

REVIEW ARTICLE

Microdialysis of Inflammatory Mediators in the Skin: A Review

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Skin microdialysis is an established method for *in vivo* sample collection from the extracellular fluid space. This method has been extensively used in studies of inflammatory reactions in the skin of animals and humans. Skin microdialysis consists of the implantation of semi-permeable probes into the upper dermis, perfusion with a physiological buffer, and the recovery of the substances that diffused from the skin into the perfusion fluid. Microdialysis allows the simultaneous assessment of the temporal variations of inflammatory mediator release in the skin as well as the monitoring of vascular and sensory functions. By the aid of this technique, potential associations can be found between functional changes and a variety of substances and mediators released at the site of interest. This allows further insights into the possible mechanisms underlying physiological and pathophysiological events in the skin, including cutaneous inflammation. This review provides a comprehensive but not exhaustive review of the use of microdialysis in studies of experimental and clinical inflammatory reactions in the skin in animals and humans. Key words: microdialysis; inflammatory mediators; inflammation; skin.

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Microdialysis was introduced approximately 40 years ago as a method for monitoring neurotransmitters in the brain of rodents (1, 2). During the last two decades, microdialysis has become a well-established method for continuously sampling substances within the extracellular fluid compartment of various tissues outside the central nervous system, including subcutaneous adipose tissue, dermis, muscle, and other organs (3–6). Microdialysis has been intensively applied in studies of inflammatory reactions in animal and human skin. Microdialysis allows the simultaneous assessment of the temporal variations in the inflammatory mediators in the skin and monitoring the vascular and sensory functions in the overlying intact skin. Hence, skin microdialysis has gained much interest as a method of studying biomarkers and the potential mechanisms underlying skin physiology and inflamma-

tion in the skin. In addition, a multitude of studies have utilised microdialysis to investigate drug penetration through the inflamed skin, a topic that is not covered in this review. This review provides a comprehensive, overview of the existing knowledge from studies that used skin microdialysis under experimental and clinical inflammatory conditions with the main emphasis on the recovery of inflammatory mediators.

PRINCIPLES OF MICRODIALYSIS

The theory of microdialysis has been described in previous reviews (7–9). Briefly, the basic components needed for a microdialysis experiment are a microdialysis probe, a perfusion pump, a perfusion fluid, and a sampling device. The microdialysis probe consists of a hollow membrane that is slowly perfused with a physiological solution. The membrane wall is permeable to water and small molecules, and mass transport occurs across the membrane depending solely on the concentration gradient. This concept implies that diffusion can occur in both directions, i.e., mediators and metabolites can be recovered from the tissue and drugs and other compounds can be added to the perfusion medium (retrodialysis) to induce inflammatory tissue reactions or to pharmacologically modulate these reactions (Fig. 1) (10).

The small-volume samples from microdialysis are often collected in microvials with or without a cooled fraction collector. Sample analysis is a critical step of microdialysis, and various methods for microdialysis analysis exist that differ in sensitivity and specificity. The analytical bioassays applied in microdialysis experiments have mainly included enzyme-linked immunosorbent assays, radioimmunoassays, spectrophotometric assays, or high-performance liquid chromatography. Other approaches for identifying the mediators in microdialysis samples are to use microsphere-based protein micro assays (5, 6), electron-spin resonance (11), immunoaffinity capillary electrophoresis, capillary electrochromatography (12), or mass spectrometry-based proteomics (13–15). Taken together, it is valuable to emphasise that appropriate development and application of an analytical method that can allow for detection of mediators within small aliquots and low concentration is a critical step in microdialysis, which requires time and step optimisation to achieve a proper set up prior to application.

