Neurogenic control of blood flow and histamine release in psoriatic skin

The manifestation of psoriasis represents an interaction between a genetically predisposed individual and environmental influences such as infections, trauma, stress, endocrine factors, sunlight and drugs. The cutaneous lesions are characterised by marked epidermal proliferation, complex alterations in epidermal differentiation, multiple biochemical, immunological, inflammatory and vascular abnormalities and a poorly understood relationship with the central and peripheral nervous system function.

***Hypothesis*: Multiple observations are compatible with the existence of a peptidergic neurogenic mechanism in the pathophysiology of psoriatic plaque. Our hypothesis was that afferent nociceptive nerve-fibre endings in lesional skin are sensitised and activated by an unknown mediator. Via an axon-reflex mechanism in afferent C and A-delta fibre endings, these nerves release neuropeptides, which themselves have dilating effects and, in addition, may induce mast-cell degranulation/histamine release causing further vasodilatation. A pathological axon-reflex mechanism may thus contribute to the high blood flow and stimulate histamine release in lesional psoriatic skin. To test this hypothesis, a series of functional studies was conducted to elucidate whether local neurogenic peptidergic mechanisms are of importance for the maintenance of the increase in blood flow and whether such mechanisms may induce histamine release in psoriatic plaque.**

***Methods*: Healthy subjects and patients with untreated psoriatic lesions were examined. The microdialysis technique was evaluated for application in dermal skin through measurements of tissue perturbations induced by the microdialysis catheter. A new calibration technique, retrodialysis calibration, was tested and used to evaluate the concentration and release of histamine in lesional and non-lesional skin before and during the local stimulation and inhibition of nociceptive afferent nerves. The short term application of topical capsaicin was used to excite such neurones. Long-term treatment with capsaicin which desensitises nociceptive fibres or local anaesthesia was used to inhibit the activity. A laser Doppler technique was used to measure changes in perfusion before and after the provocations and ^{133}Xenon clearance methodology was used to estimate skin blood flow.**

***Results*: Perfusion, blood flow, histamine concentration and histamine release increased in lesional skin as compared with non-lesional tissue. The inhibition of nervous activity by topical local anaesthesia decreased the perfusion and augmented the concentration of histamine but did not change the histamine release in lesional skin. No changes in these parameters were found in non-lesional skin. The stimulation of C-fibres by short-term capsaicin treatment increased the perfusion and the concentration and release of histamine in both lesional and non-lesional skin. Long-term capsaicin treatment reduced the perfusion in lesional skin and increased it in lesion-free skin.**

Conclusion*: The results are compatible with the hypothesis that a pathological axon reflex may contribute to the high blood flow in lesional psoriatic skin. In contrast, the increase in histamine release, is mediated either not at all or only to a minor extent by neurogenic mechanisms.** ***Key words: axon reflex; blood flow; C-nerve fibre; histamine; mast cell; microdialysis; perfusion; psoriasis; skin.

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NEUROGENIC CONTROL OF BLOOD FLOW AND HISTAMINE RELEASE IN PSORIATIC SKIN

The thesis is based on the following papers, which will be referred to by the Roman numerals I – V:

I Axon-reflex-mediated vasodilatation in the psoriatic plaque?
Krogstad AL, Swanbeck G, Wallin BG:
J Invest Dermatol 104:872 – 876, 1995

II Microdialysis methodology for the measurement of dermal interstitial fluid in humans.
Krogstad AL, Jansson PA, Gisslén P, Lönnroth P:
Br J Dermatol 134:1005 – 1012, 1996

III Increased interstitial histamine concentration in the psoriatic plaque.
Krogstad AL, Lönnroth P, Larson G, Wallin BG:
J Invest Dermatol 109:632 – 635, 1997

IV Nerve-induced histamine release is of little importance in psoriatic skin
Krogstad AL, Lönnroth P, Larson G, Wallin BG:
Br J Dermatol 139:403 – 409, 1998

V Capsaicin treatment induces histamine release and perfusion changes in psoriatic skin.
Krogstad AL, Lönnroth P, Larson G, Wallin BG:
Br J Dermatol (in press)

ABBREVIATIONS

CGRP	Calcitonin gene-related peptide
CPM	Counts per minute
DBF	Dermal blood flow
EMLA	Eutectix mixture of local anaesthetics
L/P ratio	Lactate/pyruvate ratio
LDI	Laser Doppler perfusion imager
LDF	Laser Doppler flowmeter
MC	Mast cell
PASI	Psoriasis area and severity index
PS	Permeability surface area product
SP	Substance P
VIP	Vasoactive intestinal peptide
UVB	Ultraviolet light-B
Xe	Xenon

"One learns to hope that nature possesses an order that one may aspire to comprehend"
C N Yang

Do Not use this page see separate contents page 5

Do Not use this page see separate contents page 6

1. INTRODUCTION

1.1 General aspects of psoriasis

Psoriasis is a common, chronic, intractable skin disease which affects about 2% of the Swedish population (Forssman, 1947; Hellgren, 1967). It is equally common in men and women. The skin lesions of psoriasis usually first appear between the ages of 20-50 years. The characteristic cutaneous lesions are sharply demarcated, usually round erythematous dry patches of various size covered by abundant greyish-white, imbricated scales. As the scales are removed by gentle scraping, fine bleeding points are usually seen, the so-called Auspitz sign. The plaque may be coin-shaped, geographic, annular or circinate (ringlike), figurative, gyrate, or serpiginous. Areas of the skin most commonly affected include the elbows, knees, scalp, groin and nails. The eruptions are generally symmetrically located, and may vary from a single lesion to countless patches, or may even cover the entire body with erythroderma. The symptoms which may cause discomfort to psoriatic patients include disfiguring lesions, excessive scaling, pruritus and pain.

Psoriasis is a genetically determined disease which can fit an autosomal recessive inheritance pattern (Swanbeck et al, 1994). The manifestation of the disease represents an interaction between a genetically predisposed individual and environmental influences such as infections, trauma, stress, endocrine factors, sunlight and drugs. The cutaneous lesions are characterised by marked epidermal proliferation, complex alterations in epidermal differentiation, multiple biochemical, immunological, inflammatory and vascular abnormalities and a poorly understood relationship with the central and peripheral nervous system function (Krueger et al, 1984; Baker and Fry, 1992; Elder, 1995; Christophers, 1996.)

The initial event in the development of the psoriatic lesion remains elusive. Different possibilities have been suggested in the literature: activated and abnormal keratinocytes (Barker et al, 1991), abnormal fibroblasts (Saiag et al, 1985), the activation of T-cells (Baadsgaard et al, 1990), metabolically abnormal endothelial cells (van de Kerkhof et al, 1983), cutaneous sensory nerve-substance-P-mediated inflammation (Farber et al, 1986; Naukkarinen et al, 1989), the degranulation of dermal mast cells (Brody, 1984a) and endothelial swelling and papillary vessel dilatation (Braverman and Sibley, 1982; Brody, 1984b).

Several findings indicate the involvement of nerves and an interaction with mast cells and blood vessels in the development and maintenance of the psoriatic lesions (Farber et al, 1986; Naukkarinen et al, 1989, 1991, 1993, 1996). The anatomical and functional prerequisites for an interaction of this kind are presented below.

1.2 Cutaneous vasculature and blood flow regulation

1.2.1 Normal skin

Morphology of skin vasculature The blood vessels in human skin are arranged in a microcirculatory network in the dermis, whereas the epidermis contains no blood vessels. Small vessels (arterioles and venules) form two plexuses in the dermis: an upper horizontal network in the papillary dermis, from which the capillary loops of the dermal papillae arise, and a lower horizontal plexus at the dermal-subcutaneous interface. This lower plexus is formed by perforating vessels (50-100 μm in diameter) from the underlying muscles and subcutaneous fat. From this plexus, arterioles and venules form direct connections with the upper horizontal plexus and provide lateral tributaries which supply hair bulbs and sweat glands.

Most vessels are found in papillary dermis in the upper horizontal plexus, which is composed primarily of terminal arterioles, capillary loops and postcapillary venules; the latter being the most common. The capillary loop which arises from a terminal arteriole in the horizontal papillary plexus is composed of an ascending limb, an intrapapillary loop with a hairpin turn and a descending venous limb which connects with a postcapillary venule in the horizontal plexus (Yen and Braverman 1976; Braverman and Yen 1977a; Braverman, 1989). The capillary loops are perpendicular to the surface of the skin, apart from those in the nailfold which run parallel to the surface. Each dermal papilla is supplied by a single capillary loop. In general, the walls of the blood vessels are relatively thick. The thickness is mainly due to the basement membrane layer in which elastin fibres, smooth-muscle cells or pericytes and collagen fibres are embedded. The endothelial cells form the lining of the vasculature. These are active multifunctional cells which express a number of cell surface proteins which are important in the evolution of cutaneous inflammatory disorders (for a review see Ryan, 1991; Braverman, 1997).

The terminal arterioles are the main resistance vessels in the microvasculature. Precapillary sphincters which respond to local changes in pH, temperature, oxygen and neurotransmitters regulate the blood flow through the capillary network (Strand, 1983). To supply various tissues with their specific metabolic needs, capillaries differ between organs: three different types, classified according to their function and structure, are recognised – fenestrated capillaries (kidney, glands), sinusoid capillaries (bone marrow) and continuous tube-like capillaries. The latter type is found in skin, nervous tissue and skeletal muscle (Strand, 1983). A large variation in capillary density has been observed in different tissues. In the skin, the density is about 50 capillaries/mm^3 (Rothman, 1954), but it varies at different skin locations (Pasyk et al, 1989).

The skin vasculature is large and exceeds what is necessary to meet metabolic demands. To regulate heat loss, blood can be passed through arteriovenous anastomoses, which act as shunts to short-circuit the flow between arteri-oles and venules. The dual tasks of supplying the metabolic needs and controlling body temperature are reflected by the wide range of blood flow which can be achieved (0.3 – 150 ml per 100 mg tissue per min). Blood flow in steady-state conditions is about 5 ml per 100 mg tissue per min (about 4% of the total cardiac output) in healthy skin (Sejrsen, 1971). In severe heat stress skin blood flow can comprise over 50% of the total cardiac output, which is greatly in excess of its intrinsic metabolic demands.

Neural regulation of skin blood flow The neural regulation of skin blood flow is achieved by both sympathetic and afferent sensory nerve fibres. Some sympathetic vasomotor nerves induce vasoconstriction, whereas others cause vasodilatation (for a review see Morris and Gibbins, 1997). Whether there are separate sympathetic vasodilator fibres or whether sympathetic vasodilatation is secondary to sudomotor activation is a controversial subject (Bell and Robbins, 1997), even if nerve recordings have provided some evidence of separate vasodilator impulses (Nordin, 1990; Noll et al, 1994). Direct nerve recordings in both humans and animals reveal clear differences between sympathetic traffic to different effectors, indicating

that they are controlled differentially (Jänig et al, 1983). Cutaneous sympathetic neurones innervate blood vessels, sweat glands and piloerector muscles. The vasoconstrictor neurones project to the skin in a more or less segmental fashion with their territories loosely corresponding to the dermatomes of the cutaneous sensory fibres (Langley, 1895, 1911; Jänig, 1985). Most of the noradrenergic sympathetic innervation is concentrated around the deeper dermal vessels, especially thick-walled small arteries, including AV anastomoses with a diameter of 40-120 mm which lie about 1 mm below the epidermis. Pre -and postcapillary vascular segments are also innervated by sympathetic neurones. Vasomotor neurones in the skin are activated mainly by thermoregulatory stimuli, arousal and emotional reactions. Body cooling increases the outflow of vasoconstrictor impulses, while prolonged body warming activates sudomotor and vasodilator impulses.

In addition to noradrenalin, sympathetic neurones projecting to the skin commonly contain one or more co-transmitters (such as neuro-peptide Y) which may modulate the effector response (Gibbins, 1995). The physiological importance of the co-release phenomenon in the control of blood flow in the human skin is still incompletely understood.

Cutaneous blood flow may also be influenced by the stimulation of sensory nerve fibres which release peptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP), from their endings. In contrast to sympathetic vasoconstriction, which is short-lasting, the vasodilatation induced by these fibres may last for several minutes after the end of the nerve stimulation (Lembeck and Holzer, 1979). The neuropeptides elicit a wide range of responses in many types of cell, including smooth muscle, vascular endothelium, neurones, exocrine gland cells, mast cells and cells of the immune system. Consequently, neuropeptides together with direct vasodilatory actions may also induce vasodilator responses through the stimulation of cells containing various vasodilator substances (for a review see Holzer, 1997). The anatomy and function of these nerves are described in more detail below (see Sensory innervation of the skin; 1.5).

1.2.2 Psoriatic skin

Morphological and functional changes in the cutaneous vascular system are prominent in psoriasis (Ryan, 1980). Venules and capillaries are dilated, papillary vessels elongated and convoluted (Braverman, 1972; Braverman and Yen, 1974, 1977b; Braverman and Sibley, 1982) and endothelial cell gaps are present in the capillary loops and postcapillary venules of the superficial horizontal plexus (Braverman and Yen, 1974). These large pores or endothelial cells gaps, facilitate the transport of nutrients to the rapidly proliferating epidermal cell, and explain the functional increase in trans-capillary diffusion documented in lesional skin (Bull et al, 1992). The abnormalities are located mainly in the psoriatic lesions, but they can also be seen to a lesser degree in apparently unaffected skin of psoriatic patients (Barton et al, 1992). The blood flow is increased (Nyfors and Rothenborg, 1970; Klemp and Staberg, 1983, 1985) in the anatomically altered and dilated vascular bed in psoriatic eruptions. Also the local regulation of blood flow appears to be impaired in lesional skin (Klemp, 1984). The blood flow/perfusion in psoriatic skin will be analysed in greater detail in the methodological section (4.2.6).

The mechanisms underlying the vascular abnormalities are unclear. Both angiogenetic factors which contribute to the proliferation of endothelial cells and vasodilating mechanisms induced by local autoregulatory mechanisms, histamine, cytokines, nitric oxide, hormones and/or neural factors are conceivable alternatives. It has been emphasised that dilatation and capillary gap formation are prerequisit-es for epidermal hyperplasia (Pinkus and Mehregan, 1966), which cannot occur if blood vessels are vasoconstricted (Farber and van Scott, 1979). The ultrastructural changes in the vessels are not unique to psoriasis. They may be seen also in tissue with high angiogenetic activity and increased metabolic demands (Ryan, 1991). Angiogenetic factors which cause endothelial cells to proliferate are increased in psoriatic skin (Malhotra et al, 1989). Classical antipsoriatic treatment with ultraviolet light-B (UVB) rapidly normalises papillary vessels (Braverman and Sibley, 1982). Such changes in the morphology of the capillary loop may result in less nutrient support and the epidermal proliferation might thus be dampened by the restriction of its energy sources.

The microvasculature of the dermal superficial plexus in lesional skin contains an increased amount of endothelial leucocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The cytokine responsiveness of microvascular endothelial cells is altered in psoriatic skin in a pattern corresponding to the circumscribed nature of the psoriatic plaque (Petzelbauer et al, 1994). The possibility that a vascular defect plays a major role in the pathogenesis of psoriasis has been debated (Braverman and Sibley, 1982). The accumulated evidence suggests that the vasculature is a modulator rather than an initiator of the psoriatic process, but therapy against vasodilatation and increased permeability may provide an additional approach to treatment.

1.3 Mast cells in the skin

1.3.1 General aspects

Mast cells were first described by Ehrlich (1877) as a specific cell type containing cytoplasmic granules (Fig. 1). Mast cells appear to be derived from progenitor cells in the bone marrow (Kitamura et al, 1983). They circulate as undifferentiated mononuclear cells in the peripheral circulation and subsequently migrate into tissue and mature under local influences. Human mast cells in their fully differentiated forms are widespread in the connective tissues of virtually all the organs in the body. It has been estimated that, if all the mast cells in the body were assembled together, they would form an organ the size of the spleen. The number of mast cells differs between species and tissues. Mast cells tend to be localised in perivascular structures in organs which are potential ports of entry for foreign agents (such as the skin, lungs and gut). They form a heterogeneous population of cells which differ in their development, mediator content, ultrastructure and ability to interact with the local environment (Church et al, 1994). Mast cells have the capacity to migrate and proliferate. They have been found in increased numbers in the tissues of patients with a wide variety of diseases. In clinical medicine, interest mainly focuses on mast cells (as they carry IgE receptors) in allergic diseases, but mast cells are also linked to chronic inflammatory disorders such as psoriasis, cell-mediated immunological disorders and neoplastic events. Mast-cell-specific disorders, such as urticaria pigmentosa and systemic mastocytosis, are characterised by extraordinary increases in the number of mast cells.

Mast cells are characterised by their metachromatic cyto-

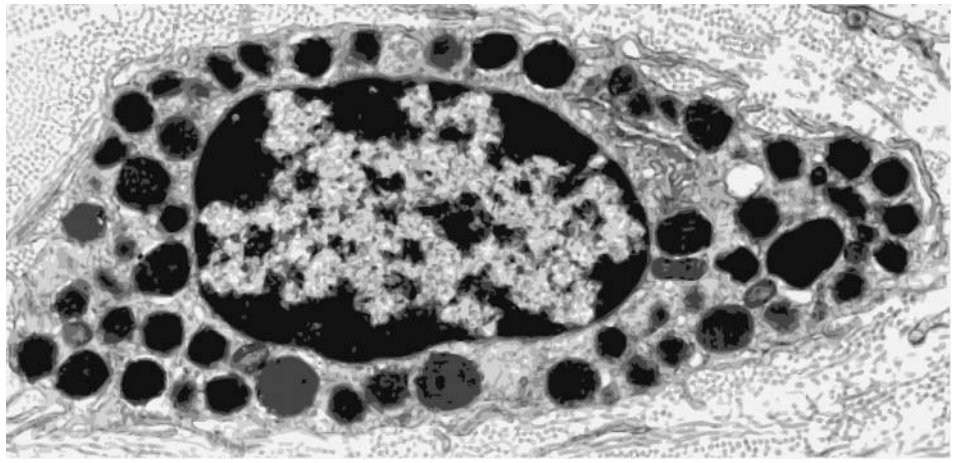

Fig. 1. Electron microscopic visualisation of human skin mast cell in breast biopsy. Note the homogeneously dense cytoplasmic granules. The centrally positioned nucleus is monolobed with partially condensed chromatin. The entire cell is encased in interstitial collagen. ×16,000. (From Dvorak and Kissell, 1991, with permission.)

plasmic granules containing various sulphated glycosaminoglycans and mediators which can be categorised into three groups (see Metcalfe et al, 1992). 1) *Preformed secretory granule mediators* are synthesised and stored until activation. They include histamine, heparin or chondroitin sulphate E, different types of proteinase, chemotactic peptide and plasminogen activator. 2) *Lipid mediators.* The activation of mast cells initiates the de novo synthesis of certain lipid-derived substances, of which the arachidonic acid metabolites are of special importance (prostaglandins, thromboxanes, leucotrienes and hydroperoxyeicosatetrae-enoic acid). 3) *Cytokines.* These are protein or glucoprotein molecules which exert their biological effects by interacting with specific cytokine receptors located on the surface of target cells. Mast cells have been shown to produce several cytokines, including interferons, interleukins and tumour necrosis factors (Plaut et al, 1989; Wodnar-Filipowicz et al, 1989; Bradding et al, 1992, 1993, 1994, 1995; Gordon and Galli, 1990).

Human mast cells (MC) are classified by their neutral protease content: MCT (localised mainly on mucosal surfaces) contain only tryptase, whereas MCTC (localised in connective tissue) also contain chymase and other proteases (Schwartz et al, 1987; Irani et al, 1986, 1989, 1991; Schechter et al, 1990). Cutaneous mast cells, which mainly consist of MCTC (Irani et al, 1989), are normally present perivascularly in the dermis and subcutaneous tissue. Their density ranges from 7,000/mm^3 to 20,000/mm^3 (Mikhail and Miller-Milinska, 1964) and is greatest at the dermo-epidermal junction (Cowen et al, 1979).

The degranulation of human mast cells can be induced by a variety of immunological and non-immunological secretagogues. The most-studied secretagogue, IgE, binds to high-affinity Fc_ϵ-receptors on mast cells. When cross-linked by anti-IgE or by specific antigen, IgE induces degranulation through the aggregation of Fc_ϵ-receptors (Ishizaka et al, 1972). Numerous other agents of potential importance in human disease also cause mast-cell degranulation. Opioids cause cutaneous mast-cell degranulation through a naloxone-sensitive receptor (Casale et al, 1984). Various complement by-products, such as C5a, C4a, and C3a, cause receptor-mediated mast-cell degranulation (el Lati et al, 1994). Inflammatory cell-derived, histamine-releasing factors produced by different types of immunocompetent cell (White et al, 1989), certain drugs or physical stimuli, such as heat, sunlight, cold, vibration and pressure, may also cause mast-cell degranulation (Casale et al, 1986; Huston et al, 1986; Kaplan, 1983; Keahey et al, 1987; Soter et al, 1979). Acute psychological stress induces corticotropin-releasing, hormone-dependent skin mast-cell degranulation (Theoharides et al, 1998), a mechanism which may be involved in dermatological diseases that are precipitated or aggravated by stress. A number of peptides, including substance P (SP), somatostatin and vasoactive intestinal peptide (VIP), are also known to induce the secretion of human skin mast cells (Eberz et al, 1987; Benyon et al, 1989; Church et al, 1989). These latter agents are potentially important as links between the nervous and the immune system.

The stimulation of mast cells induces the release of substances with a variety of biological functions. Mast-cell mediators function as growth factors for a variety of cells such as fibroblasts and endothelial cells and the stimulation of fibroblast collagen synthesis (Norrby, 1973; Marks et al, 1986; Folkman and Kagsbrun, 1987). Mast cells interact with other cells, particularly hematopoetic and mesenchymal cells (for review see Bradding, 1996). Fibroblasts have been shown to influence mast-cell differentiation and granule synthesis, whereas mast-cell mediators can stimulate the mitogenic and synthetic activity of fibroblasts (Davidson et al, 1983). Furthermore, fibroblasts and endothelial cells may interact via transgranulation (Greenberg and Burnstock, 1983). These interrelationships are thought to be important in the pathogenesis of a variety of cutaneous diseases.

