–1043.
101. Beitel RE, Dubner R. Response of unmyelinated (C) polymodal nociceptors to thermal stimuli applied to monkey's face. J Neurophysiol 1976; 39: 1160–1175.
102. Kumazawa T, Perl ER. Primate cutaneous sensory units with unmyelinated (C) afferent fibers. J Neurophysiol 1977; 40: 1325–1338.
103. Pertovaara A, Reinikainen K, Harf R. The activation of unmyelinated or myelinated afferent fibers by brief infrared laser pulses varies with skin type. Brain Research 1984; 307:341–343.

104. Parkhouse N, Lequesne PM. Quantitative objective assessment of peripheral nociceptive C fibre function. J Neurol Neurosurg Psych 1988; 51: 28–34.
105. Anderson RR, Parrish JA. Optical properties of human skin. In Reagen JD, Parrish JA, eds.: The Science of Photomedicine. New York: Plenum Press, 1982: 147–194.
106. Beitel RE, Dubner R. Fatigue and adaptation in unmyelinated (C) polymodal nociceptors to mechanical and thermal stimuli applied to the monkey's face. Brain Research 1976; 112: 402–406.
107. Bromm B, Treede RD. CO2 laser radiant heat pulses activate C nociceptors in man. Pflügers Arch 1983; 399: 155–156.
108. Melzack R, Wall PD. Pain mechanism: A new theory. Science 1965; 150: 971–978.
109. Lynn B, Perl ER. A comparison of four tests for assessing the pain sensitivity of different subjects and test areas. Pain 1977; 3: 353–365.
110. Lambelin G, Vassart-Thys D, Roba J. Cutaneous thermometry for topical evaluation of UV erythema in the guinea-pig. Arzneim Forsch Drug Res 1971; 21: 44–47.
111. Hendler E, Crosbie R, Hardy JD. Measurement of heating of the skin during exposure to infrared radiation. J Appl Physiol 1958; 12: 177–185.
112. Haimi-Cohen R, Cohen A, Carmon A. A model for the temparature distribution in skin noxiously stimulated by a brief pulse of CO2 laser radiation. J Neurosci Methods 1983; 8: 127–137.
113. Bjerring P. Intratumoural light distribution in an experimental mouse tumour irradiated by a diffuse-light irradiator compared with unilateral helium-neon light for photodynamic therapy. Int J Radiat Biol 1987; 52: 191–199.
114. Andersen PH, Bjerring P. Spectral reflectance of human skin in vivo. Photodermatology 1990; 7: 5–12.
115. Strange M, Cassady G. Neonatal transcutaneous bilirubinometry. Clin Perinatal 1985; 12: 51–62.
116. Edwards AE, Hamilton JB, Duntley SQ, Hubert G. Cutaneous vascular and pigmentary changes in castrate and eunuchoid men. Endocrinology 1941; 28: 119–128.
117. Edwards AE, Duntley SQ. Cutaneous vascular changes in women in reference to the menstrual cycle and ovariectomy. Am J Obst Gyncol 1949; 57: 501–509.
118. Banerjee S. Pigmentary fluctuation and hormonal changes. J Genet Hum 1984; 32: 345–349.
119. Findlay GH. Blue skin. Br J Dermatol 1970; 83: 127–134.
120. Kuppenheim HF, Heer RR. Spectral reflectance of white and negro skin between 440 and 1000 mu. J Appl Physiol 1959; 4: 800–806.
121. Breul L. Über die Verteilung des Hautpigments bei verschiedenen Menschenrassen. Morphol Arb 1896; 6: 691-719.
122. Buettner K. Effects of extreme heat and cold on human skin. I. Analysis of temperature changes caused by different kinds of heat application. J Appl Physiol 1951; 3: 691–702.
123. Della Corte M, Procacci P, Bozza G, Buzzelli G. A study on the cutaneous pricking pain threshold in normal man. Arch Fisiol 1965; 64: 141–170.
124. Hardy JD, Stolwijk JAJ. Tissue temperature and thermal pain. In De Reuck AVS, Knight J, eds.: Ciba foundation symposium – touch, heat and pain. London: Churchill, 1966
125. Lahaye CT, Gemert van MJ. Optimal laser parameters for port wine stain therapy: A theoretical approach. Phys Med Biol 1985; 30: 573–587.
126. Hardy JD. Method for the rapid measurement of skin temperature during exposure to intense thermal radiation. J Appl Physiol 1953; 5: 559–566.
127. Croze S, Duclaux R, Kenshalo DR. The thermal sensitivity of the polymodal nociceptors in the monkey. J Physiol (London) 1976; 263: 539–562.
128. Neisser U. Temperature thresholds for cutaneous pain. J Appl Physiol 1959; 14: 368–372.
129. Hardy JD. The pain threshold and the nature of pain sensation. In Keele CA, Smith R, eds.: The assessment of pain in man and animals. Edinburgh and London: E & S Livingstone Ltd, 1962: 170–201.
130. Keele CA. Addendum, In Keele CA, Smith R, eds,: UFAW Symposium – The assessment of pain in man and animals. London: Livingstone, 1962: 41–43.
131. Procacci P, Della Corte M, Zoppi M, Romano S, Maresca M, Voegelin MR. Pain threshold measurements in man. In Bonica JJ, Procacci P, Pagni CA, eds.: Recent advances on pain. Springfield, Illinois: Charles C Thomas Publisher, 1976: 105–147.
132. Stowell H. Human evoked responses to potentially noxious tactile stimulation I. Activ nerv sup (Praha) 1975; 17: 1–7.
133. Stowell H. Human evoked responses to potentially noxious tactile stimulation II. Activ nerv sup (Praha) 1975; 17: 94–100.
134. Wolff BB. The role of laboratory pain induction methods in the systematic study of human pain. Int J Res Acupunct Electro Ther 1977; 2: 271–305.
135. Hardy JD, Wolff HG, Goodell H. The pricking pain threshold in man as ascertained by the thermal radiation method. In Hardy JD, Wolff HG, Goodell H, eds.: Pain sensations and reactions. Baltimore: Williams & Wilkins, 1952: 86–122.
136. Smith R. The experimental investigation of pain. In Rizzi R, eds.: Atti del symposium internazionale sulla terapia di blocco della sindrome dolorosa. Consonni Vicenza, 1964.
137. McCafferty DF, Woolfson AD, McClelland KH, Boston V. Comparative in vivo and in vitro assessment of the percutaneous absorption of a local anaesthetics. Br J Anaesth 1988; 60: 64–69.
138. McCafferty DF, Woolfson AD, Boston V. In vivo assessment of percutaneous local anaesthetic preparations. Br J Anaesth 1989; 62: 17–21.
139. Juhlin L, Evers H. EMLA, a new topical anaestetic. Adv Dermatol 1990; 5: 75–92.
140. Hallén A, Ljunghall K, Wallin J. Topical anaesthesia with local anaesthetic (lidocaine and prilocaine, EMLA) cream for cautery of genital warts. Eur J Anaesthesiol 1984a; 4: 441–448.
141. Mor J, Carmon A. Laser emitted radiant heat for pain research. Pain 1975; 3: 233–237.

142. Arendt-Nielsen L, Bjerring P. The effect of topically applied anaesthetics (Emla(R) cream) on thresholds to thermode and argon laser stimulation. Acta Anaesthesiol Scand 1989; 33: 469–473.
143. Hallén B, Uppfeldt A. Does lidocaine-prilocaine cream permit pain free insertion of IV catheters in children? Anesthesiology 1982; 57: 340–342.
144. Hallén B, Olsson GL, Uppfeldt A. Pain-free venepuncture. Anaesthesia 1984; 39: 969–972.
145. Hallén B, Carlsson P, Uppfeldt A. Clinical study of a lignocaine-prilocaine cream to relieve the pain of venipuncture. Br J of Anaesth 1985; 57: 326–328.
146. Cooper CM, Gerrish SP, Hardwick M, Kay R. EMLA cream reduces the pain of venepuncture in children. Acta Oncol 1987; 26: 467–476.
147. Dybvik T, Kolflaath J. EMLA cream used as a local anesthetic before insertion of a peripheral venous catheter (Venflon) in children. Clin Allergy 1987; 17: 307–311.
148. Soliman IE, Broadman LM, Hannallah RS, McGill WA. Comparison of the analgesic effects of EMLA (eutectic mixture of local anesthetics) to intradermal lidocaine infiltration prior to venous cannulation in unpremedicated children. Anaestesiology 1988; 68: 804–806.