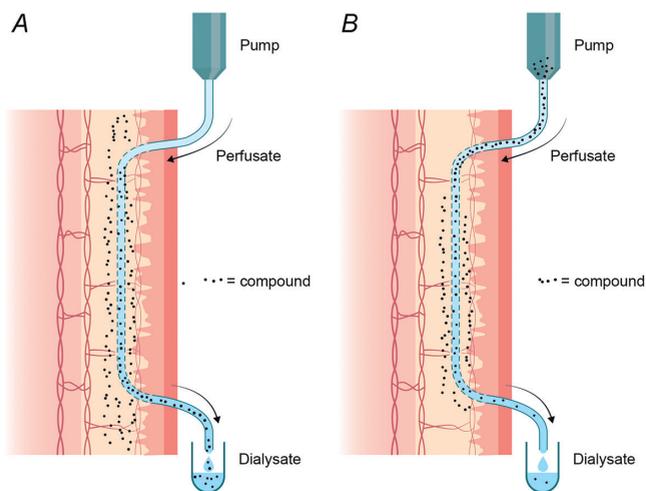


Fig. 1. Basic principles of microdialysis and retrodialysis. Part A illustrates the basic principle of microdialysis. A physiological buffer (the perfusate) is slowly flushed through the dialysis probe. Diffusible compounds within the extracellular compartment of the skin diffuse by the concentration gradient across the dialysis membrane into the lumen of the microdialysis probe, and the compounds can be collected in the fluid at the outlet (the dialysate). Part B demonstrates the principle of retrodialysis with infusion of diffusible compounds (drugs or substances) added to the perfusate. Here the compounds diffuse from the lumen of the dialysis probe into the skin where the compounds can exert their effects. Reproduced from Melgaard et al. (10) with permission from Karger Publishers.

EXPERIMENTAL STUDIES IN ANIMALS

Originally being developed for studies of neurobiology in animal brains (16, 17), microdialysis has been applied as a research method to investigate a wide range of inflammatory mediators in peripheral tissues in animals. Animal models of skin inflammation have certainly assisted in significant development, improvement and validation of the technique itself during the last decade. It is now widely acceptable that this method is a versatile, safe and valuable tool for skin inflammatory investigations in animals including a wide range of anti-inflammatory drugs for preclinical investigations in animal skin (18–21).

The majority of studies in animals have been applied in a variety of *in vivo* models. However, *ex vivo* or skin specimens have also been used for inflammatory mediator research in animal skin. For instance, superficial implantation of microdialysis probes in an isolated perfused bovine udder model has been used to study experimental inflammation. Topical application of arachidonic acid led to the release of a number of eicosanoids of which prostaglandin E2 (PGE2) was measured by microdialysis (22). The bovine udder model was also used to study inflammatory reactions to biodegradable titanium implants (23). In the latter application, the absence of notable inflammation was confirmed by measuring PGE2 and tumour necrosis factor- α (TNF- α) levels. The most common applications are discussed in the following sections. Examples of such studies are summarised in Table I.

Table I. Inflammatory mediators or substances recovered from inflammatory skin conditions by microdialysis in animal studies

Inflammatory mediator	Animal model	Reference
Adenosine	Rat	(33–36)
Albumin	Rat	(24–26)
Alpha-melanocyte stimulating hormone	Rat	(43)
Bradykinin	Rat	(139)
Calcitonin-gene related peptide	Rat	(95, 140)
Eicosanoid synthesis	Mouse	(22)
Glutamate	Rat	(42)
Histamine	Rat	(30, 31)
	Pig	(29, 32)
	Dog	(18)
Interleukin-6	Rat	(43)
Lactate	Mouse	(48)
Nitric oxide	Mouse	(39)
	Rat	(37, 38)
Prostaglandin D2	Dog	(18)
Prostaglandin E2	Rat	(37, 43)
	Cow	(22, 23)
Prostaglandin I2	Rat	(37)
Substance P	Rat	(44, 95)
Tumour necrosis factor- α	Cow	(23)

Vascular permeability

Inflammation is the body's key immune response, with oedema being a pivotal sign of inflammation. Changes in plasma protein extravasation in rat skin during inflammatory challenges have been evaluated using microdialysis (24). Dermal extravasation of ^{125}I -labeled human serum albumin and different-sized endogenous plasma proteins was studied during continuous administration of docetaxel and prostaglandin E1 in anaesthetised rats. The different pattern of protein extravasation after the administration of the vasoactive compounds indicated differential effects on the interstitial transport rate and capillary permeability. This topic was further studied with the local administration of platelet activating factor (25). Platelet activating factor influenced several aspects of protein extravasation, transcapillary fluid flux, and interstitial fluid pressure, indicating a role for platelet activating factor in the development of oedema. Microdialysis has also been successfully used for the continuous measurement of plasma protein extravasation in rat and mouse skin after various inflammatory drug challenges (26, 27). Additionally, capsaicin-evoked neurogenic plasma extravasation and the effect of topically applied acetylsalicylic acid and dipyron have been studied using the method (21).