1.3.2 Mast cells in psoriatic skin

In the dermis, the psoriatic plaque contains an increased number of mast cells in both developing and mature lesions (Steigleder GK, 1966; Cox 1976; Töyry et al, 1988; Naukkarinen et al, 1991). The mast cells are located immediately below the epidermis in the near vicinity of the dilated papillary capillaries and superficial dermal plexus (Cox, 1976; Töyry et al, 1988). When present in the epidermis, mast cells accompanied by extruded mast-cell granules and tryptase activity can be seen in mature psoriatic lesions (Brody, 1984a, Harvima et al, 1989). Mast cells in involved skin appear to be hyperreactive to various types of stimulation (Petersen et al, 1998). In early psoriatic eruptions, the first morphological observation is the

degranulation of mast cells (Brody, 1984b; Schubert and Christophers, 1985) which exhibit abnormal proliferation from the fourth day after the induction of Koebner's phenomenon (Toruniowa and Jablonska, 1988). Furthermore, the MCT subpopulation of mast cells is increased, whereas the number of MCTC cells is decreased in psoriatic lesions (Harvima et al, 1990). It has been suggested that this may lead to an imbalance in the biochemical regulatory systems (Harvima et al, 1990). The consequences of such a derangement are not known.

Little is known about the pathophysiological role of mast cells in psoriasis. Theoretically, mast-cell mediators could contribute to the development of the inflammatory psoriatic infiltrate and/or play a role in immunostimulation and inflammation in the lesion.

1.4 Histamine

1.4.1 General aspects

Histamine (1H-imidazole-4-ethanamine, molecular weight 111), the best known mast-cell product, is widely distributed in various tissues in the human body. The name derives from the Greek word for tissue, histos. Histamine is formed by the decarboxylation of the essential amino acid histidine, a reaction which is catalysed in mammalian tissue by histidine decarboxylase (Schayer, 1963). In most tissues, the bulk of the amine is concentrated and stored in the secretory granules of mast cells. In the blood, histamine is stored in the circulating counterpart of the mast cell, the basophil leucocyte. It is apparent that the tissues rapidly degrade histamine *in vivo* (Reily and Schayer, 1970). Once outside the secretory granule, histamine is sequentially catabolized by two main routes; one involves deamination mediated by diamine oxidase and the other the methylation of the imidazole ring mediated by histidine-N-methyltransferase (Schayer, 1966). The various metabolites have little or no physiological activity and are excreted in free or conjugated form in the urine.

The concentration of histamine is high in the skin, lungs and gastric mucosa. In the skin, the histamine content was found to be three and 10 microg/g in the deeper dermis and the dermo-epidermal junction respectively (Riley and West, 1956), which corresponds to the different mast-cell densities in superficial and deep layers of the human skin (Cowan et al, 1979). The analysis of histamine has been accomplished in microdialysates obtained from normal human skin (Anderson et al, 1992; Petersen et al, 1992b, 1994, 1996a, 1996b, 1997b, 1997d). In two recently published studies (Church et al, 1997; Pedersen et al, 1997c) the mean interstitial histamine concentration in unprovoked healthy skin obtained using the calibated microdialysis technique was estimated at 5 and 18 nmol/l respectively.

Histamine is the classical mediator of acute inflammation. Until recently, histamine was regarded primarily as an effector molecule in the immediate hypersensitivity response, but much evidence has been accumulated to suggest that histamine is also released in stress reactions, inflammatory responses and traumatic events. Histamine acts on a large variety of different cell types, including smooth-muscle cells, neurones, endocrine and exocrine cells, blood cells and cells in the immune system. The biological effects are mediated through the binding to and activation of specific histamine receptors. Three subtypes, designated H1, H2 and H3, have been identified. Responses mediated by these receptors are antagonised by antihistamines. The best-documented effects are vasodilatation, increased capillary permeability (H1 and H2) and gastric secretion (H2). It has been suggested that the molecule participates in the regulation of the immune system, acting on H1 and H2 receptors expressed by immunoactive cells (Rocklin, 1990), wound healing (Fitzpatrick and Fisher, 1982) and angiogenesis (Marks, 1986). Various studies indicate that H3 receptors may participate in the regulation of the activity of non-adrenergic, non-cholinergic nerves (Schwartz et al, 1990). Additionally, histamine and presumably other mast-cell mediators are able to sensitise and/or activate afferent sensory neurones (Handwerker, 1991) to release neurotransmitters which give rise to afferent nerve-mediated vasodilatation and protein exudation (Jancsó et al, 1985). An interaction between histamine and nerve fibres in the skin was first suggested by Lewis (1927), who investigated in detail the sequence of events evoked by stroking the human skin with a blunt instrument, events which were found to mimic the intradermal injection of histamine into the skin. The reaction, known as the Lewis triple response is an important mechanism in the understanding of the potential interaction between nerves, mast cells and blood flow. This interaction will be discussed in greater detail below.

1.4.2 Histamine in psoriatic skin

The histamine concentration in lesional and non-lesional psoriatic tissue and suction blisters has been reported to be similar to that in healthy skin (Juhlin et al, 1967; Ruzicka et al, 1986). In stratum papillare, fluorimetric determinations of histamine have revealed a two-fold increase (Juhlin et al, 1967) in lesional skin compared with non-lesional skin. Recently, a similar increase in the interstitial histamine concentration was detected in psoriatic eruptions compared with that in lesion-free skin (Petersen et al, 1998). Histamine production in lesional skin *in vitro* has been found to be increased five- to ten-fold (Graham et al, 1981). It has been suggested by Nielsen (1991) that histamine could participate in the induction of immunological and vascular changes observed in psoriatic skin, since both H1 and H2 receptors are expressed by immunoactive cells and vascular structures. The treatment of psoriatic patients with histamine antagonists or inhibitors of mast-cell degranulation has not been conclusive. Both significant (Kristensen et al, 1995; Petersen et al, 1998) and insignificant (van de Kerkhof et al, 1995; Zonneveld et al, 1997) improvements have been reported.

1.5 Sensory innervation of the skin

In addition to its numerous other functions, the skin is the largest and most versatile organ of sensation. The skin is innervated by around one million afferent nerve fibres (Sinclair, 1973).

The main nerve trunk entering the subdermal fat tissue divides into smaller bundles which run out in a horizontal plane to form a branching network from which fibres ascend, usually accompanying blood vessels, to form a web of interlacing nerves in the superficial dermis. The sensory nerves and their nerve endings are not fixed, immutable structures but constantly undergo degeneration and regeneration: they are thus in a dynamic state and are capable of adaptation.

Sensory endings are of two main kinds: corpuscular, which embrace non-nervous elements, and free endings, which do not (Sinclair, 1973, 1981). Corpuscular endings can, in turn, be sub-

divided into encapsulated (represented by Pacinian corpuscle, Golgi-Mazzoni corpuscle, Krause end bulb and Ruffini) and non-encapsulated receptors, exemplified by Merkel's touch spot. 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 (Cauna, 1973, 1976).

How do the miscellaneous receptors provide cutaneous sensation? The classical theory comes from von Frey, who proposed that the modality of cutaneous sensation has four qualities, each subserved by a specific type of receptor: touch by Meissner's corpuscles (in addition to hair follicles), warmth by Ruffini end-organs, cold by Krause end-bulbs and pain by free nerve endings. Certain features of the classical theory were clearly untenable. The sensory nerves and nerve endings constitute an immense complex in which the relationship between structure and function is still disputed.

With the development of modern electro-physiological techniques, it has been possible to affirm the existence of functionally-specific afferent units. An afferent unit has been defined as "the complex formed by an afferent axon, its receptor terminal or endings and any associated non-nervous structural elements" (Iggo and Young, 1975). Two major categories of afferent units have been clearly established, mechanoreceptors and thermoreceptors (Iggo 1973, 1974). A third category, pain receptors (nociceptors), responds to high-threshold mechanical or thermal stimulation and/or to chemicals, inflammation and ischemia. These sensory modalities (experienced as diffuse burning and/or pain) are mostly conducted in unmyelinated fibres (C-fibres) which have the slowest conduction velocities (<1 m/sec). The nociceptors may be divided into three main groups according to their response to various excitatory stimuli, e.g. mechanosensitive, thermosensitive and polymodal nociceptors (Sinclair, 1981). Also other subgroups of pain receptors have been identified. Recently, selective nociceptors specifically responsive to itch were detected (Schmelz et al, 1997a). Most pain receptors respond to several excitants and are thus polymodal nociceptors. The exact biochemical structure and molecular reactions leading to the generation of action potentials in the nociceptor have not yet been elucidated.

The activation of small myelinated A-delta fibres or unmyelinated C-fibres is known to induce pain at frequencies above 1Hz (Gybels et al, 1979), whereas at lower frequencies the subject often reports an itch (Torebjörk, 1974). The fact that the threshold at which the nociceptors begin to fire does not correspond with the threshold for pain perception (Bromm et al, 1984), suggests 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 (Zimmermann, 1979). This idea is in line with the finding that low frequency discharge in C-fibres of the skin in the range of 0.1 – 1 Hz resulted in vasodilatation without any sensation of warmth or pain (Lynn and Shakhanbeh, 1988). This indicates that the afferent C-fibres may have at least a dual mode of operation; as afferent sensory nerves to conduct peripheral nociceptive information to the central nervous system and as efferent nerves participating in the local regulation of vascular function via axon reflexes causing antidromic vasodilatation. The latter function will be discussed in greater detail in the following section

1.6 Interaction between afferent nerves, vessels and mast cells

1.6.1 Early studies

By tradition, the sensory nervous system was thought to function as a receptive and afferent system, which activates effector systems by reflexes and thereby enables the organism to react to changes in the external and internal environment to maintain homeostasis. This concept was questioned, however, when it was found that the antidromic stimulation of the peripheral stump of transected sensory nerves induced vasodilatation and other signs of inflammation in the cutaneous innervation zone. This observation was first made more than a hundred years ago (Stricker, 1876) and was then confirmed by many investigators. Bayliss (1901) suggested that the observed vasodilatation was produced by impulses conveyed along afferent fibres in an efferent direction and he introduced the term "antidromic" to describe this type of conduction.

A few years later, another type of vasodilatation, which was induced by the application of the chemical irritant mustard oil, was also found to be dependent on the integrity of the nerves to the region, since nerve degeneration or local anaesthesia inhibited the vasodilatation. Bruce (1913) suggested that the vasodilatation in the response to mustard oil was the result of an "axon reflex", a term introduced previously by Langley and Anderson (1894) with reference to reflexes which it was suggested took place entirely within a single neurone. A physiological role for antidromic vasodilatation was implicit in the suggestion that the same nervous elements as those responsible for antidromic vasodilatation were involved in the flare component of the inflammation (Langley, 1923).

1.6.2 The triple response

A major contribution to this field was made by Lewis (1927), who investigated in detail the sequence of events evoked by firmly stroking the human skin with a blunt instrument. Lewis called the response "the triple response" and described three major components:

1. The red reaction due to local dilatation of minute vessels in the skin
2. The flare – the widespread dilatation of neighbouring arterioles brought about by a local neural mechanism
3. The weal – a local increase in the permeability of the blood vessels.

From his observations, Lewis postulated that a cluster of axon collaterals in the skin mediated the flare by local axon reflexes through the release of a neurotransmitter onto small vessels. Noting the similarity between the triple response to noxious stimulation and the effects of histamine pricked into the skin, he proposed that the triple response was mediated by the release of a histamine-like substance which he referred to as the "H-substance". As a result of his studies, Lewis (1937a, b) proposed that a separate network existed, the "nocifensor system", which released neurotransmitters from its terminals. Lewis proposed that the process was initiated by local nerves, but that maintenance did not require the continuous flow of nerve impulses. The full importance of the concept of a specialised nocifensor system has only been recognised during the last two decades. In addition to their sensory function, the nocifensor neurones help to maintain homeostasis in the face of irritation or trauma to the tissue. This protective role is aided by appropriate changes in the microcirculation at the site of stimulation, changes which can propagate from the site of

irritation via collaterals of the afferent fibres to an enlarged area surrounding the site of the trauma.

In 1953, Celander and Folkow obtained the first experimental evidence that the sensory fibres responsible for nociception were involved in antidromic vasodilatation. In the same year, substance P was suggested as a potential transmitter (Lembeck, 1953). When pure synthetic SP became available during the 1970s, this proposal was verified indirectly (Lembeck et al, 1977). The first suggestion that mast cells might form a link between axons and blood vessels was made by Kiernan (1971) to explain the observation that electrical stimulation of the great auricular nerve of the rat caused an increase in the proportion of degranulated skin mast cells. Denervation, but not cervical sympathectomy, abolished this effect (Kiernan, 1972).

1.6.3 The use of capsaicin

Red pepper, which contains capsaicin, is commonly associated with the sensation of hot, burning and turning red, as hyperaemia ensues when tissue is brought in contact with capsaicin. From the 1940s and onwards, N Jancsó and co-workers studied reactions induced by capsaicin. They noted that capsaicin-induced responses could not be elicited in insensitive areas of the skin, i.e. in skin without nociceptive afferent neural activity. Later work confirmed that afferent neurones which are involved in local vascular control are sensitive to capsaicin, a property which has facilitated anatomical, neurochemical and functional studies of these control mechanisms. Afferent neurones are excited by acute capsaicin application, while they are desensitised, defunctionalized or even ablated by long-term treatment (Holzer, 1991). The neurogenic action of capsaicin is described in more detail in the methodological section.

Jancsó et al, (1967) were the first to provide direct evidence that the antidromic stimulation of sensory nerves elicited increased vascular permeability. Since hyperaemia and increased vascular permeability are key traits of inflammation, the phenomenon was called "neurogenic inflammation". The term thus denotes an inflammatory response by the skin, dependent on an intact nociceptive sensory innervation (Jancsó et al, 1967). Hyperaemia and increased vascular permeability facilitate the delivery of macromolecules and immunocompetent leucocytes to the tissue and promote inflammatory responses to tissue irritation and injury. Vasoactive afferent neurones thus represent a system of "first line defence" against trauma (Lembeck, 1983). Furthermore, arteriolar dilatation may be regarded as a measure to ensure that protective hyperaemia takes place not only in the challenged tissue but also in a safety margin (Holzer, 1988). In contrast, these afferents may induce hyperalgesia during chronic inflammation and thereby cause a perpetuation of the inflammatory process.

1.6.4 Peptides influencing the cutaneous circulation

The antidromal, afferent-nerve-mediated control of the vascular function is due to the release of certain vasoactive peptide transmitters from the peripheral part and endings of afferent nociceptor neurones (Szolcsányi, 1984; Chahl, 1988; Maggi and Meli, 1988; Holzer, 1988, 1992) and the binding of these peptides to juxtapositioned cells. SP was the first neuropeptide suggested as the mediator of antidromic vasodilatation and neurogenic inflammation, but other peptides have also been found in primary afferent neurones, including calcitonin gene-related peptide (CGRP), neurokinin A, somatostatin, galanin, cholecystokinin, dynorphin, pituitary adenylate cyclase-activating peptide and vasoactive intestinal polypeptide (VIP) (Holzer, 1991, 1992; Harmar and Lutz, 1994; Wallengren, 1997). These peptides coexist in varying combinations and it is likely that this heterogeneity in the chemical coding of afferent neurones reflects functional heterogeneity (Gibbins et al, 1987; Mayer and Baldi, 1991).

After synthesis in the somata of the afferent neurones, the peptides are transported towards both the central and peripheral endings where they are released in response to stimulation (Keen et al, 1982). The released transmitters have to diffuse some distance before they reach the appropriate receptors on the effector cells. In human skin, small arteries, arterioles and capillaries are innervated by thin nerve fibres (Gibbins et al, 1987; Wallengren et al, 1987) in which CGRP co-exists with SP and somatostatin (Dalsgaard et al, 1983; Hartschuh et al, 1983; Björklund et al, 1986). These peptidergic afferents also send axons into the dermis and epidermis and can be observed in close correlation to mast cells in psoriatic lesions (Naukkarinen et al, 1991, 1993, 1996) and sweat glands (Dahlsgaard et al, 1983, Tanio et al, 1987). VIP and related peptides are found in nerves which terminate more deeply in normal skin. The nerves containing VIP mainly end on arterial vessels and sweat glands (Hartschuh et al, 1983; Wallengren et al, 1987). There is rapid enzymatic degradation of interstitial neuropeptides in the tissue and the interstitial concentration of SP-like reactivity (measured in microdialysates obtained from human skin) appears to be below picomolar concentrations (Petersen et al, 1997a).

The co-existence of CGRP and SP in perivascular afferent nerve fibres, their release upon nerve stimulation, the presence of SP and CGRP receptors on cutaneous blood vessels and their high activity in dilating cutaneous blood vessels raise the possibility that they are mediators of arteriolar dilatation. There are two major mechanisms by which SP may induce vasodilatation in the skin, either by direct action on vascular smooth muscle, or indirectly by releasing secondary mediators from endothelial cells, cells of the immune system and/or from mast cells. Neuropeptides such as SP may induce the release of histamine from human skin mast cells *in vitro* and *in vivo* (Church et al, 1989; Petersen et al, 1994) and histamine might therefore act as a secondary mediator. CGRP evokes vasodilatation through direct action on vascular smooth muscle. CGRP-evoked hyperaemia in the skin remains unaltered after blocking nitric oxide synthesis in the rat (Ralevic et al, 1992) and after the application of indomethacin (Brain et al, 1985), acetylsalicylic acid (Fuller et al, 1987), histamine H-1 antagonists (Wallengren and Håkansson, 1987) and local anaesthetics (Wallengren et al, 1987).

Neuropeptides also exert a number of immunological effects by stimulating immunocompetent cells to produce cytokines (Lotz et al, 1988), prostaglandins, leucotrienes and thromboxanes (Hartung et al, 1986), and stimulating the adhesion of leucocytes to the vessel wall and their migration into the inflamed tissue of the skin (Helme and Andrew, 1985). Leucocyte-derived factors are not only able to influence vessel diameter and permeability, they can also stimulate afferent nerve fibres or augment their excitability (Ferreira et al, 1988; Cohen and Perl, 1990).

1.6.5 Peptidergic sensory nerves and neuropeptides in psoriatic skin

The epidermis and dermis of the psoriatic lesion are more densely innervated with sensory nerve fibres than either lesion-

free psoriatic or normal skin (Naukkarinen et al, 1989). Slight increases in SP- and VIP-containing nerves in the dermal papillae are found in Koebner-positive specimens, seven days after tape stripping of non-lesional psoriatic skin, and clear increases have been documented in mature psoriatic plaque (Naukkarinen et al, 1993). SP-containing fibres are also more prominent in the epidermis of lesional skin than non-lesional skin. Using immunohistochemical stains, increased SP positivity (Al'Abadie et al, 1992; Naukkarinen et al, 1989, 1993), as well as VIP positivity (Al'Abadie et al, 1992), have been demonstrated in the papillary part of psoriatic plaque.

When determined biochemically, increased levels of SP have been detected in suction blister fluid (Wallengren et al, 1987) and the lesional tissue extracts (Eedy et al, 1991) of psoriatic patients. Reduced (Pincelli et al, 1992) and unchanged (Anand et al, 1991) levels of SP have also been measured histochemically in psoriatic lesions as compared to non-lesional or normal skin. Significantly more VIP has been found in extracts of psoriatic plaque than in those of non-lesional skin (Eedy et al, 1991; Pincelli et al, 1992).

The psoriatic inflammatory infiltrate may lead to the sensitisation of afferent nociceptive C-fibre endings and an increase in their firing, which may in turn lead to an increase in the release of neuropeptides (Lynn, 1977, 1988). Since the activation of thin (A-delta or C) nerve fibres induces pain at frequencies above 1Hz (Gybels et al, 1979) whereas lower frequencies produce an itch (Torebjörk, 1974), the clinical experience that psoriatic plaque is often pruritic during exacerbation (Newbold, 1977; Gupta et al, 1988) may be explained in theory by the low-frequency activation of afferent C-fibres, which might at the same time induce local vasodilatation and histamine release. Thereby C-fibres may constitute a pathophysiological factor in the development of the psoriatic lesion. If the activity of peripheral nerve fibres plays a role in the mediation of a psoriatic lesion, the denervation of lesional skin would be expected to induce a local remission of the disease. Prompt remission of chronic plaque has also been reported after nerve sectioning, followed by the reappearance of lesions after recovery of the cutaneous sensation (Dewing, 1971; Farber et al, 1986). In pruritic psoriatic plaque, the topical application of capsaicin, which is known to deplete nerve endings of neuropeptides (Buck and Burks, 1986; Brain et al, 1993), improves the pruritus and reduces the psoriasis severity score after four weeks of treatment (Bernstein et al, 1986; Ellis et al, 1993). The symmetrical distribution of psoriatic eruptions may also indicate the involvement of neurogenic control mechanisms (Farber et al, 1986, 1990).

1.6.6 Interactions – mast cells and afferent nerve fibres

The existence of functional interactions between nerves and mast cells and the possible role of these communications in the pathogenesis of allergy and inflammation have been discussed intensively in recent decades. The morphological and functional prerequisites for interactions between mast cells and nerves are present in animal tissue (Newson et al, 1983; Skofitsch et al, 1985; Bienenstock et al, 1987; Alving, 1990; Bienenstock et al, 1991; Eedy, 1993; Foreman, 1993; McKay and Bienenstock, 1994; Marshall and Waserman, 1995).

Mast cells and histamine in neurogenic inflammations in the skin of rodents have been studied thoroughly. The salient points of these studies are that the key signs of neurogenic inflammation, vasodilatation and plasma extravasation, are significantly abrogated by histamine H1-receptor antagonists (Hägermark et al, 1978), by histamine depletion induced by compound 48/80 (Lembeck and Holzer, 1979) and in mast-cell-deficient animals (Yano et al, 1989). The stimulation of afferent nerve fibres in rodents and the topical application of capsaicin in human skin induce mast-cell degranulation (Kiernan, 1971; Bunker et al, 1991). In contrast, H1-receptor antagonists do not inhibit the flare reaction evoked by capsaicin (Barnes et al, 1986), nor does capsaicin induce histamine release in normal healthy skin (Petersen et al, 1997a). The nature of the sensory nerve-mast cell association is not known. True membrane contact is not needed, merely a sufficiently close association for the mediators to remain undegraded by enzymes before reaching their receptors on the target cells. SP has been widely studied in this context. Intradermal injections cause weal and flare reactions (Foreman et al, 1983). They release histamine from human skin mast cells in vitro (Benyon et al, 1987; Ebertz et al, 1987), from mast cells originating from different anatomic sites in the rodent in vivo (Shanahan et al, 1985) and in humans when injected intradermally (Barnes et al, 1986; Petersen et al, 1994). Human skin mast-cell degranulation may also be induced by VIP and somatostatin in vitro (Eberz et al, 1987; Benyon et al, 1989 Church et al, 1989). The doses of SP required to induce histamine release are high (around 1μmol/l); however, given the close proximity of mast cells and nerves *in vivo*, it is possible that these concentrations of neuropeptides may be obtained locally within the tissue. SP at picomolar levels has been shown to trigger electrical responses in mast cells without degranulation (Janiszewski et al, 1994), a finding which implies that the mast cell may be induced by neuropeptides to produce pro-inflammatory substances without the occurrence of classical degranulation.