149. Ohlsén L, Englesson S, Evers H. An anaesthetic lidocaine/prilocaine cream (EMLA) for epicutaneous application tested for cutting split skin grafts. Scand J Plastic Reconstr Surg 1985; 19: 201–209.
150. Goodacre TE, Sanders R, Watts DA, Stoker M. Split skin grafting using topical local anaesthesia (EMLA): A comparison with infiltrated anaesthesia. Br J Plastic Surg 1988; 41: 533–538.
151. Juhlin L, Evers H, Broberg F. A lidocaine-prilocaine cream for superficial skin surgery and painful lesions. Acta Derm Venereol (Stockh) 1980; 60: 544–546.
152. Hallén A, Ljunghall K, Wallin J. Topical anaesthesia with local anaesthetic (lidocaine and prilocaine, EMLA) cream for cautery of genital warts. Genitourin Med 1987; 63: 316–319.
153. Rosdahl I, Edmar B, Gisslén H, Nordin P, Lillieborg S. Curettage of molluscum contagiosum in children: Analgesia by topical application of a lidocaine/prilocaine cream (EMLA). Acta Derm Venereol (Stockh) 1988; 68: 149–153.
154. Bjerring P, Arendt-Nielsen L. Depth and duration of skin analgesia to needle insertion after topical application of EMLA cream. Br J Anaesth 1990; 64: 173–177. (IV).
155. Fitzgerald M. The spread of sensitization of polymodal nociceptors in the rabbit from nearby injury and by antidromic nerve stimulation. J Physiol (London) 1979; 297: 207–216.
156. Juan H. The pain enhancing effect of PGI2. Agents Actions 1979b; 9: 204–210.
157. Juan H, Lembeck F. Action of peptides and other algesic agents on paravascular pain receptors of the isolated perfused rabbit ear. Naunyn-Schmiedeberg's Arch Pharmacol 1974; 283: 151–164.
158. Lynn B. Cutaneous hyperalgesia. Br Med Bull 1977; 33: 103–108.
159. LaMotte RH, Thalhammer JG, Robinson CJ. Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: A comparison of neural events in monkey with sensory judgments in human. J Neurophysiol 1983; 50: 1–26.
160. Meyer RA, Campbell JN. Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand. Science 1981; 213: 1527–1529.
161. Campbell JN, Raja SN, Meyer RA, Mackinnon SE. Myelinated afferents signal the hyperalgesia associated with nerve injury. Pain 1988; 32: 89–94.
162. LaMotte RH. Can the sensitization of nociceptors account for hyperalgesia after skin injury?. Hum Neurobiol 1984; 3: 47–52.
163. Hallin RG, Torebjörk HE. Electrically induced A and C fibre responses in intact human skin nerves. Exp Brain Res 1973; 16: 309–320.
164. Torebjörk HE, Hallin RG. Recordings of impulses in unmyelinated nerve fibres in man; afferent C fibre activity. Acta Anaesth Scand 1978; suppl 70: 124–129.
165. White DM, Helme RD. Release of substance P from peripheral nerve terminals following electrical stimulation of the sciatic nerve. Brain Research 1985; 336: 27–31.
166. Buchthal F, Rosenfalck A. Evoked action potentials and conduction velocity in human sensory nerves. Brain Res 1966; 3: 1–122.
167. Gibson RH. Electrical stimulation of pain and touch. In Kenshalo DR, ed.: The skin senses. Springfield: Charles C Thomas, 1968: 223–260.
168. Laitinen LV, Eriksson AT. Electrical stimulation in the measurement of cutaneous sensibility. Pain 1985; 22: 139–150.
169. Nathan PW. The gate-control theory of pain. A critical review. Brain 1976; 99: 123–158.
170. Dubner R, Bushnell MC, Duncan GH. Behavioral and neural correlates of nociception. In Yokota T, Dubner R, eds.: Current topics in pain research and therapy. Amsterdam: Excerpta Medica, 1983: 45–55.
171. Lewis T. Pain. New York: Macmillan, 1942: 57–84.
172. Rosenthal SR. Histamine as the chemical mediator for cutaneous pain. J Invest Dermatol 1977; 69: 98–105.
173. LaMotte RH. Psychophysical and neurophysical studies of chemically induced cutaneous pain and itch. The case of the missing nociceptor. In Hamann W, Iggo A, eds.: Progress in brain research. Amsterdam: Elsevier Publishers, 1988; 74: 331–335.
174. Becerra-Cabal L, LaMotte RH, Ngeow J, Putterman GJ. Chemically induced itch, pain and hyperalgesia by intraepidermal injection. Soc Neurosci Abstr 1983: 1063.
175. Acachi KI, Ishii Y. Vocalization response to close-arterial injection of bradykinin and other algesic agents in guinea pigs and its application to quantitative assessment of analgesic agents. J Phamac Exp Ther 1978; 209: 117–124.
176. Simone DA, Baumann TK, Shain CN, LaMotte RH. Magnitude scaling of chemogenic pain and hyperalgesia in humans. Soc Neurosci Abst 1987; 13: 109.
177. Lawless H. Oral chemical irritation: psychophysical properties. Chem Senses 1984; 9: 143–155.
178. Simone DA, Ngeow JYF, Putterman GJ, LaMotte

RH. Hyperalgesia to heat after intradermal injection of capsaicin. Brain Research 1987b; 418: 201–203.
179. Kiistala U, Mustakallio KK. Dermo-epidermal separation with suction. Electron microscopic and histochemical study of initial events of blistering on human skin. J Invest Dermatol 1967; 48: 466–477.
180. Fruhstorfer H, Goldberg JM, Lindblom U, Schmidt WG. Temperature sensitivity and pain thresholds in patients with peripheral neuropathy. In Zotterman Y, ed.: Sensory functions of the skin in primates. Oxford: Pergamon Press, 1976: 507–517.
181. Jamal GA, Hansen S, Weir AI, Ballantyne JP. An improved automated method for the measurement of thermal thresholds. I: Normal subjects. J Neurol Neurosurg Psych 1985; 48: 354–360.
182. Hardy JD. Influence of thermal radiation on human skin. In: Proceedings of the International Photobiological Congress. Veenmum and Zonen, Amsterdam 1954: 205–225.
183. Hardy JD, Wolff HG, Goodell H. Methods for the study of pain thresholds. In Hardy JD, Wolff HG, Goodell H. eds.: Pain sensations and reactions. Baltimore: Williams & Wilkins, 1952: 52–85.
184. Beecher HK. Measurement of subjective responses: Quantitative effects of drugs. New York : Oxford University Press, 1959.
185. Lewis T. Experiments relating to cutaneous hyperalgesia and its spread through somatic nerves. Clin Sci 1935; 2: 373–423.
186. LaMotte RH, Torebjörk HE, Robinson CJ, Thalhammer JG. Time-intensity profiles of cutaneous pain in normal and hyperalgesic skin: A comparison with C-fiber nociceptor activities in monkey and human. J Neurophysiol 1984; 51: 1434–1450.
187. Hilton SM. Bradykinin formation in human skin as a factor in heat vasodilatation. J Physiol 1958; 157: 589–602.
188. Goetzl FR, Burril DY, Ivy AC. A critical analysis of algesimetric methods with suggestions for a useful procedure. Quart Bull Northw Univ Med Sch 1943; 17: 280–288.
189. Diffey BL, Oliver RJ, Farr PM. A portable instrument for quantifying erythema induced by ultraviolet radiation. Br J Dermatol 1984; 111: 663–672.
190. Bjerring P, Andersen PH. Skin reflectance spectrophotometry. Photodermatology 1987; 4: 167–171. (II).
191. Meyer RA, Walker RE, Mountcastle VB. A laser stimulator for the study of cutaneous thermal and pain sensation. IEEE Trans Biomed Eng 1976; 23: 54–60.
192. Bromm B, Treede RD. Nerve fibre discharges, cerebral potentials and sensations induced by CO2 laser stimulation. Human Neurobiol 1984; 3: 33–40.
193. Pertovaara A, Morrow TJ, Casey KL. Cutaneous pain and detection thresholds to short CO2 laser pulses in humans: Evidence on afferent mechanisms and the influence of varying stimulus conditions. Pain 1988; 34: 261–269.