Activation of protease-activated receptors (PARs) can induce vasodilation and increase the vascular permeability either directly, by stimulating endothelial cells, or indirectly, via the activation of nociceptors and the subsequent release of neuropeptides (28). The relative contribution of the 2 pathways in stimulating endogenous activators of PAR-2 (trypsin) and of PAR-1, 3 and 4 (thrombin) was studied in rat skin using dermal microdialysis. The results indicated that trypsin induced neurogenic inflammation via PAR-2 activation of

nociceptors and the subsequent neuropeptide release, whereas thrombin-induced plasma extravasation and vasodilation were mediated mainly by a non-neurogenic mechanism.

Immediate hypersensitivity

Mast cells are known to be one of the key effector cells of immediate-type allergic reactions, which upon degranulation release both preformed and newly generated mediators such as histamine, prostaglandins, leukotrienes, and pro-inflammatory cytokines. Histamine release as well as prostaglandin synthesis have been investigated following mast cell activation in guinea pigs (29), rats (30, 31), and dogs (18). Following allergenic provocation in guinea pig skin, through the subcutaneous injection of compound 48/80 as well as the intravenous administration of ovalbumin (32), histamine levels increased significantly. The effect of cyclosporin A on the allergen-induced release of histamine and prostaglandin D2 (PGD2) synthesis has been investigated in dogs (18). Whereas orally administered cyclosporine gradually reduced the allergen-induced histamine release and wheal formation over 30 days of treatment, the rate of PGD2 synthesis surprisingly remained unchanged.

Inflammatory pain

Adenosine may play a regulatory role in pain and inflammation in peripheral tissues (33). The release of adenosine in response to injections of formalin or glutamate or to spinal nerve ligation has been studied (33–36). Glutamate administration evoked peripheral adenosine release in the rat hindpaw (Fig. 2). Peripheral ionotropic glutamate receptors on unmyelinated sensory afferents were involved in this release (33). Glutamate receptors have been identified on the peripheral terminals of both primary sensory afferents and

sympathetic post-ganglionic neurons, and activation of these receptors produces peripheral sensitisation and enhances nociception (33). Following nerve injury, peripheral capsaicin-sensitive primary sensory afferent nerve terminals have shown to release notable amounts of adenosine in rat hindpaw. Sympathetic postganglionic afferents did not appear to be involved in such release. The lack of effect of inhibitors of adenosine metabolism following spinal nerve ligation (34) suggested the existence of an altered peripheral adenosine system. Mast cells did not contribute to the release of adenosine in formalin-induced inflammation (35). The ability of an adenosine kinase inhibitor and an adenosine deaminase inhibitor to modulate formalin-induced adenosine release was dependent on the concentrations of the substrate adenosine (36).

Nitric oxide (NO) and prostaglandins are key mediators of inflammatory pain. The effects of peripherally released NO on cyclooxygenase (COX) expression/activation and the production of prostaglandins in carrageenan-induced inflammation in the rat hindpaw were examined by Toriyabe et al. (37). NO activated COX-1 during the early phase of carrageenan-induced skin inflammation and it up-regulated COX-2 expression during the late phase. COX-2 expression led to the production of PGE2 and PGI2 at the site of inflammation, which exacerbated the inflammatory process. Omote et al. (38) used microdialysis to show that NO release was mediated by neuronal nitric oxide synthase (NOS) during the early phase and by both neuronal NOS and inducible NOS during the late phase of carrageenan-induced inflammation. Different compounds have been used to induce NO release, for example bradykinin in mouse skin (39).

Neurogenic inflammation has also been explored using microdialysis (40, 41). The induction of temporary pain and the sustained increase of the local skin temperature by the honeybee toxin, melittin, were