A functional interaction between mast cells and nerves in psoriatic plaque has been suggested (Farber et al, 1986; Naukkarinen et al, 1991, 1993, 1996). Morphological contact between mast cells and both SP-containing and CGRP-containing nerves in lesional psoriatic skin is much more frequent in the basement membrane zone and in the papillary dermis as compared to the corresponding areas in non-lesional skin (Naukkarinen et al, 1996). There is little experimental data which directly implicates mast cell-nerve interaction as a mechanism of disease modulation in man.

1.7 The axon-reflex hypothesis

Since the spread of flare involves nerve conduction, it is commonly thought to be the result of an axon reflex. When one axon branch is activated by an irritant stimulus, nerve impulses travel, not only centrally, but at branching points they also pass antidromically and may then reach terminals close to arterioles. If so, the release of vasodilator transmitters may cause arteriolar dilatation. The axon-reflex concept implies that the area of flare is determined by the size of neurovascular units made up by the collateral networks (receptive fields) of individual afferent nerve fibres and the area of arterioles innervated by these collateral networks (Lewis, 1927; Helme and McKernan, 1985). In the human hand, there is a good correlation between the size of the C-fibre receptive field and the area of the flare responses to electrical skin stimulation (Wårdell et al, 1993b). In other regions of the human skin, however, discrepancies between the two parameters have been observed (Lynn, 1988). This inconsistency may be related in part to the

experimental design. Different types of stimulus (capsaicin, histamine, electrical stimulation, radiant heat) may stimulate different populations of afferent nerves and thereby induce varying flare size (Treede, 1992). Inconsistent with the axon-reflex hypothesis, however, is the unexplained delay (3-5 sec) in the spread of the flare, which is slower than would be expected if the rate of spread were determined only by conduction and transmission delays in unmyelinated nerve fibres (Magerl et al, 1987).

The lack of neurophysiological evidence for the occurrence of axon reflexes and some of the inconsistencies of the axon-reflex concept have fostered alternative hypotheses to explain the spread of the flare (Lembeck and Gamse, 1982; Lynn, 1988; Lynn and Cotsell, 1991). These hypotheses assume that the flare results from electrical and/or chemical coupling between different afferent nerve fibres. The chemical coupling theory (Lynn, 1988) suggests that the spread of the flare is accomplished by a cascade of histamine release from mast cells, the activation of adjacent afferent nerve fibres by histamine, the release of vasodilator transmitters from these fibres, followed by further histamine release. However, the site of the action of histamine in the flare pathway is not entirely clear. Most reports indicate that histamine is involved in the initiation of the flare response but does not itself act as a vasodilator mediator in the flare. This inference is based on a close correlation between the peptide-induced flare and its corresponding histamine-releasing capacity from mast cells. It would thus appear that peptides induce flare only if they are able to release histamine from cutaneous mast cells. However, histamine antagonists do not inhibit the flare initiated by electrical stimulation or capsaicin (Wallengren and Håkansson, 1992), but they do inhibit the flare reactions induced by endothelin-1 (Bunker et al, 1992). The discrepancy between these findings is not well understood, but it may well be that the role of histamine in the flare response depends on the nature of the flare-inducing stimuli or on the particular afferent nerve fibres which are activated by these stimuli (Bunker et al, 1992). Mast cells appear to be involved as target cells for the released sensory neuropeptides, but the role of mast cells as constituting an essential step in the cascade of an axon-reflex arrangement has not yet been substantiated (Kowalski and Kaliner, 1988).

Whatever the mechanism of the spreading flare, the relatively sharp margin of the arteriolar dilatation implies that the propagation of excitation must be limited in some way (Lynn and Cotsell, 1991). In further delineating the mechanisms of the flare, it will be important to consider the anatomical arrangement of the terminal arterial system in the skin, because the organisation of the vascular system itself may be an important factor in determining the size of the flare (Handwerker, 1992)

1.8 The axon-reflex hypothesis in psoriatic skin – summary of observations and presentation of the working hypothesis

Multiple observations are compatible with the existence of a peptidergic neurogenic mechanism in the pathophysiology of psoriatic plaque. The prompt remission of chronic eruptions have been reported after nerve sectioning, followed by the reappearance of lesions after recovery of the cutaneous sensation (Dewing, 1971; Farber et al, 1986). The symmetrical distribution of psoriatic lesions may indicate the involvement of neurogenic control mechanisms (Farber et al, 1986). Lesional psoriatic skin contains an increased number of sensory C-fibres (Naukkarinen et al, 1989), an increased concentration of SP (Wallengren et al, 1986) and increased histochemical SP reactivity. The topical application of capsaicin, which is known to deplete the nerve endings of neuropeptides (Buck and Burks, 1986; Brain et al, 1993), improves psoriasis (Bernstein et al, 1986; Ellis et al, 1993). There are also increases in the number of mast cells and contact points between mast cells and nerves (Naukkarinen et al, 1991, 1993, 1996). Mast-cell proteases, such as tryptase and chymase, may play a role in neurogenic inflammation in psoriasis because of their neuropeptide-degrading activity, which appears to be impaired in lesional skin (Harvima et al, 1989, 1990, 1993). The finding of an increase in SP in endothelial cells in affected skin (Naukkarinen et al, 1993) may indicate an action on psoriatic vessels. The functional significance of these observations is not known.

Furthermore, the psoriatic inflammatory infiltrate may lead to the sensitisation of afferent nociceptive C-fibre endings and an increase in their firing. The clinical experience that psoriatic plaque is often pruritic during exacerbation may be explained by the low-frequence activation of afferent C-fibres, which may induce local vasodilatation and histamine release.

Against the background of this anatomical and physiological information, we suggest the possibility of local, low-frequency activation of afferent C-fibres in psoriatic plaque. Our hypothesis is that afferent nociceptive C-fibre endings in lesional skin are sensitised and activated by psoriatic inflammatory mediators and that this, via an axon-reflex mechanism, leads to an increase in the local release of neuropeptides. Action potentials in afferent C and A-delta fibre endings may therefore release SP/CGRP, which themselves have dilating effects and, in addition, induce mast cell degranulation/histamine release which causes further vasodilatation. A pathological axon-reflex mechanism may thus both contribute to the high blood flow and stimulate histamine release in lesional psoriatic skin. The axon-reflex hypothesis is presented in Fig. 2. To test this hypothesis, we have conducted a series of functional studies to elucidate whether local neurogenic peptidergic mechanisms are of pathophysiological importance for the maintenance of

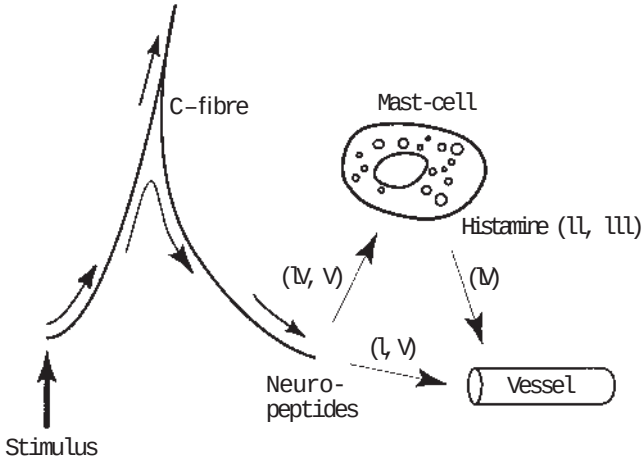

Fig. 2. A simplified schematic representation of the axon-reflex hypothesis in lesional skin. The Roman numbers refer to the papers included in this thesis. The location of the numbers indicate the mechanism examined.

the increased blood flow and whether such mechanisms may induce histamine release in psoriatic plaque

2. AIMS OF THE STUDY

In order to elucidate whether a pathological axon-reflex mechanism contributes to the high blood flow and stimulates histamine release in lesional psoriatic skin, we have tested different aspects of the axon-reflex hypothesis:

1. **The relationship between local neural activity and perfusion in psoriatic skin**
2. **Interstitial histamine concentration and release in psoriatic skin and their effects on blood flow.**
   To validate the experimental technique (the microdialysis method), measurements of histamine were preceded by a study of structural and physiological perturbations induced by the microdialysis catheter and by an evaluation of the retrodialysis calibration procedure used to estimate the true interstitial substrate concentration.
3. **The relationship between local neural activity and histamine release in psoriatic skin**

3. SUBJECTS

Patients (aged 20 – 65 y) with active untreated psoriatic plaque without antipsoriatic treatment for at least one week (Paper I) or two months (Papers III, IV and V) were recruited from the outpatient clinic at the Department of Dermatology, Sahlgrenska University Hospital. Healthy subjects (Papers I and II) were recruited from the general population in Göteborg by local advertisements. All the subjects were Caucasians. Patients and healthy controls were not taking any medication. None of the subjects had other known diseases and none of them was a smoker. On the last days before the investigation, the patients were instructed to apply neutral emollient (Essex Cream®; Schering-Plough, NJ, US) to the skin to prevent extensive scaling. Clinical characteristics of the subjects included in the different studies are presented in Table I.

Disease activity was assessed according to the Psoriasis Area and Severity Index (PASI) method (Papers III, IV and V) or percentage involvement (Paper I) by a dermatologist. In the PASI scoring system, four main body areas were assessed: the head (H), upper extremities (U), trunk (T) and lower extremities (L), corresponding to 10%, 20%, 30% and 40% of the body area respectively. The area of psoriatic involvement in these four main areas was given a numerical value (A) from 0 – 6 corresponding to 0% to 100% involvement. In each area, erythema (E), infiltration (I) and desquamation (D) were assessed on a scale of 0 to 4 (Fredriksson and Pettersson, 1978). The PASI score (0 to 72) was then calculated from the following formula:

$$\mathrm{PASI} = 0.1(\mathrm{EH}+\mathrm{IH}+\mathrm{DH})\mathrm{AH} + 0.2(\mathrm{EU}+\mathrm{IU}+\mathrm{DU})\mathrm{AU} + 0.3(\mathrm{ET}+\mathrm{IT}+\mathrm{DT})\mathrm{AT} + 0.4(\mathrm{EL}+\mathrm{IL}+\mathrm{DL})\mathrm{AL}$$

The studies were approved by the Human Ethics and Isotope Committees at the University of Göteborg. All the participants gave their informed consent.

4. METHODOLOGICAL CONSIDERATIONS

4.1 Microdialysis

4.1.1 General background

A microdialysis method for measurements of substances in the interstitial water space was introduced in 1972 by Delgado et al. The technique was further developed by Ungerstedt and Pycock (1974), for measurements in the brain tissue of experimental animals and was later adapted for measurements in human tissue (Lönnroth et al, 1987). Due to the development of in situ calibration techniques, measurements can be made of the actual concentrations of different substances in the interstitial fluid (Lönnroth et al, 1987). The microdialysis technique was introduced in dermatological research by Anderson et al (1991) and has subsequently also been used by other groups. The method has been mainly applied in two fields of dermatological research, namely the examination of the penetration of drugs through the skin (Anderson et al, 1991; Hegemann et al, 1995; Groth, 1996) and analyses of histamine concentration in healthy (Anderson et al, 1992) and diseased human skin (Andersson et al, 1995). The latter are often performed together with different types of provocation (Petersen et al, 1992b, 1994, 1995, 1996a, 1996b, 1997a, 1997b, 1997d; Petersen and Skov, 1995; Huttunen et al, 1996; Perzanowska et al, 1996).

In the biosciences, dialysis (from the Greek: to separate) means the diffusion of small molecules and water through a semipermeable membrane. A dialysis catheter (also named fibre, membrane, probe) is a hollow tubular membrane which is permeable to small molecules and water. After being placed in the tissue, the dialysis catheter is perfused by a solute (per-

Table I. *Clinical characteristics of the subjects included in the different studies*

Study	Group	Number of subjects	Sex (M/F)	Age (mean ± SD)	PASI (mean ± SD)	Age at first appearence of psoriasis (mean ± SD)
Paper I	Psoriatic	12##	10/2	47 ± 16		21 ± 12
Paper I	Control	9	6/3	37 ± 5		
Paper II	Control	15	11/4	28 ± 5		
Paper III	Psoriatic	23	16/7	42 ± 10	14 ± 6	23 ± 11
Paper IV	Psoriatic	21#	14/7	43 ± 10	16 ± 8	23 ± 11
Paper V	Psoriatic	22#	16/6	44 ± 12	13 ± 7	26 ± 13

Ten of the psoriatic patients from Paper III also participated in Papers IV and V.
After publication, another three subjects were examined. These subjects (two males and one female, mean age 48 y) are not shown in the table, but they are included in the Results section.

fusate). A concentration gradient is thereby created along the length of the membrane. Compounds in the medium surrounding the catheter, which are present in higher concentrations than in the perfusate, diffuse into the dialysis fibre and, conversely, when perfused with a higher concentration than that in the surrounding medium, substances leave the catheter and enter the medium. The tube is thus perfused with a fluid which equilibrates with the outside by diffusion in both directions. In low-rate perfusion (1 – 10 μl/min), the flux of the molecules across the membrane obeys Fick's first law

$$j = -D \times a \times dC \times dy^{-1}$$

The law states that the flux j, i.e. the number of molecules per unit of time which cross a unit area, is proportional to the concentration gradient dC/dy. D is the diffusion coefficient of a certain molecule and a the porosity, i.e. in this context the composition of the extracellular space. At zero flow rate, the concentration in the solution equals the concentration of small molecules in the interstitium after a short period of diffusion in both directions. The perfusion results in incomplete equilibration between the solution and the surrounding interstitial medium. As a result, the concentration in the dialysate is not equal to the interstitial concentration but only represents a fraction of the true interstitial concentration. This fraction (ratio or relative recovery) is defined as the relative concentration of a particular molecule in the dialysate and is expressed as a percentage of the concentration in the medium surrounding the probe:

$$(\text{dialysate concentration} / \text{interstitial concentration}) \times 100$$

The in vivo recovery is affected by a number of factors which fall into three main groups.

1. *The physical and chemical properties of the microdialysis catheter.* This includes the size of the pores in the wall of the membrane, the chemical and physical interactions between the probe material and the solutes (Hsiao et al, 1990) and the length of the dialysis membrane (Hamberger et al, 1983).
2. *The perfusion medium.* The flow rate of the perfusate (Benveniste, 1989), the tonicity and composition of the perfusion medium (should resemble and ideally be identical to the medium surrounding the probe except for the substrate of interest).
3. *Tissue morphology and physiological properties in the microdialysis area.* This includes tissue pressure, tissue tortuosity and temperature.

Both anatomical and analytical factors limit the opportunity to reach 100% recovery. A change in dialysate concentration may reflect a change in the interstitial concentration, as well as a change in the relative recovery over the microdialysis probe. Accordingly, dialysate concentrations must be corrected by probe recoveries before changes in dialysate concentrations can be interpreted as being due to changes in interstitial concentrations. Estimation of the recovery of each probe in vivo makes it possible to calculate the interstitial substrate concentration from the dialysate value.

Several experimental and theoretical methods have been developed for the quantitative estimation of the unbound extracellular concentrations from the dialysate concentrations. The flow rate method is based on a mathematical description of the relationship between the extracellular concentration and the flow rate using non-linear regression and extrapolation to zero perfusate flow leading to 100% recovery and the exact interstitial concentration (Jacobson et al, 1985). The method requires long experiments because measurements must be made with several flow rates. The slow flow method is based on the idea that a low perfusion rate (<0.1 μl/min) results in a dialysate concentration close to the tissue concentration (Wages et al, 1986). The disadvantages of this method are the low perfusion rate which makes it difficult to measure rapid changes in concentration after provocations and problems maintaining a constant low flow rate. With the difference method or the point of no-net-flux method, the compound of interest is added to the perfusate at lower and higher concentrations than the expected extracellular concentration of the same substance. There is a concentration level or point at which the concentrations inside and outside the membrane are equal and no net diffusion occurs because no concentration gradients exists. The perfusate concentration at the point of no net flux, represents the extracellular concentration (Lönnroth et al, 1987).

Retrodialysis calibration (or internal reference recovery) is based on the idea that the relative loss of the indicator substance from the microdialysis fluid to the interstitial water equals its relative recovery (Larsson, 1991; Jansson et al, 1994b; Lönnroth and Strindberg, 1995). This can be achieved by perfusing the microdialysis probe with an indicator substance which resembles the substance of interest but can be distinguished in the analysis. The indicator substance may be an (radioactive) isotope of the substance, or a different molecule with the same diffusion characteristics. The indicator substance may be part of the perfusate during the whole experiment. Accordingly, all dialysate solutes can be corrected by their own recovery. The internal reference technique has been used in human subcutaneous tissue (Lönnroth and Strindberg, 1995) but has not been evaluated for application in human skin.

4.1.2 Microdialysis – application in the present study

Microdialysis catheter and perfusate Sterile linear microdialysis membranes, composed of acrylonitrile and sodium methallyl sulfonate copolymer with a molecular weight cut-off of 50 kD, outer diameter 0.30 mm and a length available for diffusion of 30 mm (Hospal, Lyon, France) were used in all the microdialysis experiments. The catheters were perfused at a rate of 2.5 μl/min with isotonic saline and 1.5 mmol/l of glucose. The position of the microdialysis catheter in the dermis is shown in Fig. 3.

Insertion procedure Subjects were supine in a room kept at 27°C. The membranes were guided through cannulae (0.6×30 mm) inserted intracutaneously in the forearm, thigh and/or abdomen without prior local anaesthesia. After being placed in the dermis, each end of the sterile microdialysis catheter was glued with cyanoacrylate to polyethylene tubes (inner diameter 0.33 mm, length 50 mm). The dialysate outlet was led directly into the vial. The inlet was connected via polyethylene tubing to a precision pump (CMA/100, CMA/Microdialysis AB, Stockholm, Sweden) which perfused the microdialysis catheter at a constant rate. Collection of the microdialysates started 80 min after insertion. Each dialysate was collected for 10 – 20 min. The dialysates were stored at – 20°C until analysis, four to eight weeks later.

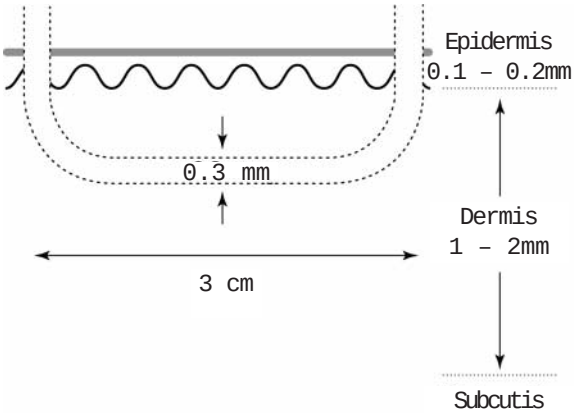

Fig. 3. Schematic model of the microdialysis catheter positioned in the dermis.

In vivo calibration and estimation of recovery The equilibration calibration method was used in Paper II to estimate dermal interstitial lactate and pyruvate concentrations. To find the concentration that led to no net flux, the microdialysis catheter was perfused by three to four different concentrations of lactate (0 – 1,200 μmol/l) and pyruvate (0 – 150 μmol/l). The net change in substrate concentration in the dialysate (i.e. dialysate minus perfusate concentration) was plotted against the perfusate concentration. Linear regression was used to estimate the substrate concentration in the perfusate which led to unchanged concentrations after microdialysis. The slope of the line corresponded to the recovery. The extracellular concentration was calculated when the correlation coefficient was 0.9 or greater.

The retrodialysis technique permits relatively rapid equilibration and is therefore a preferable method in experiments in which concentration changes are expected. Since the method had not been evaluated for measurements in human skin, the retrodialysis technique was validated for estimations of interstitial concentrations in the dermis (Paper II). The glucose concentration in plasma, which is similar to that in skin (Petersen et al, 1992a), was used as a reference and compared with the interstitial concentration obtained using the retrodialysis technique. Retrodialysis was performed in the fasting state and after the oral ingestion of 75 g of glucose. 3H-labelled glucose (approximately 100 cpm/μl) was added to the perfusate and used as an internal marker. A prerequisite for the correct estimation of recovery by retrodialysis is that the relative loss, or the efflux fraction (EF) of the indicator substance from the microdialysis probe, equals the relative recovery (r) of the substance of interest from the interstitial space (i.e. that FE = r). The efflux fraction (EF in per cent) was calculated as

$$[(C^*in - C^*out)/C^*in] \times 100$$

where C*in is the radioactivity (cpm/ml) in the perfusate and C*out is the radioactivity in the dialysate. When the recovery was estimated, the interstitial concentration of interest was calculated according to the formula

$$[(d - p) \times 1/r] + p$$

where d is the dialysate concentration and p is the known perfusate concentration.

In Papers III, IV and V the retrodialysis method was used to estimate the true interstitial histamine concentrations in lesional and non-lesional psoriatic skin. In these studies, 3H-labelled histamine (60-120 cpm per ml perfusate) was used as an internal reference.

Assessment of microdialysis catheter depth and catheter-induced structural and physiological perturbations

1) *Measurements of catheter depth.* The distance from the epidermal surface to the cannula used for insertion of the dialysis membrane was determined by ultrasound measurements (Dermascan A, Cortex Technology, Hadsund, Denmark or Toshiba SSA-270A, Toshiba, Japan) in psoriatic and healthy skin respectively (Papers II, III and V). Three measurement positions were selected, two positions about 0.5 cm from the inlet-outlet of the catheter and one above the central part of the membrane.

2) *Histological examination* Punch biopsies (3 or 4 mm in diameter) were taken from lesional psoriatic and healthy skin, without prior local anaesthesia (Papers II and III). The biopsy was taken from the middle third of a line indicating the position of the underlying catheter (which was withdrawn before the punch). The biopsy was fixed in 5% formaldehyde and embedded in paraffin wax. Three μm thick sections were cut perpendicular to the line and stained with hematoxylineosin, Van Giesen and PAS to determine the presence of inflammatory cells, extravasation of blood cells and oedema.

3) *The lactate/pyruvate (L/P) ratio* in a tissue may provide a measure of its redox state. The concentration of lactate in the extracellular water space in human dermal tissue is higher in the skin (wet weight) than in the blood (Johnson and Fusaro, 1972), despite the high cutaneous blood supply which effectively removes lactate from the skin. However, the rich blood supply of the skin is confined to the dermis, whereas the epidermis obtains its nutrients and discharges its waste products by diffusion across the dermo-epidermal interface. This may be one reason why the epidermis derives much of its energy from anaerobic glycolysis, i.e. conversion of glucose to lactate, instead of the aerobic process which converts glucose to pyruvate and then CO_2 and water.

In most tissues, the L/P ratio is about 10 – 13. In the skin, it may be even higher (Johnson and Fusaro, 1972). Ischemia induces anaerobic glycolysis with the production of lactate, which consequently increases the L/P ratio. In the present study (Paper II), this ratio was estimated to examine whether ischemia prevailed in the tissue adjacent to the microdialysis catheter.