194. Mayayo E, Trelles MA, Rigau J, Sanchez J, Sala P. Experimental effects of argon and CO2 lasers on skin and vessels. Lasers in Medical Science 1989. Proceedings of the First Plenary Workshop on Safety and Laser-Tissue Interaction; suppl 1989: 95–103.
195. Coger RW, Kenton B, Pinsky JJ, Crue BL, Carmon A, Friedman Y. Somatosensory evoked potentials and noxious stimulation in patients with intractable non-cancer pain syndromes. Psychiatry Res 1980; 2: 279–294.
196. Willer JC, Boureau F, Berny J. Nociceptive flexion reflexes elicited by noxoius laser radiant heat in man. Pain 1979; 7: 15–20.
197. Wan S, Anderson RR, Parrish JA. Analytical modeling for the optical properties of the skin with in vitro and in vivo applications. Photochem Photobiol 1981; 34: 493–499.
198. Bjerring P, Arendt-Nielsen L. Use of a new argon laser technique to evaluate changes in sensory and pain thresholds in human skin following topical capsaicin treatment. Skin Pharmacol 1989; 2: 162–167. (IX).
199. Bjerring P, Arendt-Nielsen L. Inhibition of histamine skin flare reactions following repeated topical applications of capsaicin. Allergy 1990; 45: 121–125. (X).
200. Arendt-Nielsen L, Zachariae R, Bjerring P. Quantitative evaluation of hypnotically suggested hyperaesthesia and analgesia by painful laser stimulation. Pain 1990; 42: 243–251.
201. Bjerring P, Arendt-Nielsen L, Søderberg U. Argon laser induced cutaneous sensory and pain thresholds in post herpetic neuralgia. Quantitative modulation by topical capsaicin. Acta Derm Venereol (Stockh) 1990; 70: 121–125. (XII).
202. Arendt-Nielsen L, Øberg B, Bjerring P. Analgesic efficacy of i.m. alfentanil. Br J Anaesth 1990; 65: 164–168.
203. Arendt-Nielsen L, Bjerring P, Nielsen J. Regional variations in analgesic efficacy of Emla cream – Quantitatively evaluated by argon laser stimulation. Acta Derm Venereol (Stockh) 1990; 70: 314–318.
204. Chen ACN, Chapman CR. Aspirin analgesia evaluated by event-related potentials in man: Possible central action in brain. Exp Brain Res 1980; 39: 359–364.
205. Sternbach RA. Ethical problems in human pain research. In Bonica JJ et al., eds.: Advances in pain research and therapy. New York: Raven Press, 1979; 3: 837–842.
206. Carmon A, Dotan Y, Sarne Y. Correlation of subjective pain experience with cerebral evoked responses to noxious thermal stimulations. Exp Brain Res 1978; 33: 445–453.
207. Pilowsky I, Kaufman A. An experimental study of atypical phantom pain. Br J Psychiatry 1965; 111: 1185–1187.
208. Savage CW. The measurement of sensation: A critique of perceptual psychophysics. University of California Press, Berkley, 1970.
209. Gracely RH, Dubner R. Reliability and validity of verbal descriptor scales of painfulness. Pain 1987; 29: 175–185.
210. Grabois M, Blacker HM. Chronic pain: Measurement and assessment. Int J Rehabil Res 1987; 10, suppl 5: 266–270.

211. Guck TP, Meilman PW, Skultety FM. Pain assessment index: Evaluation following multidisciplinary pain treatment. J Pain Symptom Manage 1987; 2: 23–27.
212. Ekblom A, Hansson P. Pain intensity measurements in patients with acute pain receiving afferent stimulation. J Neurol Neurosurg Psych 1988; 51: 481–486.
213. Lee EO, Jung MS. A study for assessing accuracy rates of pain rating scales (KPRS and VAS). Kanho Hakhoe Chi, 1988; 18: 239–244.
214. Gracely RH, Kwilosz DM. The descriptor differential scale: Applying psychophysical principles to clinical pain assessment. Pain 1988; 35: 279–288.
215. Stevens SS. Psychophysics, introduction to its perceptual, neural, and social aspects. New York: John Wiley & Sons, 1975.
216. Chapman CR, Benedetti C. Nitrous oxide effects on cerebral evoked potentials: Partial reversal with a narcotic antagonist. Anesthesiology 1979; 51: 135–138.
217. Benedetti C, Chapman CR, Colpitt YH, Chen AC. Effect of nitrous oxide concentration on event- related potentials during painful tooth stimulation. Anesthesiology 1982; 56: 360–364.
218. Bromm B, Scharein E. Principal component analysis of pain-related cerebral potentials to mechanical and electrical stimulation in man. Electroenceph Clin Neurophysiol 1982; 53: 94–103.
219. Wright A, Davies II. The recording of brain evoked potentials resulting from intra-articular focused ultrasonic stimulation: A new experimental model for investigating joint pain in humans. Neurosc Lett 1989; 97: 145–150.
220. Beecher KH. The measurement of pain. Pharmacol Rev 1957; 9: 59–209.
221. Wolff BB, Horland AA. Effect of suggestion upon experimental pain: A validation study. J Abnorm Psychol 1967; 72: 402–407.
222. Procacci P, Buzzelli G, Passeri I, Sassi R, Voegelin MR, Zoppi M. Studies on the cutaneous pricking pain threshold in man. Circadian and circatrigintan changes. Res Clin Stud Headache 1972; 3: 260–276.
223. Strian F, Lautenbacher S, Galfe G, Hölzl R. Diurnal variations in pain perception and thermal sensitivity. Pain 1989; 36: 125–131.
224. Davis GC, Buchsbaum MS, Bunney WE. Naloxone decreases diurnal variation in pain sensitivity and somatosensory evoked potentials. Life Sci 1978; 23: 1449–1460.
225. LaMotte RH. Intensive and temporal determinants of thermal pain. In Kenshalo DR, ed.: Sensory functions of the skin of humans. New York: Plenum Press, 1979: 327–361.
226. Torebjörk E, Ochoa J. Selective stimulation of sensory units in man. In Bonica JJ et al., eds.: Advances in pain research and therapy. New York: Raven Press, 1983; 5: 99–104.
227. Edwards AE, Duntley SQ. The pigments and color of living human skin. Am J Anat 1939; 65: 1–33.
228. Ferreira SH, Nakamura M, De Abreu Castro MS. The hyperalgesic effects of prostacyclin and prostaglandin E2. Prostaglandins 1978; 16: 31–37.
229. Campbell JN, LaMotte RH. Latency to detection of first pain. Brain Research 1983; 266: 203–208.
230. Ferreira SH, Moncada S, Vane JR. Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. Br J Pharmac 1973; 49: 86–97.
231. Gemert van MJC, Hulsbergen HJP. A model approach to laser coagulation of dermal vascular lesions. Arch Dermatol Res 1981; 270: 429–439.
232. Martin JC, van Gemert MJC, Welch AJ, Amin AP. Is there an optimal laser treatment for port wine stains. Lasers Surg Med 1986; 6: 76–83.
233. Bonner RF, Nossal R, Havlin S, Weiss GH. Model for photon migration in turbid biological media. J Opt Soc Am A 1987; 4: 423–432.
234. Buettner K. Effects of extreme heat and cold on human skin II. Surface temperature, pain and heat conductivity in experiments with radiant heat. J Appl Physiol 1951; 3: 703–713.
235. Barnes FS. Biological damage resulting from thermal pulses. In Laser applications in medicine and biology. New York: Plenum press, 1974; 3: 205–221.
236. Dawson JB, Barker DJ, Ellis DJ, Grassam E, Cotterill JA, Fisher GW, Feather JW. A theoretical and experimental study of light absorption and scattering by in vivo skin. Phys Med Biol 1980; 25: 695–709.
237. Andersen PH, Bjerring P. Reflektansspektrofotometri in vivo. Prize Project, University of Aarhus, 1986.
238. Wan S, Parrish JA, Jaenicke KF. Quantitative evaluation of ultraviolet induced erythema. Photochem Photobiol 1983; 37: 643–648.
239. Kollias N, Baqer AH. Quantitative assessment of UV-induced pigmentation and erythema. Photodermatology 1988; 5: 53–60.
240. Shelley WB, Arthur RP. The neurohistology and neurophysiology of the itch sensation in man. Arch Dermatol 1957; 76: 296–323.
241. Breit R, Kligman AM. Measurement of erythemal and pigmentary responses to ultraviolet radiation of different spectral qualities. In Urbach F, ed.: The biologic effects of ultraviolet radiation. Oxford: Pergamon Press, 1969: 267–275.