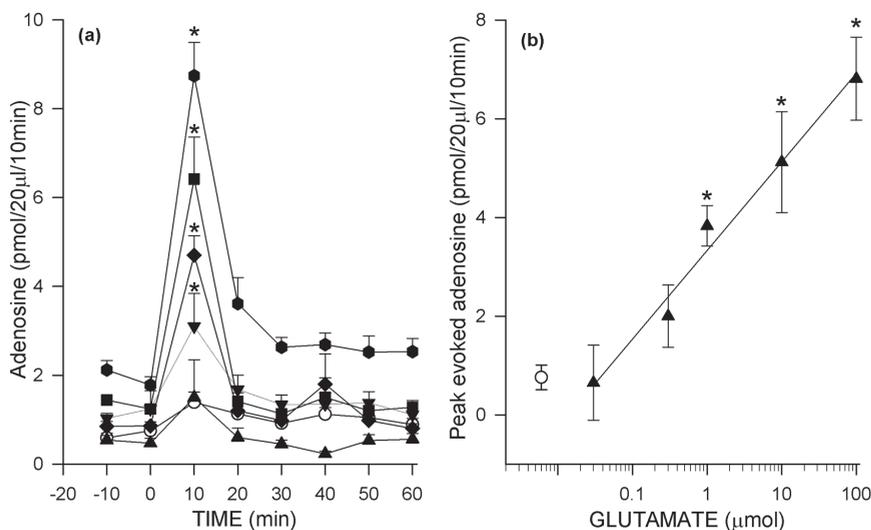


Fig. 2. (a) Time course of s.c. injection of l-glutamate into the plantar surface of the hind paw on peripheral adenosine release. L-Glutamate (L-Glu, 0.03–100 μmol) was injected at time zero in a volume of 50 μl . * $p < 0.05$ compared to basal level at time zero, one-way repeated ANOVA followed by SNK. (b) Dose-response curve for l-glutamate-evoked release of adenosine. Peak evoked release is calculated as the adenosine level at time 10 min minus the respective basal adenosine level. * $p < 0.05$ compared to saline group (one-way ANOVA followed by SNK, mean \pm SEM; $n = 4$ –5 per group, except for L-Glu 1 μmol , where $n = 12$). \circ Saline; \blacktriangle L-Glu 0.03 μmol ; \blacktriangledown L-Glu 0.3 μmol ; \blacklozenge L-Glu 1 μmol ; \blacksquare L-Glu 10 μmol ; L-Glu 100 μmol . Figure and legend are reproduced from (33) with permission from John Wiley and Sons.

suppressed by pre-treating rat skin with lidocaine. The melittin-induced increase of the skin temperature was enhanced through the activation of peripheral N-methyl-D-aspartate receptors by the locally released glutamate, contributing to the neurogenic inflammation (42).

Contact dermatitis

Irritant chemicals such as jet fuel, xylene, nonane, dodecane, and tetradecane have been used to elucidate inflammatory reactions in skin. In an attempt to understand the skin irritation cascade induced by selected aliphatic hydrocarbons, microdialysis was applied to the skin of hairless rats (43). The contents of substance P, alpha-melanocyte stimulating hormone, interleukin (IL)-6 and PGE2 were analysed after dermal exposure to nonane, dodecane and tetradecane. In this study, microdialysis proved useful as a sensitive technique for measuring inflammatory biomarker levels over time. The release of inflammatory mediators has also been quantitatively analysed in rat skin after topical exposure to jet fuel and xylene (44). Xylene, but not jet fuel, elicited a significant release of substance P. Pretreatment with a substance P antagonist significantly blocked the xylene-induced substance P release. The presence of calcitonin gene-related peptide (CGRP) was examined, but it was not detected in any of the samples.

Wounds and tissue repair

Histamine is known to be involved in wound healing and tissue repair. Guo et al. (45) analysed the histamine levels in surgical skin flaps in mast-cell deficient mice and control animals. Skin flap survival was significantly improved in mast cell-deficient mice in which the histamine and myeloperoxidase levels were lower than in control animals. Antihistamine treatment only partially reduced leukocyte infiltration in the mast cell-sufficient animals. Thus, mast cells and histamine may be involved in the accumulation of leukocytes and in tissue necrosis in the skin flaps.

Histamine may be an important mediator of oedema development after thermal injury or skin burns. Histamine release has been documented in superficial burn sites in pigs (46). A burn-depth-related increase in histamine appeared during the first 2 h post-burn, followed by a second increase at 12–24 h. The plasma histamine concentrations were not elevated at any time, indicating the ability of microdialysis to detect inflammation at the target level. Similar studies of human skin have failed to demonstrate histamine release in superficial burn lesions (47).

Wounds accumulate lactate as a consequence of both anaerobic and aerobic glycolysis following disruption of microcirculation, immune activation, and increased cell proliferation (48). Using microdialysis, Porporato et al. (48) showed that lactate improved reperfusion

and opposed muscular atrophy in ischaemic wounds on mice hind limbs. Both of these responses involved lactate-induced reparative angiogenesis, and the closure of excisional skin wounds. Recently, microdialysis has been used to study the pathophysiology of aberrant wound healing in the horse (15).