4) *Skin perfusion adjacent to the microdialysis catheter* The insertion of the microdialysis catheter induces vasodilatation adjacent to the probe (Anderson et al, 1994). This vasodilation response increases when the probe is inserted without prior local anaesthesia (Groth and Serup, 1998). In the present study, the microdialysis probes were inserted without preceding local anaesthesia. To evaluate the vascular response induced by the insertion and by the presence of the catheter in the tissue, skin perfusion was measured repeatedly adjacent to the inserted microdialysis fibre (Paper II).

4.2 Assessment of skin blood flow

4.2.1 Laser Doppler perfusion – general comments

The Doppler principle was first described by JC Doppler in 1842. The development of the laser in the 1950s made the optical Doppler effect applicable for measurements of the velocity of fluid flow. The first laser Doppler for measurements of blood

flow was made by Riva et al (1972) and technical improvements were reported by Stern in 1975, Holloway and Watkins in 1977 and Nilsson et al, in 1980.

The unique properties of laser light are its high intensity, low divergence, extreme monochromaticity and great coherence (the fact that light waves have the same frequency, phase, amplitude and direction). When laser light penetrates a tissue, some photons are absorbed, while others are backscattered. Light that is scattered by moving particles is Doppler-shifted by an amount proportional to the velocity. Consequently, in the presence of moving cells, a fraction of the light is Doppler-shifted. This fraction can be detected by the recording fibres and transformed to an electrical output signal which provides a measure of the perfusion in the tissue. In laser Doppler flowmetry, perfusion is defined as the product of the average velocity and the concentration of moving blood cells in the illuminated volume. The perfusion is expressed in volts (V) or arbitrary units (AU).

Laser Doppler flowmetry requires that several factors are taken into account when interpreting the results. The biological zero value (i.e. measurement obtained distal to suprasystolic occlusion) is often larger than the instrumental electrical zero; a phenomenon which may be related to Brownian movements of blood cells and other kinds of movement including vessel wall motions in the recorded area (Caspary et al, 1988; Öberg et al, 1990). The measurement depth, which depends on the wavelength of the laser light, the separation between transmitting and recording optical fibres and tissue characteristics, is not fully known. In lesional skin, the change in the epidermal-dermal structure and the increased blood content, could modify the penetration of the laser beam, for example.

An He-Ne laser-Doppler flowmeter measures the perfusion from the reticular and superficial parts of dermis and may include AV anastomoses. The perfusion signal corresponds fairly well to the underlying microvascular density (Rendell et al, 1998) and may distinguish between different types of microvascular vessel in the skin (Braverman et al, 1990). Measurements in adjacent skin areas reveal that there are large spatial variations in skin perfusion (Tenland et al, 1983). Measurements with the conventional "one point" laser Doppler flowmeter (LDF) which records perfusion in a small tissue volume may therefore not be representative. With the development of the laser Doppler perfusion imager (LDI), it has been possible to record the tissue perfusion pattern in large areas and to estimate a representative mean perfusion (Wårdell et al, 1993a). The spatial variation in perfusion in an image is persistant in successive recordings; i.e. the high perfusion areas in one image coincide well with the high perfusion areas in another image of the same skin site (Wårdell et al, 1994).

During the last 20 years, the method has been applied in numerous studies in dermatological research to characterise flow responses to different types of stimulus such as pharmacological agents, treatments and irritative and allergic manipulations.

4.2.2 Laser Doppler equipment used in the present study

Perfusion in lesional, non-lesional (Papers I, IV and V) and healthy skin (Papers I and II) was mapped with a laser Doppler perfusion imager (PIMI, Lisca Development AB, Linköping, Sweden) which uses a low-power He-Ne laser beam (wavelength 632.5 nm). Using a computer-controlled optical scanner, the laser light was directed step by step in a rectangular pattern over the skin surface. The scanning procedure took about four minutes for an area of skin of approximately 10×10 cm/5×5 cm (maximum/minimum step length = approximately 1.5/0.8 mm) with a distance of 17 cm between the scanner head and the skin. At each of the 4,096 measurement sites, the light penetrated the skin to a median sampling depth of about 0.2 – 0.3 mm (Wårdell et al, 1993a, Jakobsson and Nilsson, 1993). The backscattered Doppler broadened light was detected by a photodetector positioned in the scanner head. The photocurrent was processed to form an output signal proportional to tissue perfusion. To eliminate interference from ambient light, the electric light was switched off during the scanning procedure. From the perfusion values, a colour-coded image was generated and presented on a monitor. Further image processing and data analyses were made in the displayed image, which was presented on a colour plotter (HP Paint Jet, Hewlett Packard, USA).

The borders of the measured skin area were marked before measurement. These marks, which were depicted in grey (= zero perfusion) on the colour-coded image, were used as fixed points during the analysis. The mean perfusion was calculated from 100 to 200 pixels in the different studies. When the perfusion was measured repeatedly in the same area, virtually identical image areas were used to calculate the perfusion changes with time.

4.2.3 ^{133}Xenon clearance – general comments

To measure the blood flow, Kety (1951) introduced the principle of injecting radioactive isotopes of inert gases into a tissue and recording the washout curve. Since inert gases are fat-soluble, they easily penetrate capillary membranes and a rapid diffusion equilibrium is established between the tissue and the blood. This tissue-to-blood partition coefficient (called λ, Kety, 1951) is determined by estimating the physical solubility of the tracer in the specific tissue and the blood. The solubility is calculated from the content of fat, water and protein in a tissue and the solubility of the tracer in these structures. ^{133}Xenon (^{133}Xe), the most frequently-used radioactive tracer for blood flow measurements, emits β and γ irradiation and has a physical half life of 5.3 days. Measurement of cutaneous blood flow by ^{133}Xe washout method was elaborated by Sejrsen (1971). In cutaneous tissue, the ^{133}Xe partition coefficient has been estimated at 0.7 ml/g in healthy skin (Sejrsen, 1971) and at 1.2 ml/g in psoriatic eruptions (Klemp and Staberg, 1983, 1985). The recording of the ^{133}Xe washout from a tissue permits blood flow measurements in absolute units (ml per min per 100 g tissue). Blood flow (BF) is calculated according to the formula $BF = k \times \lambda \times 100$, where λ is the tissue-to-blood partition coefficient for ^{133}Xe at equilibrium and k is the slope of the washout curve. Clinical applications of the method in dermatology is reviewed by Kristensen and Petersen, (1992).

4.2.4 ^{133}Xenon clearance – application in the present study

In psoriatic and uninvolved skin (Papers III, IV and V), 0.1 ml of ^{133}Xe mixture (5 mCi/ml saline, Mallinckrodt, Petten, The Netherlands) was applied epicutaneously for five minutes under a 20 mm-thick Mylar membrane, mounted on a one-cm-wide ring-shaped adhesive surface. Clearance was monitored continuously with a 2×2 inch NaI (thallium) detector (Harshoaw QS, Nemours, France) covered by a cylindrical collimator and coupled to a multichannel analyser (Canberra model 1,000, Canberra Industries, Meriden, USA). The detec-

tor was placed 15–20 cm from the ^{133}Xe depot and only the photopeak of 81 keV was registered.

Dermal blood flow (DBF) was calculated according to the formula presented above. Partition coefficients of 0.7 ml/g and 1.2 ml/g were used in uninvolved and lesional psoriatic skin respectively and these values were assumed to be constant during the investigation. Experimental values of k were estimated as $(\ln y_1 - \ln y_2) \times T^{-1}$, where y_1 and y_2 are the ^{133}Xe counts five and 20 min after removal of the ^{133}Xe solution and T is the time interval between y_1 and $y_2 = 15$ min.

4.2.5 Comparison between ^{133}Xenon clearance and laser Doppler perfusion measurements

The laser Doppler technique has been compared with other established methods for measurements of blood flow. However, the methods partly measure different phenomena. The laser Doppler records changes in skin perfusion (i.e. the product of moving blood cells and their average velocity), whereas the blood flow measured by the clearance of ^{133}Xe expresses the total flow volume but not the amount or specific velocity of moving cells. Nevertheless, comparisons of LDF and ^{133}Xe washout measurements produce a fairly good correlation in methodological studies in the skin (Engelhart and Kristensen, 1983; Kastrup et al, 1987). Klemp and Stabeck (1985) found a linear correlation between measurements in psoriatic lesions but not in uninvolved psoriatic or healthy skin. They suggested that LDF provides a rough estimate of blood flow only in cutaneous tissue with high capillary perfusion (Klemp and Stabeck, 1985).

4.2.6 Blood flow/perfusion in psoriatic skin

The erythema in lesional skin is caused by an increase in the amount of blood (Bull et al, 1992) and an increase in blood flow (Klemp and Staberg, 1983, 1985) in the anatomically altered and dilated superficial vascular bed. The lesional blood flow obtained by the ^{133}Xe washout method is two to five times higher than that in non-lesional skin (Nyfors and Rothenborg, 1970; Klemp and Staberg, 1983, 1985). The interindividual variability ranges from about four (Nyfors and Rothenborg, 1970) to 90 ml per min per 100 g tissue (Klemp and Staberg, 1983, 1985). The difference can be partly explained by the methodological differences between the studies. Moreover, the subcutaneous blood flow is augmented in lesional but not non-lesional psoriatic skin (Klemp, 1987). Additionally, blood flow is increased in non-lesional skin compared with that in healthy skin (Klemp and Staberg, 1983, 1985).

In lesional psoriatic skin, LDF and scanning laser Doppler measurements of perfusion are increased eight-12-fold (Klemp and Staberg, 1985) and three-to five-fold respectively (Speight et al, 1993; Auer et al, 1994) compared with values in non-lesional skin. The high perfusion gradually approaches that of uninvolved skin during different types of anti-psoriatic treatment (Staberg and Klemp, 1984; Klemp and Staberg, 1985; Khan et al, 1987; Berardesca et al, 1994; Auer et al, 1994). A linear correlation has been obtained between blood flow in lesional skin and the clinical psoriasis index (Staberg and Klemp, 1984). Detailed studies of perfusion outside the visible plaque edge have identified a 2 to 4 mm rim of increased perfusion around the plaque (Speight et al, 1993), which is further increased at the active edge (Hull et al, 1989). It has been suggested that the increase in flow is the first event in the development of a plaque (Hull et al, 1989). The high perfusion in psoriatic plaque is probably due to a greater number of moving blood cells in superficial vessels and an enhanced blood flow rate. Optical effects, such as reduced scattering and increased sampling depth of the laser beam in the acantotic lesional tissue, may contribute to this increase in perfusion.

4.3 Net histamine release

The interstitial concentration of histamine depends on the supply i.e. release from intracellular sources and the clearance caused by metabolism and net release into the circulation. A constant interstitial substrate concentration is maintained as long as the clearance of histamine equals the supply. The net release is influenced by factors such as number, type and localisation of mast cells as well as the release trigging signal and the distance to the vessel, the density and permeability of vessels, the rate of degradation and the local blood flow. A reduced outflow to plasma from the interstitial compartment due to a decrease in blood flow leads to a passive increase in the interstitial substrate concentration.

Estimation of the net release of histamine makes it possible to study histamine release in relation to different types of stimulus. Calculations of the net release of substrates such as lactate have been made in subcutaneous (Jansson et al, 1994a) and dermal tissue in humans (Jansson et al, 1996). The latter study revealed a high lactate-releasing capacity in the skin indicative of an effective lactate-forming ability; a finding which is in accordance with data from animal experiments and *in vitro* studies (Johnson and Fusaro, 1972).

4.3.1 Permeability surface area product (PS)

The capillary wall is interposed between the major compartments of the extracellular space (plasma and interstitial fluid) and allows material in the stirred plasma compartment to reach the interstitial phase and vica versa. The distance dissolved substances must travel is variable. In most tissues, the longest diffusion distance is 20–50 μm. Material can cross the capillary wall by diffusion (which is the dominant mechanism), filtration and solvent drag, facilitated diffusion, active transport and vesicular transport. The capillary diffusion rates for molecules with different molecular weights, such as Cr-EDTA, sucrose, Na^+ and glucose, have been determined in various tissues. In order to convert capillary diffusion to capillary permeability, it is necessary to know the capillary surface area in the specific tissue.

The functional capillary surface area under given experimental conditions is difficult to estimate precisely. In the skin, a value of 3.7 ml per min per 100 g tissue has been obtained for Cr-EDTA (molecular weight 292, Paaske, 1976). In skeletal muscle, where the capillary structure is similar to that in the skin, the PS is about 5 and 4.6 ml per min per 100 g tissue for glucose (molecular weight 180) and Cr-EDTA respectively (Trap-Jensen and Lassen, 1970; Crone, 1963). This produces a predicted value of 4 ml per min per 100 g tissue for glucose in skin. Taking these aspects and the lower molecular weight of histamine into account, we used a PS-value of 5 ml per min per 100 g tissue in non-lesional psoriatic skin. In lesional psoriatic skin, the capillary surface area is increased about two-fold (Barton et al, 1992). An increase in the number of functioning capillaries reduces the intercapillary distance and thereby facilitates the diffusion. Additionally, the venous part of the capillary vessels in lesional skin is highly permeable (Braverman and Yen, 1974, 1977b). These structural changes

can further influence the PS value. Due to the two-fold increase in the capillary area, we anticipated the PS at 10 ml per min per 100 g tissue, but the possibility cannot be excluded that the value may be even higher. Thus, as the PS for small molecular substances has not been measured in psoriatic plaque the calculation of net histamine release should be interpreted with caution.

4.3.2 Calculations of net histamine release

Calculations of net histamine release were made according to Fick's principle; i.e. the net release of a substance from the tissue equals the extraction of the substance produced in tissue (E) multiplied by the plasma flow (Q).

$$\text{Net release} = E \times Q$$

Plasma flow was estimated from the blood flow measured by ^{133}Xe washout and compensated for the hematocrit. The extraction of histamine produced in tissue was calculated according to the formula

$$E = (I - A) \times (1 - e^{-PS/Q})$$

where I is the interstitial concentration of substrate, A is the arterial concentration and PS the capillary permeability-surface area product (Crone and Levitt, 1984). The converted expression of E was put into Fick's equation

$$\text{Net release (pmol per min per 100 g tissue)} = (I - A) \times (1 - e^{-PS/Q}) \times Q$$

Estimations of the net release of histamine have been made in the resting state (Paper III) and after different types of stimulus (Papers IV and V).

4.4 Chemical analysis

The histamine concentration in dialysates and plasma was analysed using a radioimmunoassay technique (Immunotech S.A, Marseilles, France) based on the competition between the histamine in the sample and the tracer for the binding sites on an antibody-coated tube. Histamine is too small completely to occupy the binding site on the antibody. High-affinity monoclonal antibodies directed against modified (acylated) histamine have therefore been obtained. The histamine in the sample must be derivatized in the same manner as the histamine in the iodinised tracer. This is achieved in a reproducible manner using an acylating reagent at a slightly alkaline pH. When added to the antibody-coated tubes, the sample containing the acylated histamine and the ^{125}I-acylated-histamine compete to bind to a limited number of antibody sites. After incubation, each tube is emptied to remove the unbound components. The bound radioactivity is then measured in a gamma counter. The counts per minute are inversely proportional to the concentration of histamine in the sample. The concentration is calculated on the basis of a standard curve obtained using standards supplied with the kit. The detection limit of the method is 0.2 nmol/l. The cross-reactivity of the acylated forms of methyl-histamine, histidine and serotonin is upp to 0.0069%, 0.0004% and 0.0001% respectively.

Pyruvate in dialysates and capillary blood was determined using a spectrophotometric micro method (Czok and Lampreckt, 1970).

Glucose and lactate concentrations in dialysates and plasma were analysed using immobilised enzyme sensor technology (YSI 2700 Select, YSI Inc., Yellow Springs, USA).

The radioactivity in the dialysates was measured by a liquid scintillation counter (1900 CA, Canberra Nuclear, Canberra Industries Inc., Meriden, USA).

4.5 Statistical analysis

Standard methods were used to calculate the means, standard deviations (SD) and standard error of the mean (SEM). The data are presented as the mean $\pm$ SEM (Paper I, III, IV and V) or mean $\pm$ SD (Paper II). Student's t-test for paired or unpaired data was used to evaluate statistically significant differences between groups. Differences in concentration between sequential measurements were tested with ANOVA, followed by Duncan's multiple range test. Linear regression analysis was performed according to the least squares method. Linear correlations were calculated according to Pearson. p-values of <0.05 were regarded as significant.

5. EXPERIMENTAL DESIGN AND PROCEDURE

In the following section the aim, design and experimental procedure of each paper will be presented. General comments about interventions are made in connection to the study in question.

5.1 Paper I

Aim As the first test of the axon-reflex hypothesis, a study was made of the effect on skin perfusion of the topical anaesthesia of the psoriatic lesion. The rationale was that, if the high blood flow in the plaque were an axon-reflex effect, the anaesthesia should reduce the perfusion. Since topical anaesthesia may induce circulatory effects not related to nervous inhibition, the effect of surface anaesthesia on ultraviolet light-B erythema, an erythema of known (non-neural) origin, was examined. Conduction anaesthesia of nerves to psoriatic lesions was also performed to examine whether efferent sympathetic impulses from the central nervous system could contribute to the high blood flow. In the following section the mechanisms behind the anaesthetic effects and ultraviolet light-B erythema will be briefly discussed.

5.1.1 Local and regional anaesthesia

Neural excitation is associated with the depolarisation and repolarisation of the cell membrane. The depolarisation phase results from an increase in membrane permeability to sodium ions, whereas the main determinant of the repolarization phase is an increase in potassium conductance. Peripheral nerves are enclosed in connective tissue sheets which act as barriers through which local anaesthetic agents must diffuse. Local anaesthetic agents inhibit neural excitation by impeding sodium conductance, thereby preventing membrane depolarisation. Most clinically useful local anaesthetic agents exist in a charged and uncharged (base) form in solution. The uncharged base form diffuses more readily through neural sheets, while the charged form is mainly responsible for attachment to the membrane and the ultimate blockade of neural activity. The relative proportion of charged and uncharged forms depends on the pKa of the substance, the pH of the solution and the

pH at the application site. These factors affect onset time, anaesthetic potency and duration of action.

EMLA® (eutectic mixture of local anaesthetics; Astra, Södertälje, Sweden) is based on a mixture of lignocaine and prilocaine, both of which belong to the amino-amido group of substances. After 60 min of epicutaneous application under occlusion, the mixture produces dermal analgesia for several hours (Juhlin and Evers, 1990). EMLA-anaesthetised skin can become paler than the surrounding non-treated areas (Juhlin and Ehlers, 1990), a phenomenon which is not fully understood. The pallor is not directly associated with anaesthesia (Juhlin and Rollman, 1984) and could therefore be the result of direct effects on vessels (Juhlin and Ehlers, 1990).

Application In Papers I, IV and V, lignocaine-prilocaine cream (EMLA) and EMLA-placebo cream (containing the vehicle used in the EMLA cream) were applied epicutaneously in doses of about 0.4 ml/cm^2 and the application was occluded for 60 min. To this end, self-adhesive framed chambers were cut from a 2-mm-thick hydrocolloid dressing (Duoderm®; Convatec, Uxbridge, UK) and fastened to a skin region containing psoriatic and/or uninvolved skin. The topical agents were put into the chamber created in this way and covered by an occlusive dressing (Tegaderm®, 3M Health Care Ltd, MN, US). For regional skin anaesthesia, mepivacaine (Carbocain® 20 mg/ml, Astra) was used. The absence of pin-prick sensation was taken as a criterion for adequate anaesthesia.

5.1.2 Ultraviolet light-B induced erythema

A delayed erythema emerges a few hours after exposure to UVB. Cyclo-oxygenase products of arachidonic acid metabolism have been implicated in this response (Black et al, 1978a). The concentration of prostaglandin E2 in suction blister fluid following UVB irradiation increases after two hours, reaches a maximum at 24 h and falls to control levels by 48 h (Black et al, 1980). Indomethacin, a potent inhibitor of the enzyme prostaglandin synthetase, is known to reduce the intensity of UVB-induced erythema when administered topically, intradermally or orally (Snyder and Eaglstein, 1974; Black et al, 1978b; Farr and Diffey, 1986).

Application Erythema was induced in healthy subjects by exposure to UVB using a UVB irradiator consisting of two Philips TL 20/12W fluorescence tubes. The UV-B fluence rate measured at the skin surface level was 0.77 mW/cm^2 (IL 1350, Dexter Industrial Green, Newburyport, MA, USA). For each subject, the minimal erythema dose (MED, defined as the lowest dose in a series which produced a visible erythema 24 h after exposure) was measured ventrally on the forearm or upper arm and the subjects were then exposed to two MEDs in the same region on the other arm.

5.1.3 Experimental procedure

Protocol 1) Two or three rectangular self-adhesive frames (internal size 3 × 4 cm) were fastened to a skin region containing both psoriatic and uninvolved skin on the forearm or lower leg. The chambers created in this way were filled with lignocaine-prilocaine cream (10 subjects) and placebo (eight subjects) and occluded for one hour. The third chamber was empty and occluded in the same way (six subjects). Skin perfusion was measured before application and directly after the careful removal of the creams and then sequentially with a new measurement starting every fifth minute for 30 min after removal.

Regional anaesthesia of lesional skin was achieved by infiltrating 2 ml of mepivacaine around the dorsal cutaneous branches of the ulnar and radial nerves at the wrist in two subjects with psoriatic plaque on the dorsal surface of the hand. After the publication of Paper 1, the results from three additional subjects were obtained and they are presented together with previous findings. The absence of pin-prick sensation was taken as a criterion for adequate regional anaesthesia. Lignocaine-prilocaine cream was then applied in the anaesthetised region in the same way as described above. Perfusion measurements were performed before and after regional anaesthesia and after lignocaine-prilocaine cream had been applied for one hour and removed. The experimental sequence is presented in Fig. 4.

Protocol 2) Nine healthy subjects were exposed to UVB, corresponding to two MEDs, ventrally on the forearm or upper arm. A prostaglandin-synthase inhibitor (Indomethacin gel; MSD, NJ, US; 1%, 0.15 ml) and EMLA (0.15 ml) was applied under occlusion in separate circular hydrocolloid chambers with a diameter of 1 cm in the UVB-treated area either one or six hours after exposure. EMLA placebo (0.15 ml) was applied for one hour and occlusion only was applied for six hours. The perfusion were compared after the removal of the creams, i.e., seven hours after the UVB exposure.