242. Oshiro T, Calderhead RG. Low level laser therapy: A practical introduction. New York: John Wiley & Sons, Inc. 1988; 2: 19–31.
243. Basford JR. Low energy laser treatment of pain and wounds: Hype, hope or hokum? Mayo Clin Proc 1986; 61: 671–675.
244. Basford JR. Low-energy laser therapy: Controversies and new research findings. Lasers Surg Med 1989; 9: 1–5.
245. Hökfelt T, Kellerth JO, Nilsson G, Pernow B. Substance P: Localization in the central nervous system and in some primary sensory neurons. Science 1975; 190: 889–890.
246. Leeman S, Gamse R. Substance P in sensory neurons. Trends Pharmacol Sci 1981; 2: 119–121.
247. Buck SH, Walsh JH, Yamamura HI, Burks TF. Neuropeptides in sensory neurons. Life Sci 1982; 30: 1857–1866.
248. Gamse R, Saria A. Antidromic vasodilatation in the rat hindpaw measured by laser Doppler flowmetry:

Pharmacological modulation. J Auton Nerv Syst 1987; 19: 105–111.
249. Szolcsányi J. The local efferent function of capsaicin sensitive C-nociceptors. Proc IUPS, Paris 1977; 73: 736.
250. Szolcsanyi J. Antidromic vasodilatation and neurogenic inflammation. Agents Actions 1988; 23: 4–11.
251. Cuello AC, Del Fiacco M, Paxinos G. The central and peripheral ends of the substance P-containing sensory neurones in the rat trigeminal system. Brain Research 1978; 152: 499–509.
252. Chahl LA, Ladd RJ. Local oedema and general excitation of cutaneous sensory receptors produced by electrical stimulation of the saphenous nerve in the rat. Pain 1976; 2: 25–34.
253. Lembeck F, Holzer P. Substance P as a mediator of antidromic vasodilatation and neurogenic plasma extravasation. Naunyn-Schmiedeberg's Arch Pharmacol 1979; 310: 175–183
254. Chahl LA, Szolcsányi J, Lembeck F, eds.: Antidromic vasodilatation and neurogenic inflammation. Satellite Symposium of the 29th International Congress of physiological Sciences, Newcastle, Australia 1983. Akadémia Kiadó, Budapest 1984.
255. Couture R, Cuello C. Studies on the trigeminal antidromic vasodilatation and plasma extravasation in the rat. J Physiol 1984; 346: 273–285.
256. Chahl LA. Antidromic vasodilatation and neurogenic inflammation. Pharmacol Ther 1988; 37: 275–300.
257. Foreman JC, Jordan CC. Neurogenic inflammation. Trends Pharmacol Sci 1984; 5: 116–119.
258. Lembeck F. Mediators of vasodilatation in the skin. Br J Dermatol 1983; 109, suppl 25: 1–9.
259. Jancsó N. Desensitization with capsaicin and related acylamides as a tool for studying the function of pain receptors. In Kim RKS, ed.: Pharmacology of Pain. Oxford: Pergamon Press, 1968: 33–55.
260. Meyer RA, Campbell JN, Raja SN. Antidromic nerve stimulation in monkey does not sensitize unmyelinated nociceptors to heat. Brain Research 1988; 441: 168–172.
261. Kenins P. Identification of the unmyelinated sensory nerves which evoke extravasation in response to antidromic stimulation. Neurosc Lett 1981; 25: 137–141.
262. Lynn B, Shakhanbeh J. Substance P content of the skin, neurogenic inflammation and numbers of C-fibres following capsaicin application to a cutaneous nerve in the rabbit. Neuroscience 1988; 24: 769–775.
263. Szallasi A, Blumberg PM. Neurogenic component of phorbol ester-induced mouse skin inflammation. Cancer Res 1989; 49: 6052–6057.
264. Szallasi A, Blumberg PM. Resiniferatoxin, a phorbol-related diterpentene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience 1989; 30: 515–520.
265. Holzer P, Jurna I, Gamse R, Lembeck F. Nociceptive threshold after neonatal capsaicin treatment. Eur J Pharmacol 1979; 58: 511–514.
266. Lynn B. Effect of neonatal treatment with capsaicin on the numbers and properties of cutaneous afferent units from the hairy skin of the rat. Brain Res 1984; 322: 255–260.
267. Anand P, Bloom SR, McGregor GP. Topical capsaicin pretreatment inhibits axon reflex vasodilatation caused by somatostatin and vasoactive intestinal polypeptide in human skin. Br J Pharmac 1983; 78: 665–669.
268. Helme RD, McKernan S. Neurogenic flare responses following topical application of capsaicin in humans. Ann Neurol 1985; 18: 505–509.
269. Kenins P. Responses of single nerve fibres to capsaicin applied to the skin. Neurosc Lett 1982; 29: 83–88.
270. Kenins P, Hurley JV, Bell C. The role of substance P in the axon reflex in the rat. Br J Dermatol 1984; 111: 551–559.
271. Lundblad L, Lundberg JM, Änggård A, Zetterström O. Capsaicin pretreatment inhibits the flare component of the cutaneous allergic reaction in man. Eur J Pharmacol 1985; 113: 461–462.
272. Hägermark O, Hökfelt T, Pernow B. Flare and itch induced by substance P in human skin. J Invest Dermatol 1978; 71: 233–235.
273. Bruce AN. Vaso-dilator axon-reflex. J Exp Physiol 1913; 6: 339–354.
274. Disclafani A, Wilkin JK. The axon reflex flare. Cutis 1983; 31: 523–530.
275. Lembeck F, Gamse R. Substance P in peripheral sensory processes. In Substance P in the Nervous System. London: Pitman, 1982; 91: 35–54.
276. Helme RD, McKernan S. Flare responses in man following topical application of capsaicin. In: L.A. Chahl, J. Szolcsanyi, and F. Lembeck, eds.: Antidromic Vasodilatation and Neurogenic Inflammation. Akadémia Kiadó, Budapest 1984: 303–312.
277. Carpenter S, Lynn B. Abolition of axon reflex flare in human skin by capsaicin. Proc Physiol Soc 1980; 310: 69P-70P.
278. Bjerring P, Arendt-Nielsen L. A quantitative comparison of the effect of local analgesics on argon laser induced cutaneous pain and on histamine induced wheal, flare and itch. Acta Derm Venereol (Stockh) 1990; 70: 126–131. (V).
279. Foreman JC, Piotrowski W. Peptides and histamine release. J Allergy Clin Immunol 1984; 74: 127–131.
280. Selye H. The mast cell. Washington, Butterworths, 1965.
281. Johnson AR, Hugli TE, Muller-Eberhard HJ. Release of histamine from rat mast cells by the complement peptides C3a and C5a. Immunology 1975; 28: 1067–1080.
282. Fernandez HN, Hugli TE. Partial characterization of human C5a anaphylatoxin. I. Chemical description of the carbohydrate intraepidermal accumulation of polypeptide portions of human C5a. J Immunol 1976; 117: 1688–1694.
283. Parwaresch MR, Horny HP, Lennert K. Tissue mast cells in health and disease. Path Res Pract 1985; 179: 439–461.
284. Galli SJ. New approaches for the analysis of mast cell maturation, heterogeneity, and function. Fed Proc 1987; 46: 1906–1914.
285. Bienenstock J, Tomioka M, Matsuda H, Stead RH, Quinonez G, Simon GT, Coughlin HD, Denburg JA. The role of mast cells in inflammatory processes: Evi-

dence for nerve/mast cell interactions. Int Archs Allergy Appl Immun 1987; 82: 238–243.
286. Caughey GH, Leidig F, Viro NF, Nadel JA. Substance P and vasoactive intestinal peptide degradation by mast cell tryptase and chymase. J Pharmacol Exp Ther 1988; 244: 133–137.
287. Wiesner-Menzel L, Schultz B, Vakilzadeh F, Czarnetzki BM. Electron microscopical evidence for a direct contact between nerve fibres and mast cells. Acta Derm Venereol (Stockh) 1981; 61: 465–469.
288. Newson B, Dahlström A, Enerbäck L, Ahlman H. Suggestive evidence for a direct innervation of mucosal mast cells. Neuroscience 1983; 10: 565–70.
289. Kruger L, Sampogna SL, Rodin BE, Clague J, Yeh Y. Thin-fiber cutaneous innervation and its intraepidermal contribution studied by labeling methods and neurotoxin treatment in rats. Somatosens Res 1985; 2: 335–356.
290. Celander O, Folkow B. The nature and the distribution of afferent fibres provided with the axon reflex arrangement. Acta Physiol Scand 1953; 29: 359–370.
291. Kiernan JA. The involvement of mast cells in vasodilation due to axon reflexes in injured skin. J Exp Physiol 1972; 57: 311–317.
292. Dyer RF. Endoneurial mast cells in peripheral nerves of the armadillo dermis. Cell Tissue Res 1978; 192: 97–105.
293. Skouby AP. The influence of acetylcholine, curarine and related substances on the threshold of pain stimuli. Acta Physiol 1953; 29: 340–352.
294. Bill A, Stjernschantz J, Mandahl A, Brodin E, Nilsson G. Substance P: Release on trigeminal nerve stimulation, effects in the eye. Acta Physiol Scand 1979; 106: 371–373.
295. Foreman JC, Jordan CC. Histamine release and vascular changes induced by neuropeptides. Agents Actions 1983; 13: 105–116.
296. Brain SD, Williams TJ. Substance P regulates the vasodilator activity of calcitonin gene-related peptide. Nature 1988; 335: 73–75.
297. Helme RD, Andrews PV, Watson BA. Neurogenic inflammation caused by wool fabric in the rat; Possible mediation by substance P. Neurosci Lett 1986; 66: 333–337.
298. Foreman JC, Jordan CC. Antagonism by neurotensin of the substance P-induced flare in human skin and its relationship to histamine release. Proc Phys Soc 1982; 58–59.
299. Jessell TM. Substance P in nociceptive sensory neurons. In Substance P in the nervous system. London: Pitman, 1982: 225–248.
300. Pernow B. Substance P. Pharmacol Rev 1983; 35: 85–141.
301. Jorizzo JL, Coutts AA, Eady RAJ, Greaves MW. Vascular responses of human skin to injection of substance P and mechanism of action. Eur J Pharmacol 1983; 87: 67–76.
302. Erjavec F, Lembeck F, Florjanc-Irman T, Skofitch G, Donnerer J, Saria A, Holzer P. Release of histamine by substance P. Nauyn Schmiedeberg's Arch Pharmacol 1981; 317: 67–70.
303. Juan H. Dependence of histamine-evoked nociception on prostaglandin release. Agents Actions 1981; 11: 706–710.
304. Williams TJ. Prostaglandin E2, prostaglandin I2 and the vascular changes of inflammation. Br J Pharmac 1979; 65: 517–524.
305. Goetzl EJ, Chernov T, Renold F, Payan DG. Neuropeptide regulation of the expression of immediate hypersensitivity. J Immunol 1985; 135: 802–805.
306. Vargaftig BB. The pharmacology of slow reacting substance C and of arachidonic acid. Agents Actions 1973; 3: 357–365.
307. Bisgaard H. Vascular effects of leukotriene D4 in human skin. J Invest Dermatol 1987; 88: 109–114.
308. Sandberg BEB, Iversen LL. Substance P. J Med Chem 1982; 25: 1009–1015.
309. Foreman JC, Jordan CC, Piotrowski. Interaction of neurotensin with the substance P receptor mediating histamine release from rat mast cells and the flare in human skin. Br J Pharmac 1982; 77: 531–539.
310. Pernow B. Role of tackykinins in neurogenic inflammation. J Immunol 1985; suppl. 135: 812–815.
311. Lembeck F, Gamse R, Juan H. Substance P and sensory nerve endings. In von Euler US, Pernow B, eds.: Substance P. New York: Raven Press, 1977: 169–181.
312. Zachariae H, Malmquist J, Oates JA, Pettinger W. Studies on the mechanism of kinin formation in inflammation. J Physiol 1967; 190: 81–90.
313. Maling HM, Webster ME, Williams MA, Saul W, Anderson W Jr. Inflammation induced by histamine, serotonin, bradykinin and compound 48/80 in the rat: Antagonists and mechanisms of action. J Pharmacol Exp Ther 1974; 191: 300–310.
314. Dreborg S, Agrell B, Berlin L, Einarsson R, Gherson G, Holgersson M, Halvorsen R, Lund B, Löfkvist T, Ohlsson H, Ortolani C, Stälenheim G, Wihl JA, Våla IJ. Phazet™ a new type of skin prick device: In vivo and in vitro calibration and stability studies (abstract). J Allergy Clin Immunol 1986; 77: 223.
315. Reinberg A, Sidi E, Ghata J. Circadian reactivity rhythms of human skin to histamine or allergen and the adrenal cycle. J Allergy 1965; 36: 273–283.
316. Majno G, Palade GE, Schoefl GI. Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: A topographic study. J Biophys Biochem Cytol 1961; 11: 607–626.
317. Hulström D, Svensjö E. Intravital and electron microscopic study of bradykinin-induced vascular permeability changes using FITC-Dextran as a tracer. J Pathol 1979; 129: 125–133.
318. Tóth-Kása I, Jancsó G, Bognár A, Husz S, Obál JR. Capsaicin prevents histamine-induced itching. Int J Clin Pharm Res 1986; 6: 163–169.
319. Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind PW. Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. Br J Pharmac 1986; 88: 74l-745.
320. Riley JF, West GB. The presence of histamine in tissue mast cells. J Physiol 1953; 120: 528–537.
321. Zachariae H. Skin histamine. Spectrofluorometric studies on normal and diseased skin. Copenhagen: Munksgaard, 1965.
322. Juhlin L. Localization and content of histamine in

normal and diseased skin. Acta Derm Venereol (Stockh) 1967; 42: 218–226.
323. Uvnäs B, Åborg CH, Bergendorff A. Storage of histamine in mast cells. Evidence for an ionic binding of histamine to protein carboxyls in the granule heparin-protein complex. Acta Physiol Scand 1970; 78, suppl 336: 1–26.
324. Uvnäs B, Åborg CH, Lyssarides L, Thyberg J. Cation exchanger properties of isolated rat peritoneal mast cell granules. Acta Physiol Scand 1985; 125: 25–31.
325. Roszkowski W, Plaut M, Lichtenstein LM. Selective display of histamine receptors on lymphocytes. Science 1977; 195: 683–685.
326. Beer DJ, Rocklin RE. Histamine-induced suppressor-cell activity. J Allergy Clin Immunol 1984; 4: 439–452.
327. Payan DG, Brewster DR, Missirian-Bastian A, Goetzl EJ. Substance P recognition by a subset of human T lymphocytes. J Clin Invest 1984; 74: 1532–1539.
328. Busse WW. Histamine: Mediator and modulator in inflammation. In Glyunn LE, Houck JC, Weissmann G, eds.: Handbook of Inflammation. Amsterdam: Elsevier Biomedical Press, 1979; 1: 1–45.
329. Mannaioni PF, Fantozzi R, Giannella E, Masini E. Pathophysiological significance of the distribution of histamine receptor sub-types: A proposed dual role for histamine in inflammation and type I hypersensitivity reactions. Agents Actions 1988; 24: 26–34.
330. Garbarg M, Quach TT, Rose C. Histamine receptors in the brain: Characterization by binding studies and biochemical effects. Adv Biochem Psychopharmacol 1980; 21: 169–182.
331. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 1983; 302: 832–837.
332. Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, Schunack W, Schwarz JC. Highly potent and selective ligands for histamine H3-receptors. Nature 1987; 327: 117–123.
333. Ichinose M, Belvisi MG, Barnes PJ. Histamine-H3-Receptors Inhibit Neurogenic Microvascular Leakage in Airways. J Appl Physiol 1990; 68: 21–25.
334. Juan H. Prostaglandin release by histamine due to stimulation of H1-receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1979; 308: R 27.
335. Manzini S, Ballati L, Geppetti P, Rubini I, Meini S, Perretti F. Arachidonic Acid-Induced Bronchomotor Responses Are Partially Mediated by Release of Sensory Neuropeptides from Capsaicin-Sensitive Structures. Br J Pharm 1989; 98: 1077–1079.
336. Okamoto H, Nakano K. Regulation of Interleukin-1 Synthesis by Histamine Produced by Mouse Peritoneal Macrophages per-Se. Immunology 1990; 69: 162–165.