5.2 Paper II

Aim As a second test of the axon-reflex hypothesis, we planned to measure the concentration and release of histamine in psoriatic skin; parameters which can be calculated from the content of histamine in dermal microdialysates. Certain aspects of the microdialysis technique that were of importance for the interpretation of the microdialysis studies had not been evaluated in

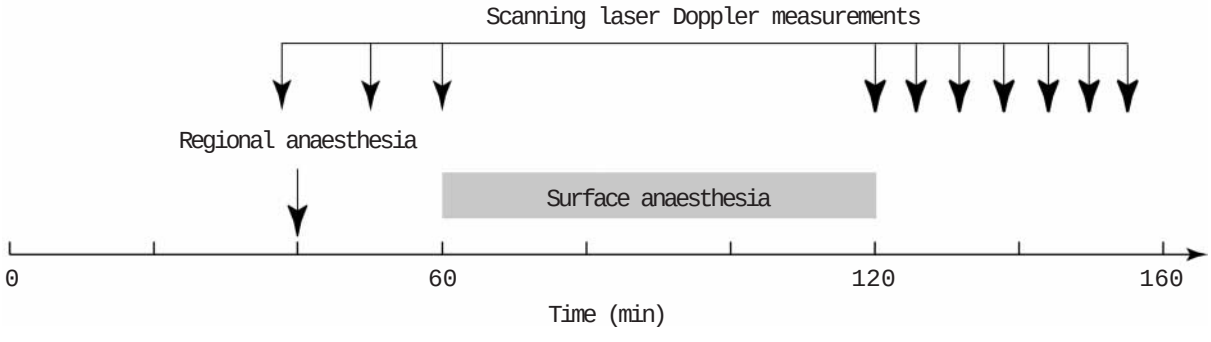

Fig. 4. Effects of topical anaesthesia on perfusion: The experimental sequence. Arrows indicate the time of the intervention or measurement.

the skin. The study described in this paper was therefore performed to characterise the structural and physiological perturbations induced by the microdialysis catheter and to validate the retrodialysis calibration method.

5.2.1 Experimental procedure

Subjects were investigated after an overnight fast to ensure stable glucose, lactate and pyruvate concentrations in plasma and tissue. In each subject, two or three microdialysis catheters were placed in the skin on the left dorsal forearm and uni- or bilaterally on the abdomen, 6 cm lateral to the umbilicus. Catheter depth was measured in six periumbilical and three forearm positions. Tissue perfusion was recorded 20-40-60-90 min, four and eight hours after catheter insertion ($n=8$) and compared to the skin perfusion 3 cm lateral to the probe in the respective image. Thirteen punch biopsies were taken from the microdialysis area 10 h after insertion in five subjects. The dialysate and plasma lactate, pyruvate and glucose concentrations were measured repeatedly in the fasting state for about four hours (for details, see Fig. 5). The equilibration calibration method was used to estimate dermal interstitial concentrations of lactate ($n=15$) and pyruvate ($n=5$). The lactate/pyruvate ratio surrounding the catheter at different depths was calculated.

The interstitial glucose concentration was estimated using the retrodialysis calibration technique, both in the steady state ($n=10$) and during a two-hour period after a standardised oral glucose intake (OGTT) of 75 g ($n=7$). To validate the retrodialysis method, the interstitial glucose concentrations were compared with the simultaneously obtained plasma glucose levels, which were assumed to be equal to the interstitial values.

5.3 Paper III

Aim In this study, the concentration/release of histamine in lesional skin was measured and compared with similar data from non-lesional skin. The rationale was that the presence of an abnormal axon reflex in psoriasis might lead to an increase in the concentration/release of histamine in the plaque.

5.3.1 Experimental procedure

Three microdialysis catheters were positioned in each of 23 subjects; two centrally and/or peripherally in psoriatic lesion(s) and one in adjacent lesion-free skin in the forearm (five subjects), the thigh (five subjects) or the abdomen (13 subjects). Catheter depth and the epidermal + dermal thickness were measured and punch biopsies were taken from the catheter areas ($n=6$). For the analysis of histamine, five (14 subjects) or two (nine subjects) dialysate samples were obtained from each catheter at 20-min intervals. DBF was recorded using the ^{133}Xe-washout technique in lesional and non-lesional skin ($n=9$). Arterialized venous plasma samples were taken ($n=9$) for measurements of histamine concentration. DBF and plasma and interstitial histamine concentrations were used to estimate the net histamine release in lesional and non-lesional psoriatic skin ($n=9$). The experimental sequence is presented in Fig. 6.

5.4 Paper IV

Aim Histamine release may be elicited by a number of agents. The rationale of this study was to examine whether the increase in histamine concentration/release found in Paper III could be reduced by the inhibition of the postulated axon-reflex mechanism in psoriatic plaque.

Local anaesthesia decreases the perfusion in lesional skin and could thereby influence the release of histamine into the blood stream and thus affect the interstitial concentration. To examine whether changes in blood flow influence the interstitial concentration of histamine, adrenalin was applied iontophoretically to separate microdialysis areas.

5.4.1 Iontophoresis

The passive diffusion of polar (ionic) compounds through the stratum corneum, the main barrier to the percutaneous

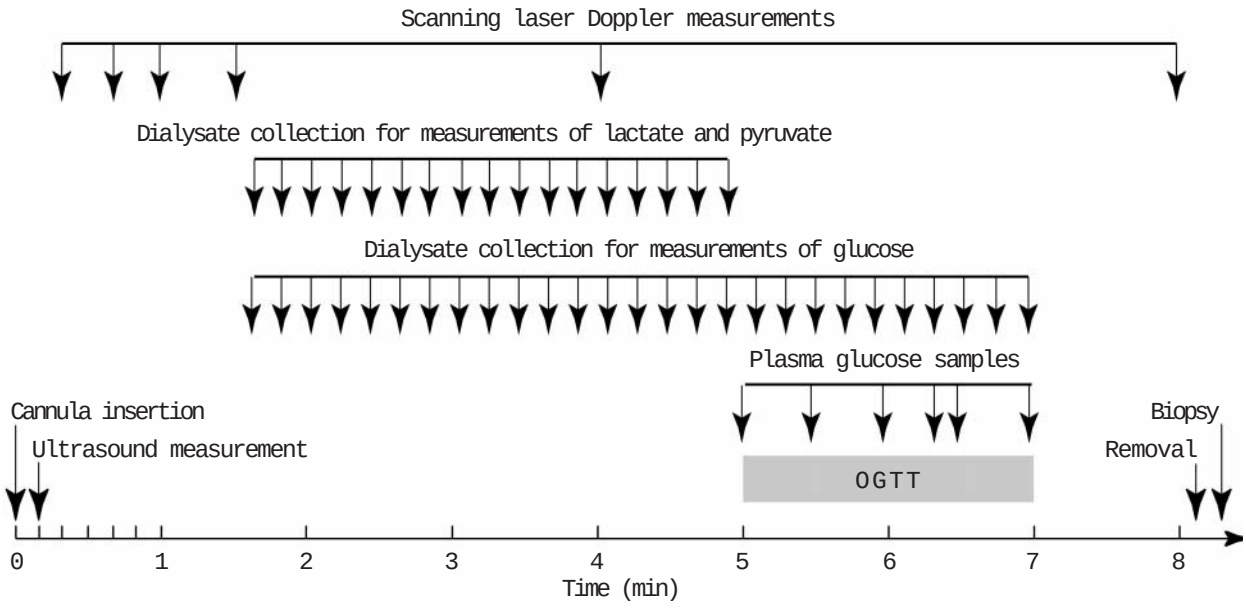

Fig. 5. Control of the microdialysis procedure: The experimental sequence. Arrows indicate the time of the intervention or measurement. OGTT is an oral glucose test.

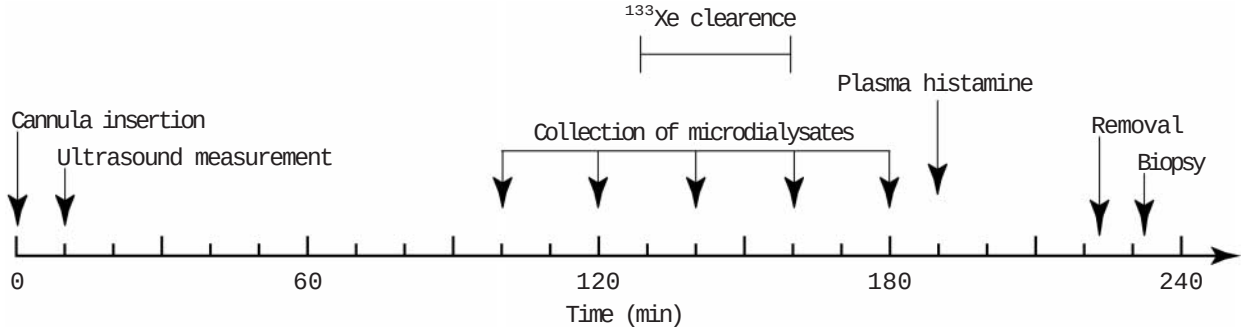

Fig. 6. Dermal histamine concentration and release: The experimental sequence. Arrows indicate the time of the intervention or measurement. Arrows connected to microdialysis sampling indicate the time of sample exchange.

absorption of a drug or chemical, is often slow and incomplete (Glass et al, 1980). Iontophoresis is the use of an electromotive force to enhance drug penetration through the stratum corneum. The electrical potential gradient used in iontophoresis may alter the permeability of the skin by inducing changes in the arrangement of lipid, protein and water molecules in the stratum corneum (Chien et al, 1989). Iontophoretic transport may occur through discrete pores in the skin (Grimnes, 1984; Cullander and Guy, 1991) or through hair follicles and sweat gland ducts which act as diffusion shunts, offering paths of reduced resistance for iontophoretic transport (Burnette and Marrero, 1986). Iontophoretic drug delivery increases with increasing drug concentrations and applied current and decreases in the presence of counterion salts such as potassium chloride (Behl, 1989). Moreover, an intact stratum corneum appears to be important for delivery (Behl, 1989).

Application Adrenalin tartrate (0.1%) in sterile, deionized water was applied iontophoretically with a current of 0.4 mA for one minute using the PF 480 PerIont Micro-pharmacology System (Perimed AB, Järfälla, Sweden). The cathode was placed 5 cm distally. The maximum quantity of adrenalin supplied to the skin was calculated at 0.045 mg.

5.4.2 Experimental procedure

In 12 subjects, two microdialysis catheters were inserted whereas three were inserted in nine subjects; one or two in psoriatic lesion(-s) and one in adjacent lesion-free skin on the forearm, thigh and abdomen. In three subjects, catheters were put into psoriatic skin only. Dialysates were collected at 20-minute intervals. After the collection of two to four samples, lignocaine-prilocaine cream was applied epicutaneously under occlusion for 60 min in the microdialysis catheter area in non-lesional (eight subjects) and lesional (19 subjects) skin. Perfusion in the microdialysis area was measured before and after topical anaesthesia ($n=10$). ^{133}Xe clearance and perfusion were measured in similar skin areas adjacent to the catheters before anaesthetic treatment ($n=10$) and a perfusion/blood flow ratio was calculated. In anaesthetised skin, DBF was not measured and DBF was therefore calculated using this ratio and the obtained perfusion value. Arterialized venous blood samples were taken from the cubital vein for the analysis of plasma histamine concentrations ($n=10$). The DBF and histamine concentrations in plasma and interstitium were used to estimate the net release of histamine before and after anaesthetic treatment in lesional skin.

To examine whether changes in blood flow influence the interstitial concentration of histamine, adrenalin was applied iontophoretically to separate microdialysis areas of forearm psoriatic skin in three randomly-selected patients. Perfusion and interstitial histamine concentrations were measured before and repetitively during a period of two hours after iontophoresis. The experimental sequence is presented in Fig. 7.

5.5 Paper V

Aim In this study, we investigated the effect of the topical application of capsaicin on histamine release and perfusion in psoriatic plaque. The rationale was that, if a local neural mechanism were active in the psoriatic plaque, we would expect capsaicin to cause acute increases in blood flow and histamine release, whereas prolonged capsaicin treatment should reduce blood flow to below the basal level.

5.5.1 Capsaicin – neurogenic effects

The majority of the afferent nerve fibres which induce neurogenic inflammation and dilatation in the skin can be manipulated pharmacologically by capsaicin, the active ingredient in hot peppers. When used as a spice, the hot sensation caused by peppers is due to the capsaicin-induced excitation of afferent nerve endings in the oral mucosa. Similar excitatory effects occur when the agent is applied to the skin, in the airways and in many visceral organs. With regard to sensory modality, capsaicin-sensitive afferent nerve fibres include chemoceptors, chemonociceptors, polymodal nociceptors and some warmth receptors (Holzer, 1991).

Capsaicin has two effects on afferent neurones; the acute administration of low doses of the drug stimulates afferent neurones, whereas systemic or repetitive doses cause the long-lasting defunctionalization of afferent neurones (Jancsó et al, 1987; Szolcsányi, 1990; Holzer, 1991). The extent of the injury depends on the dosage, the route of administration, the animal species and the age of the animal. In humans, the topical application of capsaicin to the skin produces a burning pain sensation, marked hyperalgesia and vasodilatation. After occlusive or repeated application, the skin becomes completely insensitive to capsaicin. At this time, vasodilatation is absent and cannot be induced by further capsaicin application, electrical stimulation or other types of provocation which normally evoke C-fibre-mediated dilatation (Bjerring and Arendt-Nielsen, 1990; Holzer, 1991). This excitoxic action of capsaicin on primary afferent neurones is mediated by specific binding sites (Szállási and Blumberg, 1990) associated with non-selective cation channels in the cell membrane and the intracellular accumulation of Ca^{2+} and Na^{+} is thought to be the major factor which determines the

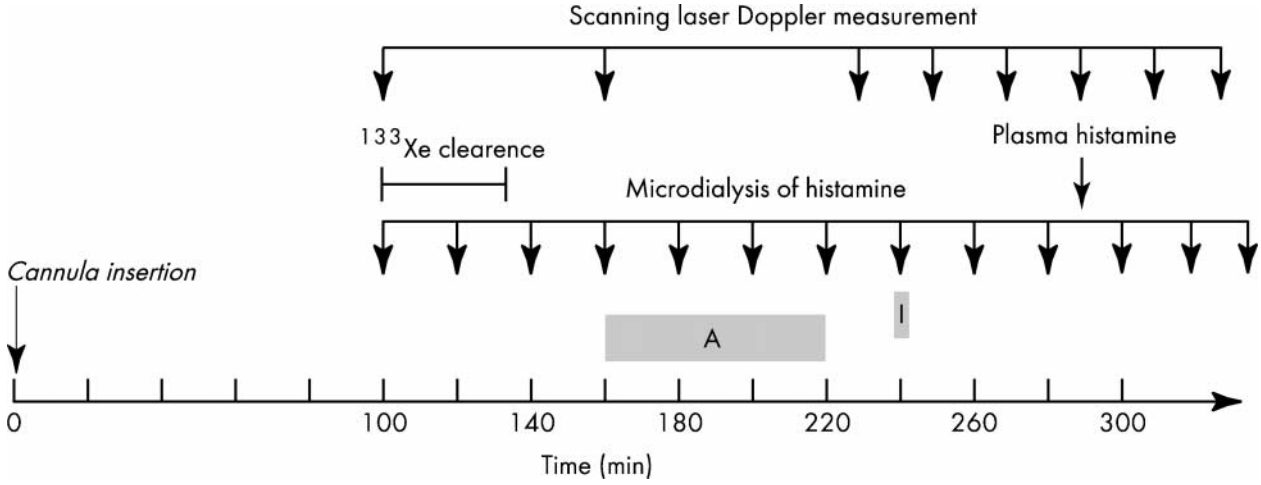

Fig. 7. Effects of topical anaesthesia on histamine release: The experimental sequence. Arrows indicate the time of the intervention or measurement. Arrows connected to microdialysis sampling indicate the time of sample exchange. A, topical anaesthesia; I, iontophoresis of adrenalin.

neurotoxicity of capsaicin (Holzer, 1991). Capsaicin induces SP release in rodent tissue, but it is unclear whether a similar release occurs in healthy human skin (Petersen et al, 1997a).

Application Capsaicin (0.75 to 1%) in Essex cream was applied epicutaneously to lesional and non-lesional skin. To this end, rectangular self-adhesive, 2-mm-thick Duoderm frames (internal size 1×2.5 cm) were fastened to the skin. The chambers were filled with 1 ml capsaicin ointment and covered by an occlusive dressing.

5.5.2 Electrical stimulation of the skin

Low-frequency repetitive electrical stimulation of the skin induces local long-lasting vasodilatation, which spreads outside the stimulated area. Presumably, this vasodilatation is induced by the release of neuropeptides evoked by the recruitment of polymodal afferent nociceptive C-fibres under the stimulating probe and antidromic impulse propagation into branches of the stimulated axon. The response cannot therefore be evoked in skin infiltrated by a local anaesthetic agent (Wårdell et al, 1993b), or in skin pretreated with capsaicin. In the present study, electrical stimulation was used to control C-fibre function in lesional and non-lesional skin before and after the epicutaneous application of capsaicin.

Application A circular surface cathode 6 mm in diameter was wetted with water and held perpendicular to the skin surface. The anode, a circular silver plate (40 mm in diameter) embedded in cotton gauze and steeped in water, was placed 10 cm from the cathode. A train of square-wave pulses (pulse duration 1 ms, frequency 2 Hz, number of pulses 20 current 20 mA, surface area of stimulation probe 0.38 cm^2) was delivered to the skin from an electrical stimulator (Grass S40, Grass Instrument Company, MA, USA) connected to a constant-current generator (Grass CCU1A) and an isolation unit (Grass SIU05).

5.5.3 Experimental procedure

Protocol 1) Two microdialysis catheters were positioned in each subject, one in lesional skin and one in adjacent lesion-free skin on the forearm, thigh and abdomen ($n = 12$ in lesional and $n = 10$ in non-lesional skin; in two subjects, one catheter was placed in lesional skin only). Catheter depth was measured in six periumbilical and six forearm positions. After collecting two microdialysis samples, capsaicin was applied epicutaneously in the lesional and non-lesional sampling areas for two hours. Scanning laser Doppler measurements were made before and 50 min after application. Before these measurements, the capsaicin was carefully removed and it was then reapplied directly after the measurements. ^{133}Xe clearance and perfusion were measured in similar skin areas adjacent to the catheters before treatment ($n = 10$) and a perfusion/blood flow ratio was calculated. In capsaicin-treated lesional skin, ^{133}Xe clearance was not measured. DBF was therefore calculated from the scanning measurement in capsaicin-treated skin and the perfusion/blood flow ratio obtained before treatment. Arterialized venous blood samples were taken from the cubital vein for analysis of the plasma histamine concentration ($n = 10$). The DBF and histamine concentrations in plasma and interstitium were used to estimate the net release of histamine before and after capsaicin treatment in lesional skin. The experimental sequence is illustrated in Fig. 8.

Protocol 2) Three rectangular Duoderm frames (internal size $1 \times 2{,}5$ cm) were fastened to the skin on the forearm ($n = 10$); two were applied to lesional skin and one to non-lesional skin. Perfusion was measured prior to and directly after the electrical stimulation of the skin in the middle of each chamber. One ml of capsaicin or placebo (containing the vehicle used in the capsaicin cream) was applied randomly in either of the two chambers positioned in lesional skin. An equal amount of capsaicin was applied to uninvolved skin. The chambers were occluded. After one hour, the creams were removed carefully and perfusion was measured. The application procedure was then repeated and the chambers were occluded for 24 h, before removal of the cream and new perfusion measurements. To check whether the capsaicin desensitisation of afferent C-fibres was complete and vascular axon reflexes had been abolished, electrical skin stimulation was repeated, followed by new measurements of perfusion. The experimental sequence is presented in Fig. 9.

6. RESULTS

6.1 Paper I

6.1.1 Surface anaesthesia, but not conduction anaesthesia, reduced the perfusion in psoriatic plaque

Basal skin perfusion was 4.5 ± 0.7 V in psoriatic plaque and 1.4 ± 0.30 V in unaffected skin 0.5 – 3 cm away from the plaque ($n = 10$, $p < 0.001$) (Fig. 10). After surface anaesthesia with

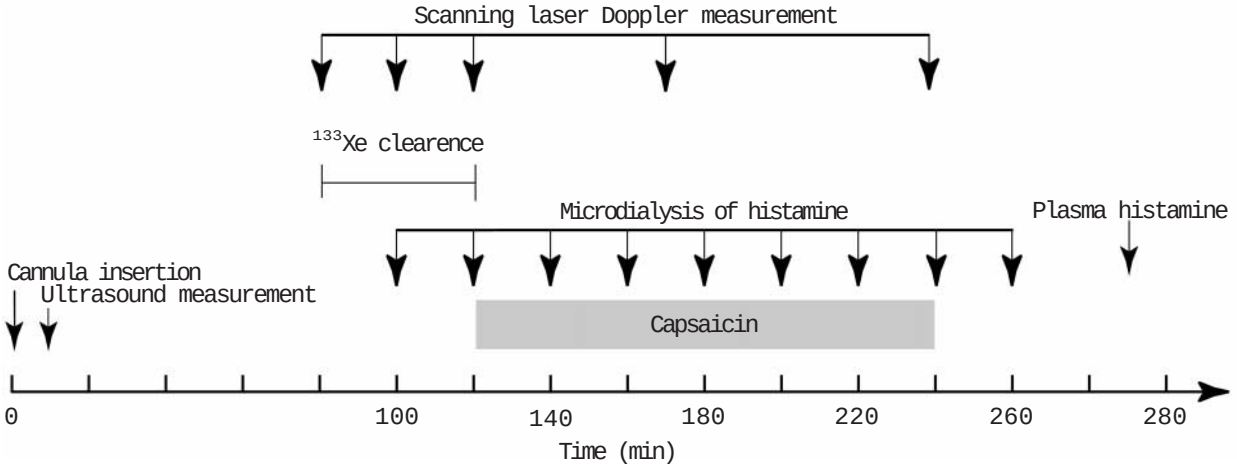

Fig. 8. Effects of topical capsaicin on histamine release: The experimental sequence. Arrows indicate the time of the intervention or measurement. Arrows connected to microdialysis sampling indicate the time of sample exchange.

EMLA cream, the psoriatic plaque was usually considerably paler than the surrounding untreated plaque area and skin perfusion was markedly reduced (Fig. 11). The maximum reduction in perfusion (to $59.4 \pm 6.1\%$ of the control value, which was set at 100%) always occurred in the first measurement after removal of the EMLA cream; there was then a successive return towards control levels during a period of approximately 30 min. Directly after the removal of the EMLA placebo cream, one subject displayed local blanching and reduced perfusion in the psoriatic lesion. Compared with the EMLA effect, however, the reduction was brief and in the entire material there was no significant change in perfusion compared with the basal level (Fig. 11). The difference in flow reactions between EMLA and EMLA-placebo was statistically significant for 20 min after the removal of the creams. Occlusion of a psoriatic plaque region alone led to a 10–20% increase in perfusion compared with the control level. The effect was statistically significant directly after the removal of the occlusion but not thereafter.