337. Bradley KC. The sympathetic nervous-system and pain. Advances in pain research and therapy 1990; 13: 115–122.
338. Linde B, Gainer JL. Disappearance of 133-xenon and 125-iodide and extraction of 86-rubidium in subcutaneous adipose tissue during sympathetic nerve stimulation. Acta Physiol Scand 1974; 91: 172–179.
339. Engel D. The influence of the sympathetic nervous system on capillary permeability. J Physiol 1941; 99: 161–181.
340. Engel D. The influence of the sympathetic nervous system on capillary permeability. Res Exp Med (Berl) 1978; 173: 1–8.
341. Helme RD, Andrews PV. The effect of nerve lesions on the inflammatory response to injury. J Neurosci Res 1985; 13: 453–459.
342. Brown JH, Mackey HK, Riggilo DA, Schwartz NL. Studies on the acute inflammatory response. II. Influence of antihistaminics and catecholamines on formaldehyde-induced edema. J Pharm Exp Ther 1967; 160: 243–248.
343. Jancsó N, Jancsó-Gábor A, Szolcsányi J. The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. Br J Pharmacol Chemother 1968; 32: 32–41.
344. Cervero F, McRitchie HA. Neonatal capsaicin does not affect unmyelinated efferent fibers of the autonomic nervous system: Functional evidence. Brain Research 1982; 239: 283–288.
345. Pipkorn U, Andersson M. Topical dermal anaesthesia inhibits the flare but not the weal response to allergen and histamine in the skin-prick test. Clinical Allergy 1987; 17: 307–311.
346. Hanig JP, Hui FW, Sun CL. The role of H1 and H2 agonists and antagonists and morphine in antinociception (abstact). Fed Proc 1981; 40: 282.
347. Juhlin L, Rollman O. Vascular effects of a local anaesthetic mixture in atopic dermatitis. Acta Derm Venereol (Stockh) 1984; 64: 439–440.
348. Covino BG, Vassallo H. Local anaesthetics. Mechanisms of action and clinical use. New York: Grune & Stratton, 1976.
349. Juhlin L. Vascular skin reaction in atopic dermatitis. Acta Derm Venereol (Stockh) 1962; 42: 218–219.
350. Ramsay C. Vascular changes accompanying white dermographism and delayed blanche in atopic dermatitis. Br J Dermatol 1969; 81: 37–42.
351. Bjerring P, Andersen PH, Arendt-Nielsen L. Vascular response of human skin after analgesia with EMLA cream. Br J Anaest 1989; 63: 655–660. (VI).
352. Joó F, Szolcsányi J, Jancsó-Gábor A. Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. Life Sci 1969; 8: 621–626.
353. Fitzgerald M. Capsaicin and sensory neurones – a review. Pain 1983; 15: 109–130.
354. Szallasi A, Joo F, Blumberg PM. Duration of desensitization and ultrastructural changes in dorsal root ganglia in rats treated with resiniferatoxin, an ultrapotent capsaicin analog. Brain Research 1989; 503: 68–72.
355. Waddell PJ, Lawson SN. The C-fibre conduction block caused by capsaicin on rat vagus nerve in vitro. Pain 1989; 39: 237–242.
356. Biggs DF, Ladenius RC. Capsaicin selectively reduces airway responses to histamine, substance-P and vagal stimulation. Eur J Pharm 1990; 175: 29–33.
357. Bernstein JE, Swift RM, Soltani K, Lorincz AL. In-

hibition of axon reflex vasodilatation by topically applied capsaicin. J Invest Dermatol 1981; 76: 394–395.
358. Bjerring P, Arendt-Nielsen L. Inhibition of argon laser induced pricking pain by repeated applications of topical capsaicin. Proceedings of the 13th Annual Meeting of the Scandinavian Association for the Study of Pain 1989: A46.
359. Szolcsányi J. Effect of pain-producing chemical agents on the activity of slowly conducting afferent fibres. Acta Physiol Acad Scient Hungaricae 1980; 56: 86.
360. Theriault E, Otsuka M, Jessell T. Capsaicin-evoked release of substance P from primary sensory neurons. Brain Research 1979; 170: 209–213.
361. Smith JG, Crounse RG, Spence D. The effects of capsaicin on human skin, liver and epidermal lysosomes. J Invest Dermatol 1970; 54: 170–173.
362. Lelkes Z, Benedek G, Fekete M, Szikszay M. Impaired opiate mechanisms after capsaicin pretreatment. Acta Physiologica Hungarica 1987; 69: 519–522.
363. Wall PD, Fitzgerald M. Effects of capsaicin applied locally to adult peripheral nerve. I. Physiology of peripheral nerve and spinal cord. Pain 1981; 11: 363–377.
364. Jessell TM, Iversen LL, Cuello AC. Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Research 1978; 152: 183–188.
365. Gamse R, Petsche U, Lembeck F, Jancsó G. Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Research 1982; 239: 447–462.
366. Saria A, Martling CR, Theodorsson-Norheim E, Gamse R, Hua X, Lundberg JM. Coexisting peptides in capsaicin-sensitive sensory neurons: Release and actions in the respiratory tract of the Guinea-pig. Acta Physiologica Hungarica 1987; 69: 421–424.
367. Saria A, Lundberg JM, Skofitsch G, Lembeck F. Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. Arch Pharmacol 1983; 324: 212–218.
368. Bernstein JE, Hamill JR, Soltani K. Bradykinin, substance P, prostaglandin E2 and papain induced flare suppressed by capsaicin. Clin Res 1981; 29: 787.
369. Carpenter SE, Lynn B. Vascular and sensory responses of human skin to mild injury after topical treatment with capsaicin. Br J Pharmac 1981; 73: 755–758.
370. Konietzny F, Hensel H. The effect of capsaicin on the response characteristic of human C-polymodal nociceptors. J Therm Biol 1983; 8: 213–215.
371. Jancsó G, Obal JF, Toth-Kasa I, Katoma M, Husz S. The modulation of cutaneous inflammatory reactions by peptide-containing sensory nerves. Int J Tis Reac 1985; 7: 449–457.
372. Juan H, Lembeck F, Seewann S, Hack U. Nociceptor stimulation and PGE release by capsaicin. Naunyn-Schmiedeberg's Arch Pharmacol 1980; 312: 139–143.
373. Gamse R, Holzer P, Lembeck F. Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br J Pharmac 1980; 68: 207–213.
374. Chiba T, Masuko S, Kawano H. Correlation of mitochondrial swelling after capsaicin treatment and substance P and somatostatin immunoreactivity in small neurons of dorsal root ganglion in the rat. Neurosci Lett 1986; 64: 311–316.
375. Hayes AG, Tyers MB. Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. Brain Research 1980; 189: 561–564.
376. Gamse R, Wax A, Zigmond RE, Leeman SE. Immunoreactive substance P in sympathetic ganglia: Distribution and sensitivity towards capsaicin. Neuroscience 1981; 6: 437–441.
377. Szolcsányi J. Capsaicin type pungent agents producing pyrexia. In Milton AS, ed.: Handbook of experimental Pharmacology. Berlin: Springer, 1982; 60: 437–478.
378. Buckley TL, Brain SD, Williams TJ. Ruthenium red selectively inhibits oedema formation and increased blood flow induced by capsaicin in rabbit skin. Br J Pharm 1990; 99: 7–8.
379. Malamed SF. Diphenhydramine hydrochloride, its use as a local anesthetic in dentistry. Anes Progr 1973; 20: 76–82.
380. Mattern JB, Gander R. Antihistamines as local anesthetic agents for allergic patients. J Colo Dent Assoc 1968; 3: 26–28.
381. Roberts EW, Loveless H. The utilization of diphenhydramine for production of local anesthesia: Report of a case. Texas Dental J 1979; aug: 13–15.
382. Rumore MM, Schlichting DA. Analgesic effects of antihistaminics. Life Sci 1985; 36: 403–416.
383. Weller CP, Ibrahim I, Sulman FG. Analgesic profile of tranquillizers in multiple screening tests in mice. Arch Int Pharmacodyn Ther 1968; 176: 176–192.
384. Langford FD, Holmes PA, Emele JF. Objective method for evaluation of analgesic/anti-inflammatory activity. J Pharm Sci 1972; 61: 75–78.
385. Morichi R, Pepeu G. A study of the influence of hydroxyzine and diazepam on morphine antinociception in the rat. Pain 1979; 7: 173–180.