In the unaffected skin of psoriatic patients, surface anaesthesia with EMLA did not affect the perfusion (control perfusion 1.5 ± 0.1V; 1.6 ± 0.3V after EMLA treatment, $n = 10$). There was no change in perfusion in the psoriatic plaque upon the application of conduction anaesthesia in the nerves to the plaque area (Fig. 12).

6.1.2 Indomethacin, but not surface anaesthesia, reduced perfusion in UVB erythema

In experiments with UVB treatment, there was clear erythema in the skin of the healthy subjects seven hours after irradiation and perfusion had increased from 1.6 ± 0.05 to 4.6 ± 0.2V ($n = 9$, $p < 0.001$). The application of EMLA and EMLA-placebo creams for one hour (starting six hours after UVB exposure) had no significant effects on skin perfusion (Fig. 13). In contrast, after the application of indomethacin, the UVB-treated skin became paler and perfusion was reduced to $68 \pm 4\%$ of the control level (which was set at 100%).

If treatments were instead applied for six hours (starting one hour after UVB exposure), EMLA cream induced a 26% (to $126 \pm 10\%$) increase in perfusion compared with the control level, whereas indomethacin still caused a 34% (to $66 \pm 6\%$) decrease in perfusion. Occlusion alone had no significant effect.

6.2 Paper II

6.2.1 Depths of catheters

The upper surface of the cannula was positioned 0.8 ± 0.3 mm (range 0.4–1.5 mm, $n = 9$) under the skin surface. The depth of three arm catheters was 0.6 ± 0.2 mm, while that of six abdom-

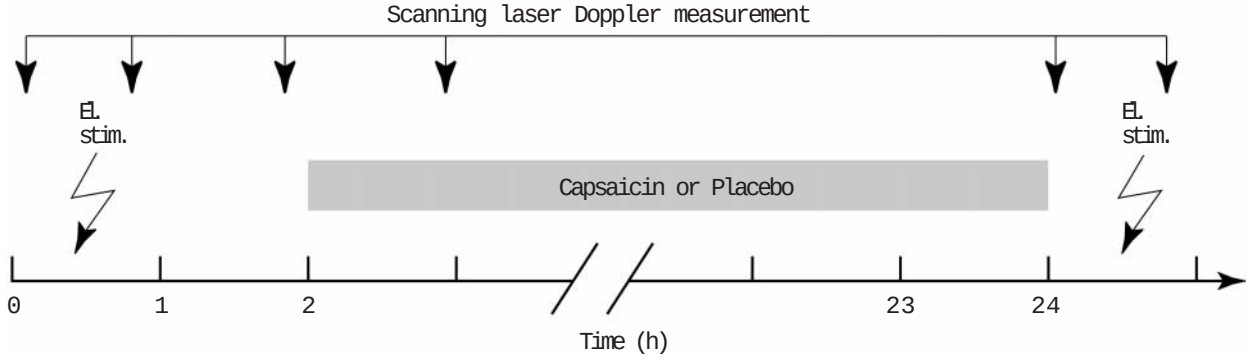

Fig. 9. Effects of 24 hours of topical capsaicin on blood flow: The experimental sequence. Arrows indicate the time of the intervention or measurement. El.stim., electrical stimulation.

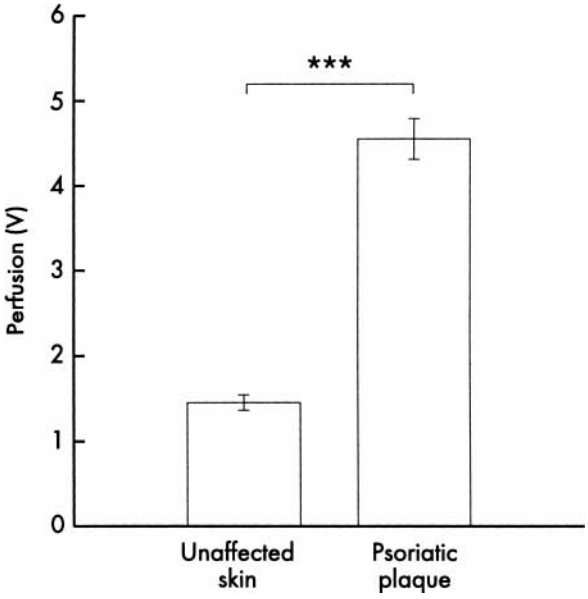

Fig. 10. Higher perfusion in psoriatic plaque compared with unaffected skin. Mean perfusion expressed in V in psoriatic and unaffected skin in the same subjects ($n=10$). ***$=p<0.001$. Error bars, SEM.

inal catheters was 0.9 ± 0.4 mm. In all cases, the insertion cannula was judged to be placed in the dermal tissue.

6.2.2 Histological examination revealed extravasation of erythrocytes

Two punch biopsies were taken too superficially. In the remaining 11 biopsies, no apparent increase in inflammatory cells or oedema was found. Localised extravasation of erythrocytes was common in the tangentially-cut sections.

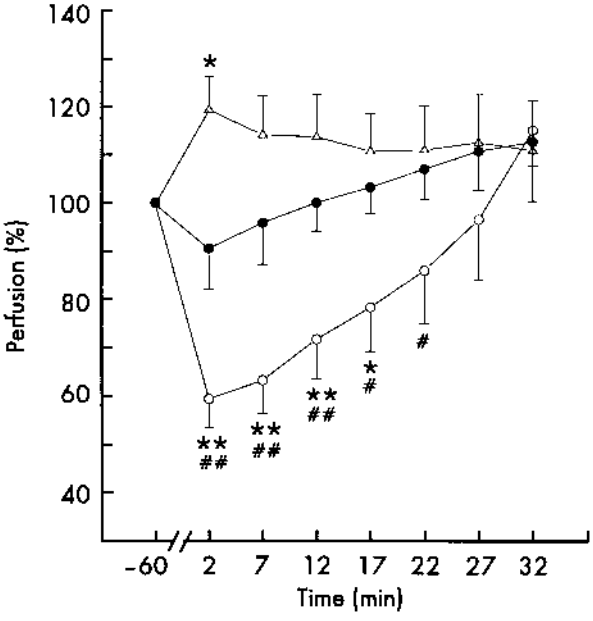

Fig. 11. Perfusion in psoriatic plaque after EMLA, EMLA-placebo and occlusion. Perfusion (as percentage of control) after 60 minutes' application of surface anaesthesia with EMLA (open circles; $n=10$), EMLA-placebo (closed circles; $n=8$), and skin occlusion (open triangles; $n=6$). Significant changes from control: **$=p<0.01$ and *$=p<0.05$. Significant differences between EMLA and EMLA-placebo; ##$=p<0.01$ and #$=p<0.05$. Error bars, SEM.

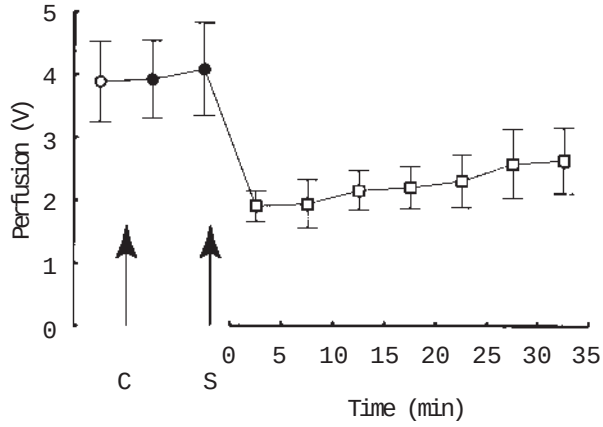

Fig. 12. Conduction anaesthesia of lesional skin caused no reduction in perfusion. Comparison of the effects of conduction and surface anaesthesia on skin perfusion (mean$\pm$SEM) in five subjects. The results show control perfusion (open circle), perfusion 10 and 20 minutes after conduction anaesthesia (closed circles) and perfusion values after additional surface anaesthetic treatment for one hour (open squares). The arrows indicate the time of application of conduction anaesthesia (C) and surface anaesthesia (S) respectively. Zero time was set at the removal of surface anaesthesia.

6.2.3 Increased skin perfusion ambient to microdialysis catheters

Insertion of the catheters caused a transient increase in ambient tissue perfusion, without complete normalisation during the experiments. About 20–30 min after insertion, there was a three-fold increase in the mean perfusion in the scanned areas, corresponding to the central part and the inlet/outlet

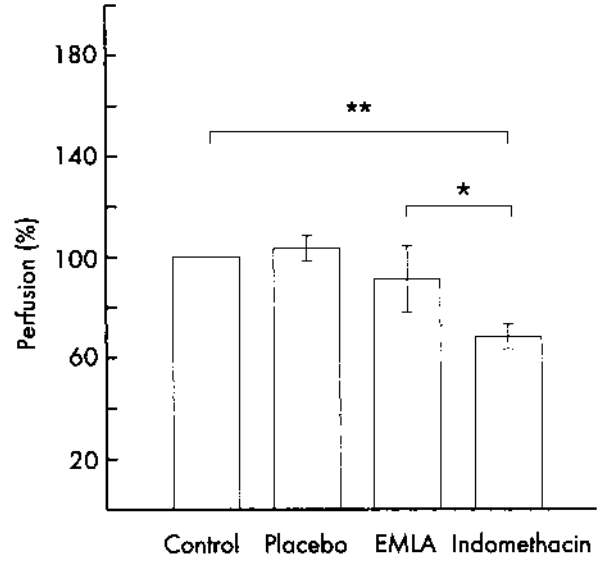

Fig. 13. Indomethacin but not EMLA reduced the perfusion in the UVB erythema after one hour of application. Perfusion in UVB-induced erythema (control$=100\%$) after the application of EMLA, EMLA-placebo and indomethacin. The creams were applied for one hour between the sixth and seventh hour after irradiation and measurements were made seven hours after a UVB dose of two MEDs ($n=9$). *$=p<0.05$, **$=p<0.01$. Error bars, SEM.

area of the microdialysis catheters, followed by new steady-state perfusion (60–90 min after insertion) ranging from 115 to 125% of the skin perfusion 3 cm lateral to the probe set at 100% ($n=8$, $p<0.05$). This perfusion level persisted throughout the experiment.

6.2.4 Increased interstitial concentration of lactate and pyruvate but normal L/P ratio

The equilibration calibration of lactate and pyruvate produced a close linear relationship between the net change in the substrates in the dialysate and the concentrations of the respective substances in the perfusate prior to dialysis (Fig. 14). Lactate and pyruvate recoveries were estimated at $46\pm10\%$ and $59\pm9\%$ respectively. Up to a two-fold intra/interindividual variation in recovery was found, irrespective of skin location. Insignificant differences in mean recoveries were obtained between the abdominal and forearm skin areas.

The mean concentrations of lactate were $1{,}171\pm228$ and 781 ± 180 μmol/l in skin and plasma respectively ($p<0.001$). No significant difference was found between the mean interstitial lactate concentrations measured concomitantly in the arm ($1{,}408\pm108$ μmol/l) and abdominal ($1{,}239\pm317$ μmol/) catheters ($n=8$). The pyruvate concentrations were 105 ± 14 μmol/l and 116 ± 15 μmol/l in the skin and plasma.

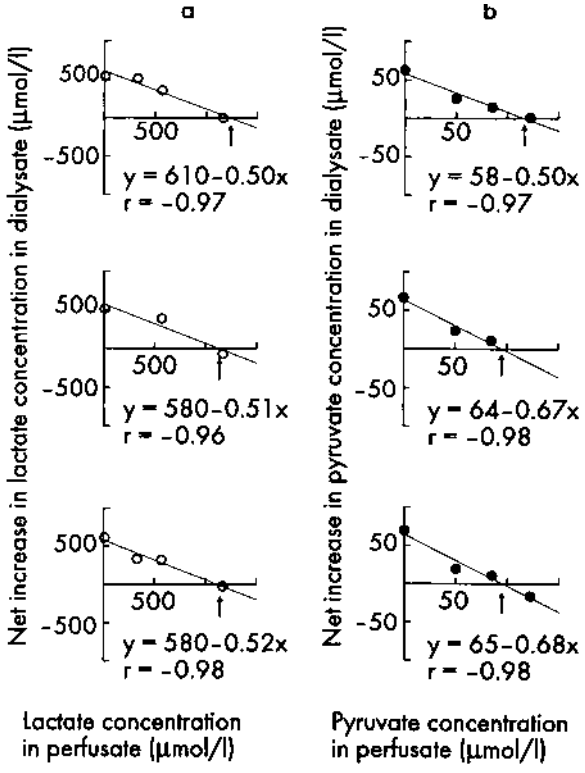

Fig. 14. Net changes in lactate (a) and pyruvate (b) concentrations in the dialysate plotted against the respective perfusate concentrations in one subject. Each regression line represents data from catheters on the left (top) and right (middle) side of the umbilicus and on the forearm (bottom), placed 0.6, 0.6 and 0.4 mm under the surface of the skin respectively. The equations and correlation coefficients (r values) are shown under the respective regression line. Relative recovery is given by the slope coefficient of the line (numerical values not shown). At $y=0$ (arrow), the point of no net flux, there is no net change in lactate and pyruvate concentrations and the true interstitial concentration (x value) can be estimated.

Data are summarised in Table II. The mean lactate/pyruvate ratio (L/P ratio) in the interstitial fluid was 12 ± 0.7 in five subjects (12 catheter positions) and 7 ± 1.3 in plasma. The interstitial lactate and pyruvate concentrations were higher when sampled from the more superficially positioned catheters and linear correlations were obtained between the interstitial lactate and pyruvate concentrations and the position depths of six catheters, with r values of -0.89 and -0.84 respectively ($p<0.05$). In these few positions, no linear correlation was found between catheter depth and interstitial L/P ratio or between the depth and calculated recoveries.

6.2.5 The retrodialysis calibration method produced reliable interstitial data

The mean initial glucose recovery (obtained from the first microdialysis sample) was estimated at $36\pm9\%$ (range 28–55%). During steady-state, the mean glucose concentration was 5.2 ± 0.8 and 4.9 ± 0.4 mmol/l in interstitial fluid and plasma respectively ($n=10$, Table II). Based on the assumption that the true glucose value in skin should be equal to that in plasma, the relative methodological error was calculated at 6.2%. A continuous decline in the efflux fraction of radioactively labelled glucose was observed in the samples subsequently obtained from the four hour steady-state period ($p<0.05$, $r=-0.63$). Consequently, if recovery was estimated from each efflux fraction value, the mean interstitial glucose concentration artefactually increased to 5.7 ± 1.2 mmol/l (which produced a relative methodological error of 16.2%). The retrodialysis technique was reliable during an OGTT, revealing interstitial glucose concentrations similar to those in arterialized venous plasma, with a minor time delay in the change in the interstitial fluid ($n=7$, Fig. 15).

6.3 Paper III

6.3.1 Histological data showing local extravasation of erythrocytes in lesional skin

The mean thickness of the epidermis + dermis in the microdialysis area was 1.9 ± 0.1 mm and 1.3 ± 0.1 mm in lesional and non-lesional skin respectively ($n=6$). The mean catheter depth was 1.0 ± 0.1 mm in lesional skin and 0.7 ± 0.1 mm in non-lesional skin ($n=6$). There was extravasation of erythrocytes indicative of small bleedings but no apparent increase in inflammatory cells.

6.3.2 Increased histamine concentration in lesional skin

Mean retrodialysis recovery was calculated at $76\pm1\%$ with similar values in lesional ($75\pm1\%$) and non-lesional skin ($77\pm2\%$), range 54-86% ($n=20$). The histamine concentrations were 32 ± 3 (range 13-81) nmol/l in lesional skin, 13 ± 1

Table II. *Recovery and concentration of glucose, lactate and pyruvate (mean ± SD) in the interstitial fluid and plasma in the fasting state*

Substances	n	Recovery (%)	Interstitial fluid concentration (μmol/l)	Plasma concentration (μmol/l)
Pyruvate	5	59 ± 9	105 ± 14***	116 ± 15
Lactate	15	46 ± 10	1171 ± 228***	781 ± 180
Glucose	10	36 ± 9	5200 ± 800***	4900 ± 400

n, number of subjects; *** = $p<0.001$ interstital fluid v plasma.

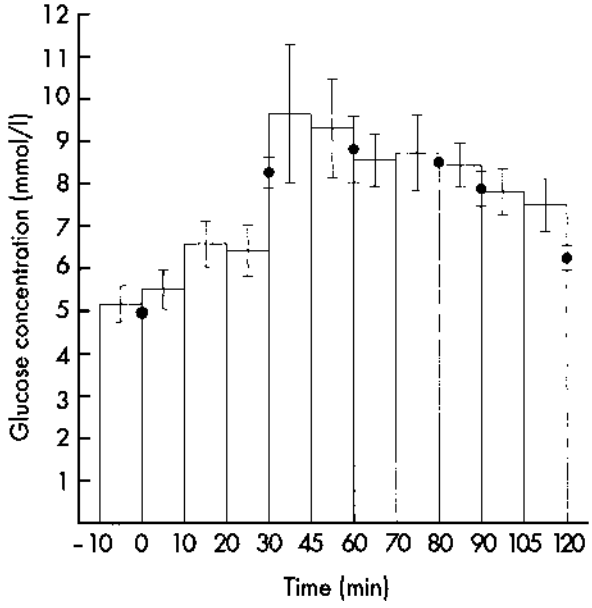

Fig. 15. Good correspondence between changes in glucose concentration in plasma and interstitium after OGTT. Glucose concentration (mean ± SEM) in plasma (filled circles) and dermal interstitium (histogram) before and after the ingestion of 75 g of glucose (OGTT) at zero time ($n = 7$). Note the irregular time interval on the x-axis.

(range 7-30) nmol/l in uninvolved skin ($n = 23$, $p < 0.001$) (Table III), and 3 ± 1 nmol/l ($n = 9$) in plasma. There were no significant differences between consecutive measurements of histamine in neither psoriatic nor uninvolved skin. A positive correlation was obtained between the histamine levels in lesional and non-lesional skin (r = 0.61, $p = 0.002$). The histamine concentration in psoriatic eruptions was 45 ± 11 nmol/l ($n = 5$) on the forearm and 41 ± 5 nmol/l ($n = 5$) on the thigh. When combined, these values were significantly different ($p < 0.004$) from the concentration in abdominal skin (25 ± 2 nmol/l, $n = 13$). The corresponding values in unaffected skin were 13 ± 1, 16 ± 4 and 16 ± 3 nmol/l respectively ($p = 0.2$).

There were no significant correlations between histamine concentrations in lesional/non-lesional skin and PASI score, age, catheter depth or number of years since the first appearance of psoriasis. Similar concentrations were obtained in dialysates from central and peripheral parts of the same psoriatic plaques (25 ± 2 and 27 ± 2 nmol/l respectively, $n = 14$). The histamine concentration was, however, significantly higher in the more erythematous lesions (erythema = 2: histamine concentration 25 ± 4 nmol/l, $n = 11$; erythema = 3: histamine concentration 40 ± 5 nmol/l, $n = 12$, $p < 0.05$).

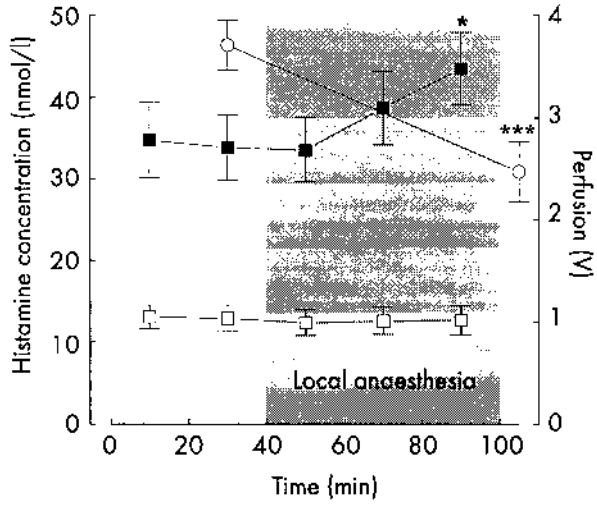

Fig. 16. Surface anaesthesia increased histamine concentration in lesional skin. The histamine concentrations (mean ± SEM) at 20-min intervals based on mean dialysate values in lesional (filled squares, $n = 19$) and non-lesional psoriatic skin (open squares, $n = 8$) before and during anaesthetic treatment. Note that each mean value is positioned in the middle of the sampling period. In the microdialysis area of lesional skin, the perfusion (open circles) was measured before and after anaesthetic treatment ($n = 10$). *$p < 0.05$; **$p < 0.001$.

6.3.3 High release of histamine in lesional skin

On the basis of the ^{133}Xe measurements, DBF was calculated at 12 ± 1 ml per min per 100 g in lesional skin and 2 ± 1 ml per min per 100 g in non-lesional abdominal skin, with the net release of histamine at 210 ± 30 in involved skin and 18 ± 3 pmol per min per 100 g in uninvolved skin respectively ($p < 0.001$, $n = 9$) (Table III).

6.4 Paper IV

6.4.1 Increased histamine concentration but unchanged histamine release in anaesthetised lesional skin

The mean retrodialysis recovery was calculated as 72 ± 1%, range 51–86%, with similar values in lesional (71 ± 2%, $n = 21$) and non-lesional skin (73 ± 2%, $n = 18$). The histamine concentration was 34 ± 4 nmol/l ($n = 21$) in lesional skin, 14 ± 1.5 nmol/l ($n = 18$) in non-lesional skin and 2.8 ± 1 nmol/l ($n = 10$) in plasma.

After topical anaesthesia for 60 min, the histamine concentration increased from 34 ± 5 to 44 ± 4 nmol/l ($n = 19$, $p < 0.05$) in lesional skin (Fig. 16). Net histamine release from affected skin, however, showed similar values before (198 ± 30 pmol

Table III. *Skin thickness, depth of catheter, dermal blood flow, recovery of histamine and concentration and net release of histamine in the dermal interstitial fluid in lesional and non-lesional skin (mean ± SEM)*

Parameters	n	Lesional	Non-lesional
Thickness epidermis + dermis (mm)	6	1.9 ± 0.1	1.3 ± 0.1
Depth of catheter (mm)	6	1.0 ± 1	0.7 ± 0.1
Recovery of histamine (%)	20	75 ± 1	77 ± 2
Histamine concentration (nmol/l)	23	32 ± 3***	13 ± 1
Histamine release (pmol/min/100 g)	9	210 ± 30***	18 ± 3
Blood flow (ml/min/100 g)	9	12 ± 1***	2 ± 1

n, number of subjects; *** = $p < 0.001$ lesional v non-lesional skin.

Table IV. *Effects of surface anaesthesia on histamine concentration and release in lesional psoriatic skin (mean ± SEM)*

	n	Non-anaesthetised skin	Anaesthetised skin
Histamine concentration (nmol/l)	19	34 ± 4*	44 ± 4
Histamine release (pmol/min/100 g)	10	198 ± 30	205 ± 29
Blood flow (ml/min/100 g)	10	14.5 ± 5***	9 ± 1

*** = $p < 0.001$ and * = $p < 0.05$ non-anaesthetised v anaesthetised skin.

per min per 100 g) and after anaesthesia (205 ± 29 pmol per min per 100 g, $n = 10$). The data are summarised in Table IV. No significant differences were found between histamine concentrations in consecutive dialysates in uninvolved skin ($n = 8$) (Fig. 16) or between consecutive dialysates from unanaesthetised lesional skin ($n = 8$). The release of histamine in non-lesional skin was estimated at 20 ± 5 pmol per min per 100 g ($n = 10$).

6.4.2 Topical anaesthesia reduced perfusion/blood flow in lesional skin

The mean perfusion was 3.7 ± 0.2 V and blood flow was 14 ± 5 ml per min per 100 g in lesional skin, while it was 0.9 ± 0.1 V and 3 ± 1 ml per min per 100 g tissue in non-lesional skin ($n = 10$, $p < 0.001$ for both). In anaesthetised lesional skin, the perfusion decreased to 2.5 ± 0.3 V ($p < 0.001$, Fig. 16) and blood flow decreased to 9 ± 1 ml per min per 100 g tissue ($p < 0.01$, Table IV). Individual values for blood flow and perfusion in lesional psoriatic skin tended to correlate (r = 0.55, $n = 10$, $p = 0.1$).

6.4.3 Iontophoresis of adrenalin into lesional skin induced vasoconstriction and an increase in interstitial histamine concentration

The iontophoresis of adrenalin into affected skin caused an increase in the histamine concentration from 34 ± 10 to a maximum of 49 ± 12 nmol/l ($n = 3$) and a reduction in the perfusion values from 3.4 ± 0.6 to 1.9 ± 0.8 V ($n = 3$) 40 min after application. The relative histamine and perfusion values are presented in Fig. 17. A significant linear correlation ($p = 0.013$, $r = -0.69$) was obtained between the interstitial histamine concentrations and perfusion values.

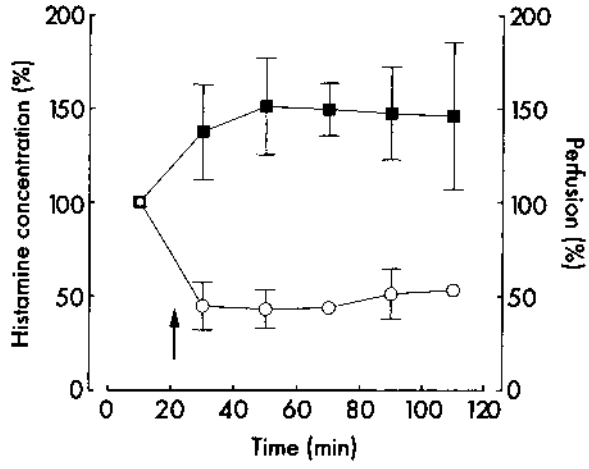

Fig. 17. Iontophoretically-applied adrenaline in lesional skin reduced the perfusion and increased the histamine concentrations. Adrenalin was applied iontophoretically (arrow) into psoriatic skin in the microdialysis area in three subjects. Perfusion (open circles) and histamine concentrations (filled squares) were measured before and after treatment. Values (percentage of control) are mean ± SD.

6.5 Paper V

6.5.1 Capsaicin-induced histamine release in psoriatic skin

The mean depth of the catheters was 0.9 ± 0.3 mm in lesional and 0.7 ± 0.2 mm in lesion-free skin ($n = 6$). In the control situation, the histamine concentration was 38 ± 6 nmol/l ($n = 12$) in lesional skin, 15 ± 2 nmol/l ($n = 10$) in non-lesional skin ($p < 0.001$) and 3 ± 0.5 nmol/l in plasma (Table V). The mean recovery of histamine was 68 ± 2 ($n = 12$) in lesional skin and 69 ± 2 ($n = 10$) in non-lesional skin. After 20 min of capsaicin treatment, a flare was observed outside the treated area in both affected and unaffected skin. Concomitantly, the mean interstitial histamine concentration increased from 38 ± 6 to 45 ± 6 nmol/l in lesional skin and from 15 ± 2 to 19 ± 2 nmol/l in non-lesional skin ($p < 0.05$ for both) and stabilised at this level with only small variations in subsequent dialysates. The basal net release of histamine was calculated at 202 ± 30 pmol per min per 100 g in affected skin and 20 ± 5 pmol per min per 100 g in lesion-free skin ($p < 0.001$). After 50 min of capsaicin

Table V. *Effects of capsaicin treatment on blood flow histamine concentration and histamine release in lesional and non-lesional psoriatic skin (mean ± SEM)*

		n	Before capsaicin	After capsaicin
Histamine concentration (nmol/l)	Lesional	12	38 ± 6*	45 ± 6
	Non-lesional	10	15 ± 2*	19 ± 2
Histamine release (pmol/min/100 g)	Lesional	10	202 ± 30*	262 ± 32
	Non-lesional	10	20 ± 5*	48 ± 9
Blood flow (ml/min/100 g)	Lesional	10	13 ± 1*	17 ± 2
	Non-lesional	10	3.4 ± 1***	10 ± 2

*** = $p < 0.001$ and * = $p < 0.05$ before v after capsaicin treatment.

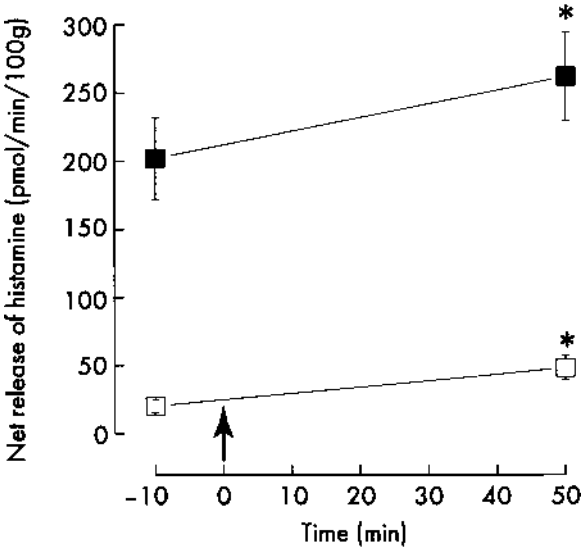

Fig. 18. Capsaicin increased net release of histamine in lesional and non-lesional skin. Histamine release (mean ± SEM, $n = 10$) in lesional (filled squares) and non-lesional skin (open squares) before and after 50 min of capsaicin treatment. The arrow indicates the time of capsaicin application. *indicates a significant change ($p < 0.05$) from control values.

treatment, the release increased to 262 ± 32 (30% increase, $p < 0.05$) and 48 ± 9 pmol per min per 100 g (140% increase, $p < 0.05$) in lesional and non-lesional skin respectively (Table V, Fig. 18). No correlation was found between depth of the catheter and the release of histamine after capsaicin treatment.

6.5.2 Electrical stimulation and acute capsaicin treatment elicited axon-reflex-mediated vasodilatation in lesional and non-lesional psoriatic skin

Electrical stimulation elicited axon-reflex-mediated vasodilatation with an increase in perfusion from 3.3 ± 0.5V to 4.1 ± 0.5V (25% increase, $p < 0.05$) in psoriatic skin and from 1.0 ± 0.6 V to 2.0 ± 0.3V (100% increase, $p < 0.001$, $n = 10$, Fig. 19a) in unaffected skin. Capsaicin treatment for 50–60 min caused a significant increase in laser Doppler perfusion in lesional skin (from 3.5 ± 0.3 V to 4.4 ± 0.3 V, 25% increase, $p < 0.05$, $n = 22$) and lesion-free skin (from 0.9 ± 0.03 to 3.3 ± 0.3V, $n = 20$, 270% increase, $p < 0.001$, Fig. 19b). Blood flow increased from 13 ± 1 to 17 ± 2 ml per min per 100 g in affected skin (30% increase, $p < 0.05$) and from 3.4 ± 1 to 10 ± 2 ml per min per 100 g in unaffected skin (195% increase, $n = 10$, $p < 0.001$).

6.5.3 Capsaicin treatment for 24 h reduced perfusion in lesional skin

Capsaicin treatment for 24 h caused a 15% decrease in perfusion from basal values ($p < 0.05$) in lesional skin and a 20% increase in unaffected skin ($p < 0.05$). Electrical stimulation in the capsaicin-treated uninvolved skin produced no significant changes in perfusion ($p = 0.44$). In the lesional psoriatic skin, however, electrical skin stimulation evoked a 12% flow increase in perfusion ($p = 0.05$).

7. DISCUSSION

7.1 Blood flow/perfusion in psoriatic skin (Papers I, IV and V)

Morphological and functional changes in the cutaneous vascular system are prominent in psoriasis (Braverman 1972; Braverman and Yen, 1974, 1977b; Braverman and Sibley, 1982) and our present finding of an increase in basal blood flow/perfusion in psoriatic plaque agrees with earlier results (Klemp and Staberg, 1983). In previous studies using percutaneous/intracutaneous ^{133}Xenon clearance methods (Nyfors and Rothenborg, 1970; Klemp and Staberg, 1983, 1985), a two- to five-fold increase in blood flow in psoriatic plaque was reported, which agrees with our figures of a three-fold increase in perfusion and a five-fold increase in blood flow obtained using the ^{133}Xe clearance technique. Our absolute blood flow value in lesional skin of 12–15 ml per min per 100 g is smaller than (Klemp and Staberg, 1983) or similar to (Nyfors and Rothenborg, 1970) the blood flow levels in the previous studies. In part, the large variation in dermal blood flow reported between studies in the literature can be explained by the differences in methodology.

When using the rate of washout of a freely diffusable tracer for calculations of blood flow, it is necessary to know the coefficient for the partition between tissue and blood in the tracer (see under methods). In psoriatic skin, the relative content of lipids is increased and that of water decreased compared with healthy skin, which results in a tissue-to-blood partition coefficient of 1.2 (Klemp and Staberg, 1983, 1985). A slight increase in lipogenic rate has also been found in non-lesional psoriatic skin (Cooper et al, 1976), i.e. the keratin lipids of the stratum corneum may be increased in uninvolved psoriatic skin. If so, the tissue-to-blood partition coefficient may be slightly higher than the value for normal skin of 0.7 used in the present study. The result of this would be that our value for dermal blood flow in non-lesional skin would represent an underestimation (Klemp and Staberg, 1983).

The undisturbed washout curve of ^{133}Xe following epicutaneous labelling has a biexponential course, due to a high washout rate from the dermis and a slow clearance rate from the subcutaneous adipose tissue (Sejrsen, 1971). The different washout rates can be calculated by graphic separation of the two components to estimate the dermal part of the washout curve exactly. In the present study, only the initial part of the washout curve was recorded and graphic separation was therefore not possible. Since the slow clearance washout curve influences the dermal washout rate, this may also lead to underestimation of DBF.

The three-fold increase in the scanning laser Doppler perfusion compared with non-lesional skin is in agreement with previous values obtained using a similar technique (Auer et al, 1994; Speight et al, 1993). In a study using the conventional "point" laser Doppler flowmeter, a 12.8-fold increase in perfusion was found (Klemp and Staberg, 1985). This value may be less reliable because the microvasculature in the skin is not uniformly distributed and, perfusion values obtained from the single-point laser Doppler technique therefore often differ markedly between adjacent skin areas (Tenland et al, 1983). This variability is underlined by the large differences between individual pixels seen in our original scanning perfusion records. Another factor, which could influence the variability is differences in the thickness of the plaques. To minimise this

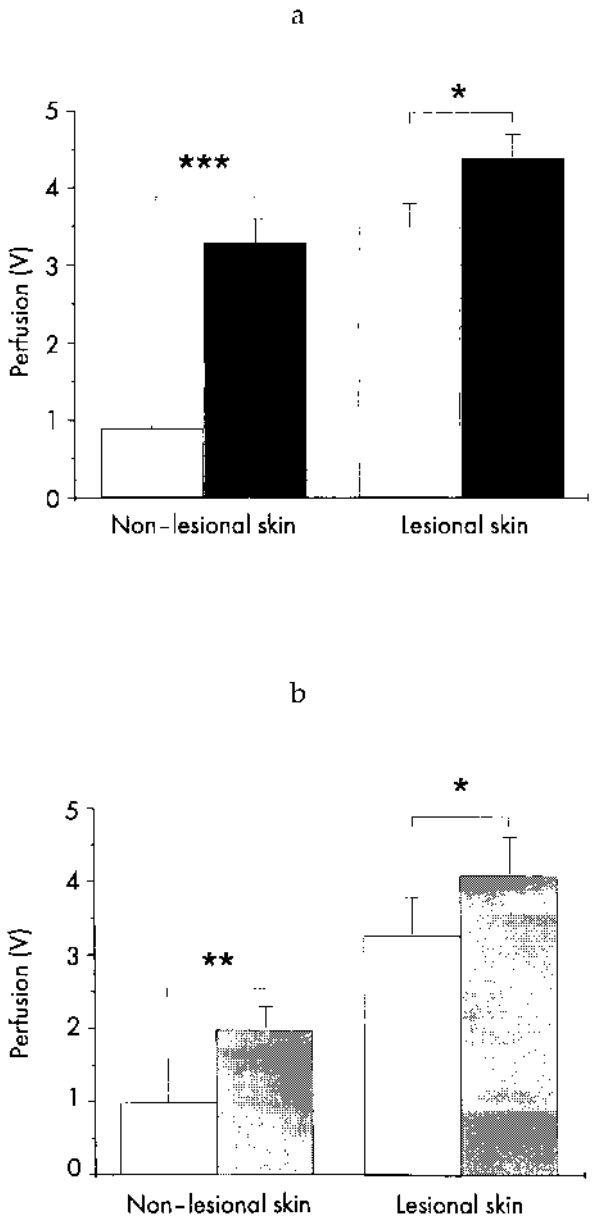

Fig. 19a and b. Capsaicin and electrical stimulation increased the perfusion in psoriatic skin. a) Perfusion in lesional and non-lesional skin (mean ± SEM, $n = 20$) before (open bars) and after topical capsaicin treatment for 50 – 60 min (filled bars). b) Perfusion in lesional and non-lesional skin (mean ± SEM, $n = 10$) before (open bars) and after electrical stimulation of the skin (stippled bars). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

effect, we used neutral ointment on the plaques for one week before the experiment. As the standard errors of perfusion values are small (cf. Fig. 10), the psoriatic plaques are likely to have been of comparable thickness. A third reason for the variability between pixels (but not mean values) may be that the laser Doppler imager records perfusion at each measurement site for less than 0.1 second; i.e. a small part of the pulse wave and spontaneous vasomotion circuit. These variations are, however, thought to influence the output signal to a minor extent (Wårdell et al, 1994). Although a longer measurement time at each site would be preferable, this would prolong the total duration of the measurements and impair the time resolution. For example, if perfusion were recorded for one second at each site, approximately one hour would be required to cover the whole area, which would make it impossible to follow the time course of a change in perfusion.

If we assume a medium sampling depth of 0.2 – 0.3 mm, a major part of the laser Doppler output signal ought to derive from blood cell motion in papillary vessels and in arterioles and venules in the superficial dermal plexus. The degree of acantosis and the quantity of scales in psoriatic skin influences the medium sampling depth in lesional skin. It is therefore conceivable that different strata in the vascular network contribute to the perfusion value in lesional and non-lesional/healthy skin respectively. Topical treatment with anaesthetics or capsaicin creams may also interact with the penetration of light. The possibility cannot be excluded that the creams, despite being removed before measurement, may change the optical properties of tissue and thereby interact with the output signal. The expected effect of epicutaneous emollient application is enhanced penetration of the laser beam and, to some extent, an increase in the perfusion signal. If so, the inhibitory effects of anaesthesia and 24 h of capsaicin on perfusion would be even more pronounced.

When a cuff has been inflated to suprasystolic values on an extremity perfusion measurements performed in a skin area distal to the cuff show a perfusion signal larger than the instrumental electrical zero (Tenland et al, 1983). Different opinions exist as to whether this biological zero value, which usually is about 5 – 15% of the measurement value (Wahlberg et al, 1992) should be subtracted from the measurement (Caspary et al, 1988). Since local vasodilatation, concentration of blood cells and/or oedema formation affect the biological zero value, it is difficult to predict in experiments in which vascular changes are provoked. In the present study, biological zero values were not obtained. However, with a predicted biological zero value of about 15% of the recorded perfusion, the relative changes in perfusion would usually have been even larger in lesional skin.

Comparisons of LDF and ^{133}Xe washout measurements in the skin show a fairly good correlation (Engelhart and Kristensen, 1983; Kastrup et al, 1987). Although both measurements produced considerably higher values in affected than unaffected skin in the present study, the correlation between them did not reach statistical significance in either lesional or non-lesional skin. The reason may be the small number of observations ($n = 10$) and, in larger material, significance was reached with a similar degree of correlation in psoriatic eruptions but not in lesion-free skin (Klemp and Staberg, 1985).

7.1.1 Mechanisms behind the increased blood flow in psoriatic skin

The mechanisms underlying the increased blood flow in lesional psoriatic skin are unclear, but possible alternatives include an increase in blood in the anatomically large superficial vessel system and/or active vasodilating mechanisms induced by local autoregulatory mechanisms, cytokines and other immunological mediators, hormones or neural factors.

Neurogenic mediators Conduction anaesthesia of the nerves innervating a psoriatic plaque did not affect the mean perfusion in the plaque. The fact that perfusion increased in some subjects and decreased in others after conduction anaesthesia may be related to differences in the thermoregulatory state between patients. Even though only five patients were examined in this way, the result makes it unlikely that efferent sym-

pathetic impulses from the central nervous system are important for the high blood flow in the plaque. On the other hand, the finding that surface anaesthesia of the plaque evoked a marked reduction in blood flow is compatible with the idea that a local neurogenic mechanism contributes to the high blood flow. It is well known that mechanical or electrical stimulation of the skin leads to axon-reflex vasodilatation in a local area around the stimulus. Because axon-reflex vasodilatation does not occur in skin anaesthetised with EMLA cream (Wårdell et al, 1993b), the flow reduction after surface anaesthesia observed in the present experiments would be the expected response if the high blood flow in the psoriatic plaque were due to an ongoing axon reflex.

Our findings of local increases in perfusion induced by electrical stimulation or one hour of capsaicin application are in agreement with an intact peptide-induced vasodilator mechanism in both lesional and non-lesional skin. This vasodilatory ability of C-fibres is, however, inhibited by prolonged topical capsaicin treatment (Jancsó et al, 1987). This agrees with our results in unaffected skin showing that 24 h of capsaicin treatment inhibited axon-reflex-mediated vasodilatation. In affected skin, on the other hand, electrical stimulation still induced a 12% increase in perfusion, indicating an incomplete capsaicin-induced inhibition of C-fibre nerves. One possible reason for the incomplete inhibition of the C-fibres could be that the high perfusion in lesional skin led to the rapid washout of capsaicin. Another alternative could be incomplete penetration by capsaicin through the acantotic lesional skin. However, all patients reported a burning sensation when capsaicin was applied to the plaque and this therefore speaks against this possibility. A third alternative may be related to differences in the neurogenic response to capsaicin between affected and unaffected psoriatic skin (Glinski et al, 1991) due to differences in the degradation of substance P and antinociceptive opioids by neutral proteases (Glinski et al, 1994). Irrespective of the mechanism, a 15% reduction in basal perfusion in lesional skin was observed after 24 h of capsaicin compared with a 20% increase in non-lesional skin. Had the capsaicin-induced inhibition of C-fibres in the lesion been complete, it seems likely that the reduction in perfusion in the plaque would have been more pronounced. The findings are thus compatible with the hypothesis that C-fibres may contribute to the high blood flow in lesional skin and they provide a possible explanation as to why repetitive topical capsaicin treatment reduces the pruritus, erythema and combined psoriasis severity score in psoriatic lesions (Bernstein et al, 1986; Ellis et al, 1993).

Inflammatory mediators The EMLA-induced reduction in perfusion in the plaque need not necessarily be mediated via nerve fibres; direct effects on interstitial inflammatory cells or vascular endothelial cells could contribute. The effect of the topical anaesthesia would then be due to the membrane stabilisation of these cells, resulting in the reduced release of some vasodilating factor. Several vasodilating substances, such as prostaglandins, nitric oxide and histamine, are known to be released from these cells and the possibility cannot be excluded that this release is blocked or reduced by the membrane-stabilising effect of the anaesthetic agents in the EMLA cream. If so, it is unlikely that prostaglandins are involved, as the perfusion of the prostaglandin-mediated UVB erythema remained unchanged after EMLA treatment but was reduced in indomethacin-treated skin. In agreement with this, topically-applied indomethacin has been found to have no influence on capsaicin-induced neurogenic inflammation (Herbert et al, 1993). Blanching of the skin has been observed after EMLA treatment (Juhlin, 1984), but this was not confirmed in the present study of unaffected psoriatic skin. Whether the discrepancy reflects the different methodological approaches, differences in penetration through skin or non-specific EMLA effects is unknown.

In addition, histamine is known to cause cutaneous vasodilatation in humans (Lewis, 1927). Our finding of an increase in histamine concentration in lesional skin compared with non-lesional skin and a higher concentration in the more erythematous lesions, is therefore compatible with the idea that histamine may contribute to an increase in blood flow in psoriatic plaque. However, we also found that topical anaesthesia induced vasoconstriction in the face of increasing histamine concentrations, and histamine is therefore highly unlikely to be an important factor behind the high blood flow in the plaque. Presumably, the histamine concentration in the tissue does not reach the level necessary to induce vasodilatation, which was recently reported to be 100 nmol/l (when histamine was infused through a microdialysis catheter) in normal skin (Petersen et al, 1997d). A concentration level of this kind was not obtained after one hour of capsaicin treatment, which also indicates that histamine is of minor importance for the regulation of the high blood flow in lesional skin.

The psoriatic eruptions are erythematous and are often referred to as "salmon pink". The erythema is due to an increase in blood cells within the subpapillary plexus and/or to an increase in visibility of the microvasculature. The redness does not necessary signify any increase in the rate of blood flow through the skin. The dilated capillary loops in lesional skin contain an increased number of blood cells/mm^3 (Bull et al, 1992). Because the perfusion is defined as the product of the average velocity and the concentration of moving cells, it could be argued that increased concentrations of blood cells within the large superficial psoriatic vessels might be a major contributor to the higher perfusion obtained in lesional skin. However, this is not plausible as the radioactive washout results in prior studies (Klemp and Staberg, 1983, 1985) and the present study clearly indicate that blood flow is also considerably increased in lesional skin.