386. Cormia FE, Kuykendall V. Experimental histamine pruritus II. Nature, physical and environmental factors influencing development and sensitivity. J Invest Dermatol 1953; 20: 429–446.
387. Kowalski ML, Kaliner MA. Neurogenic inflammation, vascular permeability and mast cells. J Immunol 1988; 140: 3905–3911.
388. Beck PW, Handwerker HO. Bradykinin and serotonin effects on various types of cutaneous nerve fibres. Pflügers Arch 1974; 347: 209–222.
389. Iversen LL, Hanley MR, Sandberg BEB, Lee CM, Pinnock RD, Watson SP. Substance P receptors in the nervous system and possible receptor subtypes. In Substance P in the nervous system. London: Pitman, 1982; 186–205.
390. Regoli D, Escher E, Drapeau G, D'Orleans-Juste P, Mizrahi J. Receptors for substance P. III. Classification by competitive antagonists. Eur J Pharmacol 1984; 97: 179–189.

391. Watson SP. Are the proposed substance P receptor sub-types, substance P receptors? Life Sci 1984; 35: 797–808.
392. Lembeck F, Donnerer J, Barthò L. Inhibition of neurogenic vasodilation and plasma extravasation by substance P antagonist, somatostatin and (D-Met2,Pro5) enkephalinamide. Eur J Pharmacol 1982; 85: 171–176.
393. Piotrowski W, Devoy MAB, Jordan CC, Foreman JC. The substance P receptor on rat mast cells and in human skin. Agents Actions 1984; 14: 420–424.
394. Pascual DW, Blalock JE, Bost KL. Antipeptide antibodies that recognize a lymphocyte substance P receptor. J Immunol 1989; 143: 3697–3702.
395. Laurenzi MA, Persson MA, Dalsgaard CJ, Ringden O. Stimulation of human B lymphocyte differentiation by the neuropeptides substance P and neurokinin A. Scand J Immunol 1989; 30: 695–701.
396. Doherty NS, Wong S. Pain and inflammation. Agents Actions 1988; 24: 120–122.
397. Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol 1983; 131: 1631–1615.
398. Bar-Shavit Z, Goldman R, Stabinsky Y, Gottlieb P, Fridkin M, Teichberg VI, Blumberg S. Enhancement of phagocytosis – a newly found activity of substance P residing in its N-terminal tetrapeptide sequence. Biochem Biophys Res Commun 1980; 94: 1445–1451.
399. Eglezos A, Andrews PV, Boyd RL, Helme RD. Effects of capsaicin treatment on immunoglobulin secretion in the rat – further evidence for involvement of tachykinin- containing afferent nerves. J Neuroimmun 1990; 26: 131–138.
400. Danek A, O'Dorisio SM, O'Dorisio TM, George JM. Specific binding sites for vasoactive intestinal polypeptide on nonadherent peripheral blood lymphocytes. J Immunol 1983; 131: 1173–1177.
401. Gamse R, Posch M, Saria A, Jancsó G. Several mediators appear to interact in neurogenic inflammation. Acta Physiol Hung 1987; 69: 343–54.
402. Gibbins IL, Furness JB, Costa M, MacIntyre I, Hillyard CJ, Girgis S. Co-localization of calcitonin gene related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci Lett 1985; 57: 125–130.
403. Williams TJ. A proposed mediator of increased vascular permeability in acute inflammation in the rabbit. J Physiol 1978; 281: 44P-45P.
404. Williams TJ, Jose PJ. Mediation of increased vascular permeability after complement activation. Histamine-independent action of rabbit C5a. J Exp Med 1981; 153: 136–153.
405. Wedmore CV, Williams TJ. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature 1981; 288: 646–650.
406. Björk J, Hedqvist P, Arfors KE. Increase in vascular permeability induced by leucotriene B4 and the role of polymorphonuclear leukocytes. Inflammation 1982; 6: 189–200.
407. Fogh K. Lipooxygenase products of arachidonic acid in psoriasis, atopic dermatitis, and experimentel arthritis. Thesis, University of Århus, Copenhagen: Lægeforeningens Forlag, 1990.
408. Yancey KB, Hammer CH, Harvath L, Renfer L, Frank MM, Lawley TJ. Studies of human C5a as a mediator of inflammation in normal human skin. J Clin Invest 1985; 75: 486–495.
409. Ternowitz T, Bjerring P, Andersen PH, Schröder JM, Kragballe K. Methotrexate inhibits the human C5a-induced skin response in patients with psoriasis. J Invest Dermatol 1987; 89: 192–196. (VII).
410. Ternowitz T, Andersen PH, Bjerring P, Fogh K, Schröder JM, Kragballe K. 15-Hydroxyeicosatetraenoic acid (15-HETE) specifically inhibits the LTB4-induced skin response. Arch Dermatol Res 1989; 281: 401–405. (VIII).
411. Swerlick RA, Yancey KB, Lawley TJ. Analysis of the role of mast cells in C5a induced inflammation in human skin (abstr.). J Invest Dermatol 1986; 87: 170.
412. Kazimierczak W, Peret M, Maslinski C. The action of local anesthetics on histamine release. Biochem Pharmacol 1976; 25: 1747–1750.
413. Worm AM. Plasma proteins and extracellular water in extensive skin disease. Thesis, University of Copenhagen. Copenhagen: Lægeforeningens Forlag, 1982.
414. Black MM. A modified radiographic method for measuring skin thickness. Br J Dermatol 1969; 81: 661–666.
415. Tanner JM, Whitehouse RH. The Harpenden skinfold caliper. Am J Physiol Antropol 1955; 13: 743–746.
416. Southwood WPW. The thickness of skin. Plast Reconstr Surg 1955; 15: 423–429.
417. Dykes PJ, Marks R. Measurements of skin thickness: A comparison of two in vivo techniques with a conventional histometric method. J Invest Dermatol 1977; 21: 418–429.
418. Alexander H, Miller DL. Determining skin thickness with pulsed ultra sound. J Invest Dermatol 1979; 72: 17–19.
419. Serup J. Non-invasive quantitation of psoriasis plaques – measurement of skin thickness with 15 MHz pulsed ultrasound. Clin Exp Dermatol 1984; 9: 502–508.
420. Serup J. Diameter, thickness, area, and volume of skin-prick histamine weals. Measurement of skin thickness by 15 MHz A-mode ultrasound. Allergy 1984; 39: 359–364.
421. Serup J, Staberg B, Klemp P. Quantification of cutaneous oedema in patch test reactions by measurement of skin thickness with high-frequency pulsed ultrasound. Contact Dermatitis 1984; 10: 88–93.
422. Tikjøb G, Kassis V, Søndergaard J. Ultrasonic B-scanning of the human skin. An introduction of a new ultrasonic skin-scanner. Acta Derm Venereol (Stockh) 1984; 64: 67–70.
423. Søndergaard J, Serup J, Tikjøb G. Ultrasonic A- and B-scanning in clinical and experimental dermatology. Acta Derm Venereol (Stockh) 1985; Suppl. 120: 76–82.
424. Marks R. Mechanical properties of the skin. In Goldsmith LA, eds.: Biochemistry and physiology of the

skin. New York-Oxford: Oxford University Press, 1983: 1237–1254.
425. Serup J, Northeved A. Skin Elasticity in psoriasis. In vivo measurement of tensile distensibility, hysteresis and resilient distension with a new method. Comparison with skin thickness as measured with high frequency ultrasound. J Dermatol 1985; 12: 318–324.
426. Bjerring P. Skin Elasticity measured by dynamic admittance – a new technique for mechanical measurements in patients with scleroderma. Acta Derm Venereol (Stockh) 1985; suppl. 120: 84–87.
427. Argenbreit LW, Forbes PD. Erythema and skin blood content. Br J Dermatol 1982; 569–574.
428. Lewis T. Standard colours for use in the study of vascular reactions of the human skin. Heart 1929; 15: 1–3.
429. Winder CV, Wax J, Burr V, Been M, Rosiere CE. A study of pharmacological influences on ultraviolet erythema in guinea-pigs. Arch Int Pharmacodyn Ther 1958; 116: 261–292.
430. Bjerring P, Arendt-Nielsen L. Quantitative inhibition of human axon reflexes by epicutaneous application of capsaicin and local analgesics. Proceedings of the 18th Annual Meeting of the Society for Neuroscience 1988; A15.11.
431. Brunsting LA, Sheard C. The color of the skin as analysed by spectrophotometric methods. J Clin Invest 1929; 7: 575–593.