7.2 Histamine concentration and release in psoriatic skin (Papers II, III, IV and V)

The main known source of histamine in the skin is the connective tissue mast cells distributed around hair follicles, sebaceous glands, sweat glands and small vessels (Cowen et al, 1979). In addition, histamine may be released in the skin from circulating basophilic leucocytes. One possible explanation for the relatively high histamine level in lesion-free skin (about 15 nmol per litre) compared with plasma (3 nmol per litre) may be that there is normally continuous mast-cell degranulation and/or leakage of histamine from unactivated cells. Another alternative could be that the insertion of the dialysis membrane and/or the dialysis procedure itself induces mast-cell degranulation. In healthy subjects, Anderson et al (1992) reported an initial trauma-induced increase in histamine levels, thought to be mediated directly by the damage caused by the catheter insertion and/or indirectly via an axon-reflex mechanism. However, one to two hours after the catheter insertion, constant levels of histamine have been found in normal skin

(Anderson et al, 1992), which agrees with our findings of steady levels 80 min after the insertion trauma, in both lesional and non-lesional skin. Whether this stable level is related to the continued presence of the dialysis membrane in the tissue is unclear.

We found a positive correlation between the histamine concentrations in non-lesional and lesional skin which may indicate a coupling between histamine release and mast-cell numbers and/or mast-cell activation in both types of skin. On the other hand, with a mast-cell density in the lesional skin that is only double that in lesion-free psoriatic skin (Schubert and Christophers, 1985; Töyry et al, 1988), our finding of a 10-fold increase in histamine release in the lesion seems more likely to be due to an inherent exaggerated histamine release from the mast cells than to continuous mechanical irritation from the microdialysis catheter. This result is in accordance with the finding of a five-to ten-fold increase in histamine production in lesional skin *in vitro* (Graham et al, 1981). The assumption of an increase in mast-cell activity in lesional psoriatic skin agrees with reports of extruded mast-cell granules and tryptase activity in the epidermis of typical psoriatic lesions (Brody 1984a; Harvima et al, 1989) and of mast-cell degranulation as an early and constant feature of acute eruptive guttate (Brody, 1984a,b) and early relapsing psoriasis (Schubert and Christophers, 1985).

The increased concentration and release of histamine in lesional skin may explain why treatment with H2 antagonists appears to improve the psoriatic lesions (Kristensen et al, 1995; Petersen et al, 1998). Recently, the H2 antagonist ranitidine was shown to normalise mast-cell hyperreactivity in lesional skin (Petersen et al, 1998), which may indicate that binding to H2 receptors inhibits the release of signals leading to the hyperreactivity. However, since histamine is in all probability released together with other preformed mast-cell mediators which may also influence the psoriatic inflammatory infiltrate, the co-release phenomenon could explain why exacerbation or no beneficial effect has also been reported after antihistamine treatment (Navaratnam and Gebauer, 1990; van de Kerkhof et al, 1995; Zonneveld et al, 1997). Moreover, it has been suggested that the mode of action of anthralin in psoriasis involves the prevention of mast-cell degranulation, thereby inhibiting the release of histamine and other mast-cell mediators sustaining the psoriatic process (Brody, 1986). These treatment reports are in agreement with the idea that skin mast cells and their products may be involved in the psoriatic inflammatory process (Brody, 1984a, 1984b; Kristensen et al, 1995; Petersen et al, 1998).

In theory, the interindividual differences in interstitial histamine concentration may be due to variations in catheter depth, insertion trauma and/or intraindividual variations in mast-cell numbers and reactivity. In accordance with previous results (Andersson et al, 1996), we found no correlation between the dermal catheter depth and the histamine concentration. This may indicate that the interstitial histamine is equally distributed in the part of dermis in which the catheters were positioned (0.4 to 1.7 mm from the epidermal surface), even though the bulk of the mast-cell infiltration in lesional skin is located immediately below the epidermis in the near vicinity of the dilated papillary capillaries and the superficial dermal plexus (Cox, 1976; Töyry et al, 1988). The regional differences in histamine levels with higher histamine concentrations on the forearm/thigh than on the abdomen may therefore not be related to the measurement depth but may reflect a higher number (Zachariae, 1964) or activity of cutaneous mast cells in the extremities. It could be argued that it is not possible to measure differences in substrate concentration levels at various depths in the skin using the microdialysis membrane, as it occupies a considerable part of the dermal thickness. However, the finding in healthy skin that interstitial lactate and pyruvate concentrations were higher in dermal skin near the epidermis and were correlated to the depth of the catheter indicates that the methodology permits measurements of concentration levels at different dermal levels.

The insertion of microdialysis catheters in the skin caused local bleedings in the tissue along the catheter in both healthy and lesional psoriatic skin. Theoretically, a major bleeding and/or inflammation adjacent to the probe might reduce the rate of diffusion of interstitial substances and make measurements of rapid changes in concentration difficult. However, rapid changes in interstitial glucose concentration were observed in healthy skin in the present and previous studies (Petersen et al, 1992a) and values similar to those in plasma were demonstrated. This constitutes indirect evidence that the tissue trauma caused by the catheter did not seriously influence the microdialysis results. It also verifies the fact that the retrodialysis technique, which has been used in other tissues (Jansson et al, 1994b; Lönnroth and Strindberg, 1995), produces reliable interstitial glucose values in healthy human skin. Given that the range of recovery values was small and similar in non-lesional and lesional skin, it seems unlikely that bleedings interfered significantly with the dialysis process in lesional skin. The similar recovery in affected and unaffected skin is in agreement with recent findings showing that recovery is not influenced by vasodilatation (Petersen et al, 1997c). Due to the accumulation of labelled glucose near the microdialysis membrane, restricted diffusion may prevail in the dermal interstitial fluid (Lönnroth and Strindberg, 1995). If so, it is unlikely that this interferes with the recovery, which was estimated from the first microdialysate in the series of samples.

Calculations of recovery are needed in microdialysis studies to calculate absolute concentrations of compounds in the extracellular space. Skin histamine levels can be calculated using the flow rate method (Petersen et al, 1997c) and the equilibration calibration method (Church et al, 1997). The potential problem of increasing the drainage of extracellular compounds with increasing perfusion rates up to 5 µl/min appears to be of minor importance (Petersen et al, 1997c) and is in accordance with the stable values obtained over two to four hours in our study with a perfusion rate of 2.5 µl/min. The retrodialysis method used in the present study produced accurate calculations of extracellular glucose provided that the efflux fraction (or recovery) was estimated from the first in the series of sequentially obtained samples. It is highly unlikely that the radiolabelled histamine added to the perfusate influenced histamine concentration or histamine release in the skin. The unvarying level of interstitial histamine reported in unprovoked lesional and non-lesional skin, also speaks against such a possibility. Using the equilibration calibration method satisfactory regression lines ($r > 0.9$) were obtained running concentrations of histamine up to 15 nmol/l in the perfusate (Church et al, 1997), which exceeds the concentration of the radioactive tracer in the present experiments.

Artefactual high interstitial substrate concentrations may be created if blood flow is impeded close to the catheter, whereas a

small increase in flow does not appear to interfere with the microdialysis measurements (Benveniste et al, 1987). As the skin perfusion is obtained from the superficial parts of the dermis (0.2 – 0.3 mm), it could be argued that the small increase in perfusion recorded over the microdialysis area in healthy skin, does not exclude the existence of ischaemic conditions in the immediate vicinity of the microdialysis membrane (positioned at a mean depth of 0.8 mm). This is unlikely since the interstitial lactate/pyruvate ratio (L/P ratio) indicated non-ischaemic environments in the microdialysis region at various dermal depths. Under ischaemic conditions, L/P ratios reach levels well beyond 20 (Johnson and Fusaro, 1972). In lesions where the blood flow is much higher than in healthy skin, it is unlikely that the catheter induced any ischaemia in adjacent tissue.

The interstitial concentration of histamine depends on the release of histamine and the clearance caused by metabolism and net outflow into the circulation. A constant interstitial substrate concentration is maintained as long as the clearance equals the release. The release is influenced by factors such as the distance to the vessel, the rate of degradation, the density and permeability of vessels and the local blood flow (Crone and Levitt, 1984). A reduced outflow to plasma from the interstitial compartment due to a decrease in blood flow causes a passive increase in the interstitial substrate concentration. This is illustrated by our finding of an increase in histamine concentration following the iontophoretic application of adrenalin into psoriatic skin. We suggest that the effect of topical anaesthesia in psoriatic plaque (a 20% increase in interstitial concentration and a 30% reduction in perfusion) in the face of an unchanged net release of histamine can be explained by a similar mechanism. Theoretically, the iontophoretic application of adrenalin may stimulate mast-cell degranulation. This has been shown in animal models (Moroni, 1977) but not in human skin. The electrical current used for iontophoresis may also induce transient degranulation of mast cells (Kowalski and Kaliner, 1988) and thereby contribute to the increase in histamine. However, this is unlikely to explain our results with an increase in stable histamine concentration for two hours after iontophoresis (cf. Fig. 17).

Previous studies have shown that a combination of microdialysis and ^{133}Xe clearance permits the assessment of both the subcutaneous (Jansson et al, 1994a) and cutaneous (Jansson et al, 1996) release of substrates. It should be noted that the estimated histamine release was based on a constant PS value of 10 ml per min per 100 g in lesional skin. The PS value, which is the product of permeability (P) and capillary surface area (S), has not been evaluated in psoriatic lesions. Since the venous part of the capillary vessels is highly permeable (Braverman and Yen, 1974, 1977b) and the endothelial volume is increased approximately two-fold (Barton et al, 1992), the PS value may have been underestimated in the present experiments. Since the application of capsaicin induces vasodilatation, it is possible that S and thereby the PS value increased during treatment. If an increase did occur, it is, however, unlikely that it would affect the interpretation of our results. Even if the PS were to increase from 10 to 20 ml per 100 g per min during capsaicin stimulation (which is unlikely), the release would only increase from 262 to 280 pmol per min per 100 g in lesional, capsaicin-treated skin. This would still be a fairly minor change compared with that induced by 1 mmol/l of intracutaneously applied SP in normal human skin, which induced a 3.5-fold increase in histamine concentration (Petersen et al, 1994).

7.2.1 Mechanisms behind the release of histamine in lesional skin

In accordance with the increased concentration and net release of histamine, mast cells in lesional skin have been reported to be hyperreactive to secretagogues (Petersen et al, 1998). The reason for the increase in the net release and hyperreactivity of mast cells in psoriatic lesional skin is not known. We have considered two main possibilities. Firstly, mast cells in lesional skin may be hyperreactive due to alterations in the mast cells themselves and/or differences in the pattern of distribution between mast-cell subpopulations with a different sensitivity to secretagogues. There is little evidence in the literature to support the presence of pathologically transformed mast cells in lesional skin. However, in contrast to normal skin, which mainly contains MCTC, the MCT subtype is the dominant mast cell at the dermo-epidermal junction in lesional skin, whereas chymase-positive cells are markedly diminished (Harvima et al, 1989, 1990). Unlike skin tryptase, chymase can be inhibited by plasma inhibitors (Schechter et al, 1989) and the inflammatory reaction in psoriasis is thus associated with an influx of plasma inhibitors which may inactivate chymase but not tryptase. Due to this imbalance in lesional skin, the ability to degrade potential mast-cell-stimulating peptides may be impaired (Harvima et al, 1990). It has been suggested that the activity of proteases is of importance for the modulation of the neurogenic inflammatory response and the imbalance between these proteases may therefore constitute one factor behind the hyperreactivity and increased histamine release. Secondly, the net release of histamine could be induced by substances which prime, activate and/or degranulate mast cells. Both immunological and non-immunological secretagogues have been reported to induce degranulation of mast cells in human skin.

Neurogenic mechanisms If low-frequency, continuous local nerve activity were present in psoriatic plaque (a self-regenerating axon reflex), a mechanism of this kind might stimulate histamine release. The present study reveals, however, that the release of histamine in lesional skin was not reduced by topical anaesthesia, thereby suggesting that mainly non-neurogenic factors are responsible for the histamine release in psoriatic plaque. It could be argued that one hour of anaesthetic treatment is too short for a measurable reduction in mast-cell release to occur if nerves were of importance. This is unlikely because, firstly, SP is quickly degraded (Caughey et al, 1988) and a continuous supply therefore appears to be necessary to maintain the activation and, secondly, histamine clearance from the skin is rapid, with a half life of only a few minutes (Petersen et al, 1994). Other reasons for the lack of neurogenically-mediated histamine release could be that the topical anaesthesia caused an incomplete block of neural activity, or that C-fibre nerves release neuropeptides despite being anaesthetised. These possibilities are difficult to exclude. However, in healthy skin, the vasodilatation mediated by neuropetides released from C-fibres was completely abolished after one hour of topical anaesthesia (Wårdell et al, 1993b).

Although the inhibition of nerve activity did not reduce histamine release in the present study, the activation of sensory C-fibres with capsaicin increased both the concentration and the release of histamine in lesional and non-lesional skin. Capsaicin itself does not release histamine from mast cells *in vitro,* but

capsaicin treatment may indirectly cause mast-cell degranulation *in vivo* (Bunker et al, 1991), via the release of neuropeptides from nociceptive sensory neurones (Holzer, 1988). The transduction of this signalling pathway elicits a transient excitation, manifested as a burning sensation and vasodilatation in both affected and unaffected psoriatic skin. These capsaicin-induced effects suggest that capsaicin may induce some histamine release from mast cells in psoriatic skin, presumably through the release of neuropeptides. The net increase in histamine release was small compared with the large changes in concentration caused by provocation with anti-IgE (Petersen et al, 1996a) or externally-injected SP in normal human (Petersen et al, 1994) and lesional psoriatic skin (Petersen et al, 1998). It is therefore unlikely that the excitation of C-fibres is an important mechanism contributing to the 10-fold increase in histamine release in psoriatic skin. This assumption agrees with the finding that the intra- or epicutaneous application of capsaicin to healthy skin did not affect the cutaneous interstitial histamine concentration (Huttunen et al, 1996, Petersen et al, 1997a). Our results are in accordance with findings showing that micromolar concentrations of SP were needed to induce histamine release from dispersed human skin mast cells (Church et al, 1989). This concentration exceeds the concentration of SP found in normal human skin (Wallengren et al, 1987). Even though the concentrations of neuropeptides are increased in psoriatic plaque, and presumably even further increased in the narrow space between the nerve fibre and the mast cell, it seems unlikely that such levels are reached unless extensive nervous activation prevails.

Although micromolar concentrations of SP are needed to induce histamine release in healthy skin (Petersen et al, 1994), it was found that picomolar concentrations could prime mast cells to subsequent activation (Janiszewski et al, 1994). Our finding of a small capsaicin-induced release of histamine in psoriatic skin may indicate that dermal mast cells are primed and hyperresponsive, due to neuropeptides being continuously released by the low-grade activation of C-fibres. If so, the degranulating ability of inflammatory mediators, which are increased in lesional skin, may be reinforced.

The observation that mast-cell degranulation occurs before inflammatory infiltration (Brody 1984b; Schubert and Christophers, 1985) favours a non-immunological activation, at least during the development of the plaque. This would also agree with the presence in psoriatic plaque of an increased number of neurofilament-positive nerve fibres in near apposition to mast cells (Naukkarinen et al, 1991, 1993, 1996) and increased concentrations of the neuropeptides substance P and vasoactive intestinal polypeptide (Naukkarinen et al, 1989, Anand et al, 1991; Pincelli et al, 1992).

Inflammatory mediators Anaphylatoxin C5a, which is increased in psoriatic scales (Takematsu et al, 1986) and is known to release histamine from skin mast cells (el Lati et al, 1994), may contribute to the histamine release in lesional skin. Inflammatory cytokines such as tumour necrosis factor-α (van Overveld et al, 1991), interleukin-1 (Subramanian and Bray, 1997) and/or other proteins (Kubota, 1992) may also be of importance for the histamine release in psoriatic plaque. The inflammation in affected skin constitutes a complicated pattern of inflammatory cells, cytokines and other potential modulators in an interacting network which may influence mast-cell activity (Fig. 20).

The observation that the histamine concentration was similar in the peripheral and central parts of the lesion, speaks against a higher release of histamine from mast cells in the periphery of lesions where the disease often progresses. This finding does not, however, exclude the possibility that the histamine concentration and mast-cell hyperreactivity may vary at different stages of the disease.

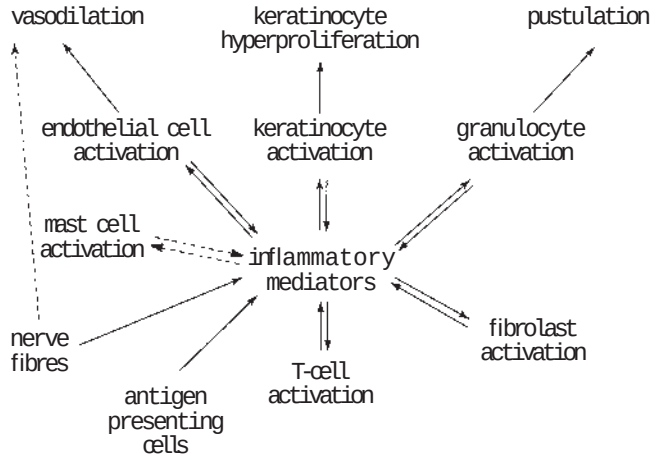

Fig. 20. A simplified schematic model of possible interactions between the cellular constituents thought to be involved in the pathogenesis of psoriasis. Note the suggested interaction between mast cells and inflammatory mediators and the nerve-mediated vasodilatation (dotted arrows).

7.3 The axon-reflex mechanism in lesional psoriatic skin (Papers I, III, IV and V)

In these papers, the axon-reflex hypothesis in the psoriatic lesion was tested by studying the effects of C-fibre activity on two variables; skin perfusion/blood flow and histamine release. The results differed. With regard to skin perfusion, topical (but not conduction) anaesthesia caused a marked reduction in the perfusion. The perfusion was also reduced by prolonged capsaicin treatment whereas there was an increase in skin treated with an inactive cream; these changes occurred in spite of an incomplete capsaicin-induced blockade of C-fibre activity. Thus, taken together, the findings are compatible with the idea of a continuous local neurogenic mechanism enhancing blood flow in the lesion, but they provide no clue to the mechanism initiating the abnormal nerve activity (Fig. 21). As histamine stimulates afferent C-fibre nerve endings (Schmelz et al, 1997a), one possible initiating mechanism could be that the high histamine concentration in lesional skin induces the neural activation.

On the other hand, with regard to histamine release, no significant change occurred after topical anaesthesia and acute application of capsaicin resulted in only a small increase. Thus, the reduction of nerve traffic had no effect and strong C-fibre activation only a minor influence on the histamine release. The findings reveal that activity in thin nerve fibres has little or no effect on histamine release from cutaneous mast cells and consequently the axon-reflex hypothesis cannot be evaluated by measuring histamine concentration or release.

Although the present study provides indirect support for an axon-reflex mechanism underlying the high blood flow in psoriatic plaque, more direct evidence is required to confirm the concept. One approach would be to measure the neuropeptides

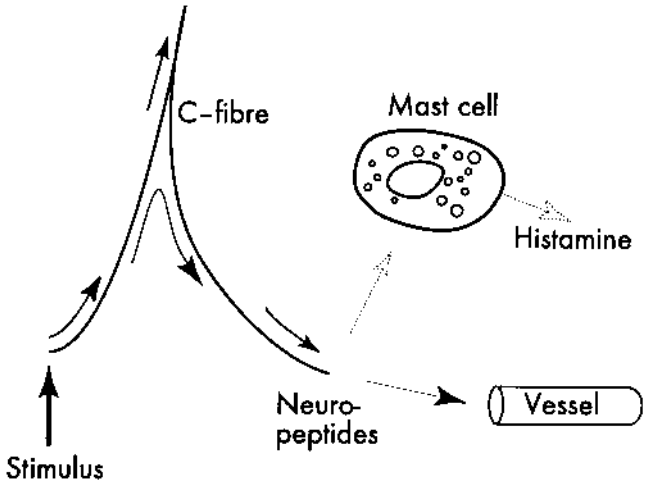

Fig. 21. Schematic illustration of the modified axon-reflex hypothesis in lesional psoriatic skin. Note that the antidromic activity in nociceptive fibres has little to no effect on histamine release from cutaneus mast cells (dotted arrows).

SP and CGRP directly in dialysates from lesional skin. The analysis of the small amount of these peptides in microdialysates from the skin is difficult but has recently been achieved (Schmelz et al, 1997b), hence such a study may be possible. The most direct way of obtaining evidence for or against an abnormal axon-reflex activity in lesional skin would be recordings of nerve traffic in single afferent C-fibres using the microneurographic technique. This approach is also technically very demanding but if combined with neuropeptide measurements it may provide a more definitive answer whether axon-reflex activity prevails in lesional skin, than that obtained in the present thesis.

8. GENERAL CONCLUSIONS

1. The relationship between local neural activity and perfusion in psoriatic skin (Papers I and V):

- The skin perfusion was unaffected by conduction anaesthesia, but surface anaesthesia of the plaque evoked a marked reduction in blood flow. The perfusion in UVB-irradiated skin, used as a control for non-specific phenomena, was reduced after the local application of indomethacin but was unaffected or increased after surface anaesthesia.
- The topical application of capsaicin for 24 h in lesional skin decreased the perfusion.

Conclusion: Afferent unmyelinated nerve fibres may contribute to the high blood flow in psoriatic plaque, via an on-going axon-reflex mechanism.

2. Interstitial histamine concentration and release in psoriatic skin and their effects on blood flow (Papers II, III and IV):

- Tissue aberrations induced by the microdialysis catheter were demonstrated in lesional psoriatic and healthy skin. These perturbations did not seriously interfere with the microdialysis results.
- The retrodialysis technique produced reliable interstitial values in steady state and during rapid changes in the interstitial glucose concentration.
- The interstitial histamine concentration was increased two-fold in psoriatic lesions compared with unaffected skin.
- The net histamine release was increased ten-fold in psoriatic lesions compared with unaffected skin.
- An increase of histamine concentration induced by iontophoretic application of adrenalin was associated with a decrease of blood flow (perfusion).

Conclusion: Application of the microdialysis technique in the dermis produced reliable interstitial data. Mast cells may play a role in the pathophysiology of the psoriatic lesion through the release of histamine (and/or other co-released mediators). The enhanced blood flow in affected skin is not due to the high interstitial histamine concentration.

3. The relationship between local neural activity and histamine release in psoriatic lesions (Papers IV and V):

- Local anaesthesia of the psoriatic lesion did not affect the cutaneous release of histamine.
- The stimulation of capsaicin-sensitive nociceptive nerves enhanced the net histamine release in psoriatic skin to a minor extent.

Conclusion: Afferent nociceptive nerves are of minor importance for the exaggerated histamine concentration and release found in lesional skin.

To summarise, these results are compatible with the hypothesis that a pathological axon reflex may contribute to the high blood flow in lesional psoriatic skin. The high release of histamine is mediated either not at all or only to a minor extent by neurogenic mechanisms.

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