432. Buckley WR, Grum F. Reflection spectrophotometry. Arch Dermatol 1961; 83: 249–261.
433. Ryatt KS, Feather JW, Dawson JB, Cotterill JA. The usefulness of reflectance spectrophotometric measurements during psoralens and ultraviolet: A therapy for psoriasis. J Am Acad Dermatol 1983; 9: 558–562.
434. Andersen PH, Bjerring P. Noninvasive computerized analysis of skin chromophores in vivo by reflectance spectroscopy. Photodermatol Photoimmunol Photomed 1990; 7: 249–257.
435. Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation. Br J Dermatol 1984; 111: 673–682.
436. Nilsson GE, Tenland T, Öberg PÅ. A new instrument for continuous measurenent of tissue blood flow by light beating spectroscopy. IEEE Trans Biomed Eng 1980; 27: 12–19.
437. Tenland T. On laser Doppler flowmetry. Methods and microvascular applications. Linköping University. Linköping (Dissertation), 1982
438. Huff SE, Taylor HL. Observations on peripheral circulation in psoriasis. Arch Dermatol Syphiol 1953; 68: 385–388.
439. Thune P. Plethysmographic recordings of skin pulses. Acta Derm Venereol (Stockh) 1970; 50: 263–269.
440. DiLorenzo PA, Brown DW, Walker SH, Dern PL, Goltz RW. Technetium-99m pertechnetate disppearance studies in normal and psoriatic skin. J Invest Dermatol 1971; 56: 39–43.
441. Nyfors A, Rothenborg HW. Cutaneous blood flow in psoriasis measured by 133Xenon clearance. J Invest Dermatol 1970; 54: 381–385.
442. Hutchison KJ, Overton TR, Biltek KB, Nixon R, Williams MB. Skin blood flow during histamine flare using the clearance of epicutaneous applied Xenon-133 in diabetic and non-diabetic subjects. Angiology 1983; 34: 223–230.
443. Holloway GA, Watkins DW. Laser Doppler measurement of cutaneous blood flow. J Invest Dermatol 1977; 69: 306–309.
444. Tooke JE, Östergren J, Fagrell B. Synchronous assessment of human skin microcirculation by laser Doppler flowmetry and dynamic capillaroscopy. Int J Microcirc Clin Exp 1983; 2: 277–284.
445. Engelhart M, Kristensen JK. Evaluation of cutaneous blood flow responses by 133Xenon washout and a laser-doppler flowmeter. J Invest Dermatol 1983; 80: 12–15.
446. Nilsson GE, Otto U, Wahlberg JE. Assessment of skin irritancy in man by laser Doppler flowmetry. Contact Dermatitis 1982; 8: 401–406.
447. Pierau FK, Szolcsányi J. Neurogenic inflammation: Axon reflex in pigs. Agents Actions 1989; 26: 231–232.
448. Aschheim E, Farber EM. Blood-tissue exchange in psoriatic skin. Acta Derm Venereol (Stockh) 1966; 46: 310–131.
449. Karpman HL. Current status of thermography. Angiology 1970; 21: 103–109.
450. Cosh JA, Ring EFJ. Thermography and rheumatology. Rheumatol Phys Med 1970; 10: 342–348.
451. Comstock W, Ochoa J, Marchettini P. Neurogenic warming of human hand provinces by activation of the unmyelinated population of single skin nerve fascicles. Soc Neurosci Abstr 1986; 12: 331.
452. Lafitte F, Chavoin JP, Bonafé JL, Cornil A, Brunetaud JM, Costagliola M. Large port-wine stains: Continuous argon laser treatment with neuroleptanalgesia. Lasers in Medical Science 1988; 3: 103–110.
453. Lanigan SW, Cotterill JA. Objective assessments of port wine stains: Response to temperature change. Br J Dermatol 1988; 118: 803–809.
454. Janscó G, Kiraly E, Jancsó-Gábor A. Pharmacological induced selective degeneration of chemosensitive primary sensory neurones. Nature 1977; 270: 741–743.
455. Lynn B. The immediate and long-term effects of applying capsaicin to cutaneous nerves. Acta Physiol Hungarica 1987; 69: 287–294.
456. Saria A, Lundberg JM, Hua X, Lembeck F. Capsaicin-induced substance P release and sensory control of vascular permability in the guinea-pig ureter. Neurosc Lett 1983; 41: 167–172.
457. Colpaert FC, Donnerer J, Lembeck F. Effects of capsaicin on inflammation and on the substance P content of nervous tissues in rats with adjuvant arthritis. Life Sci 1983; 32: 1827–1834.
458. Nielsen JC, Bjerring P, Arendt-Nielsen L, Petterson KJ. A double-blind, placebo controlled, cross-over comparison of the analgesic effect of ibuprofen 400 mg and 800 mg on laser-induced pain. Br J clin Pharmac 1990; 30: 711–715.
459. Jancso G, Lynn B. Possible use of capsaicin in pain therapy. Clin J Pain 1987; 3: 123–126.
460. Watson CPN, Evans RJ. Postherpetic neuralgia, a review. Arch Neurol 1986; 43: 836–840.

461. Bernstein JE, Bickers DR, Dahl MV, Roshal JY. Treatment of chronic postherpetic neuralgia with topical capsaicin. J Am Acad Dermatol 1987; 17: 93–96.
462. Bernstein JE, Korman NJ, Bickers DR, Dahl MV, Millikan LE. Topical capsaicin treatment of chronic postherpetic neuralgia. J Am Acad Dermatol 1989; 21: 265–270.
463. Loeser JD. Herpes zoster and postherpetic neuralgia. Pain 1986; 25: 149–164.
464. Watson CPN, Evans RJ, Watt VR, Birkett N. Postherpetic neuralgia: 208 cases. Pain 1988; 35: 289–297.
465. Watson CPN, Evans RJ, Watt VR. Post-herpetic neuralgia and topical capsaicin.
466. Jancso G, Husz S, Simon N. Impairment of axon reflex vasodilatation after herpes zoster. Clin Exp Dermatol 1983; 8: 27–31.
467. Nilsson J, von Euler AM, Dalsgaard CJ. Stimulation of connective tissue cell growth by substance P and substance K. Nature 1985; 315: 61–63.
468. Rosell S, Björkroth U, Xu JC, Folkers K. The pharmacological profile of a substance P (SP) antagonist. Evidence for the existence of subpopulations of SP receptors. Acta Physiol Scand 1983; 117: 445–449.
469. Berridge MJ, Dawson RMC, Downes CP, Heslop JP, Irvine RF. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. Biochem J 1983; 212: 437–482.
470. Berridge MJ, Irvine RF. Inositol triphosphate, a novel second messenger in cellular signal transduction. Nature 1984; 312: 315–321.
471. Björkstén B, Jung B, Tågsjö, Groth O. Delayed hypersensitivity responses in children after local cutaneous anesthesia. Acta Pædiatr Scand 1987; 76: 935–938.
472. Wybran J, Appelboom T, Famaey JP, Govaerts A. Suggestive evidence for receptors for morphine and methionine-enkaphelin on normal human blood T lymphocytes. J Immunol 1979; 123: 1068–1070.
473. Van Epps DE, Saland L. Beta-endorphin and met-enkephalin stimulate human peripheral blood mononuclear cell chemotaxis. J Immunol 1984; 132: 3046–3053.
474. Farber EM, Reusch MK, Naukkarinen A, Wastek GJ, Karasek MA. The psychoneural pathogenesis of psoriasis. In Orfanos CE, Stadler R, Gollnick H, eds.: Dermatology in five continents. Proceedings of the XVII world congress of dermatology. Berlin: Springer, 1988: 574–580.
475. Zachariae R, Bjerring P, Arendt-Nielsen L. Modulation of type I and type IV delayed immunoreactivity using direct suggesting and guided imagery during hypnosis. Allergy 1989; 44: 907–912.
476. Wallengren J, Möller H. The effext of capsaicin on some experimental inflammations in human skin. Acta Derm Venereol (Stockh) 1986; 66: 375–380.
477. Lynn B. Capsaicin: Actions on nociceptive C-fibres and therapeutic potentials. Pain 1990; 41: 61–69.
478. Arvier PT, Chahl LA, Ladd RJ. Modification by capsaicin and compound 48/80 of dye leakage induced by irritants in the rat. Br J Pharmac 1977; 59: 61–68.