EXPERIMENTAL STUDIES IN HUMANS

Inflammatory skin reactions can be induced by exposure to chemical compounds, physical damage, ultraviolet radiation, or topical irritants. Inflammation can also be induced by infusion of a variety of agents with the perfusate (retrodialysis), including cholinergic agonists (10, 49, 50). Besides the *in vivo* models, similar to animal studies, skin specimen's models exist for human skin *ex vivo* investigation. Intact skin specimens obtained from surgical patients were found to be an excellent resource for studies of histamine release in human skin without the requirement of dispersing the mast cells (51, 52). The chemokines monocyte chemoattractant factor-1, RANTES, and macrophage inflammatory protein-1 α cause the release of histamine from human basophils and dispersed rodent mast cells, but their effect on human skin mast cells remains unclear. Petersen et al. (51) used microdialysis of human skin specimens to show that these chemokines did not directly affect histamine release or interfere with IgE-mediated mast-cell activation.

Microdialysis has been applied to investigate cytokine and/or neuropeptide release in inflammation due to ultraviolet B (UVB) exposure (53), ultraviolet exposure-based inflammation with or without thermal injury (5), and in allergic contact dermatitis in human skin (54). For example, the release of prostaglandins, leukotrienes, monohydroxyeicosatetraenoic acids, and other mediators has been shown in a UVB model (55).

In the following sections, examples of experimental inflammatory studies of human skin will be presented (see also Table II).

Vascular permeability

Leakiness of vessels is a key feature of inflammation. Plasma extravasation has been studied in detail in immediate hypersensitivity and neurogenic inflammation (40, 56, 57). NO is a potent vasodilator that is involved in local inflammatory responses. Direct measurements of NO release have been obtained in the human skin (58). Numerous studies have used the local delivery of NO-synthase inhibitors by retrodialysis to investigate the role of NO in the vasoactive effects of acetylcholine, sodium nitroprusside, histamine, and bradykinin (50, 59). The NO synthase inhibitor N-nitro-L-arginine-methyl ester inhibited substance P-induced vasodilation and protein extravasation, but it only slightly inhibited CGRP-induced vasodilation. These results

Table II. *Inflammatory mediators or substances recovered from inflammatory skin conditions by microdialysis in experimental human studies*

Inflammatory mediator	Reference
Angiogenin	(53)
Beta-thromboglobulin	(102)
Bradykinin	(84)
Brain-derived neurotrophic factor	(98)
Calcitonin gene-related peptide	(56, 96)
Eosinophilic cationic protein	(73)
Granulocyte colony-stimulating factor	(5)
Histamine	(76–78, 83, 88, 100)
Hyaluronan	(105)
Interleukin-1 α	(70)
Interleukin-1 β	(5, 53, 70)
Interleukin-2	(5, 53)
Interleukin-4	(53)
Interleukin-5	(5, 53)
Interleukin-6	(5, 53, 70)
Interleukin-7	(5)
Interleukin-8	(5, 53, 70)
Interleukin-10	(5, 53)
Interleukin-12	(5, 53)
Interleukin-13	(5)
Interferon- γ	(5, 53, 70)
Interferon- γ inducible protein 10	(53)
Leukotriene B4	(73)
Leukotriene C4	(75)
Macrophage inflammatory protein-1 β	(5)
Monocyte chemoattractant protein-1	(5)
Monocyte inflammatory protein-1 β	(5)
Monohydroxyeicosatetraenoic acids	(55)
Myeloperoxidase	(73)
Nerve growth factor	(5, 98)
Nitric oxide	(58, 59, 90, 101, 141)
Prostaglandin D2	(73, 92)
Prostaglandin E2	(101, 141)
Prostaglandin F2 α and isoprostanes	(55)
Substance P	(56, 95)
Transforming growth factor- β 1	(53)
Tryptase	(56, 73, 83)
Tumour necrosis factor- α	(5, 53, 70, 97)
Uric acid	(104)
Vascular endothelial growth factor	(53)

indicated that substance P-induced vasodilation and protein extravasation is at least partly mediated by NO, whereas CGRP-induced vasodilation appears to be NO-independent (60). CGRP is degraded by a neutral endopeptidase that can be blocked by phosphoramidon. The role of CGRP in acetylcholine-induced vasodilation has been investigated by delivering phosphoramidon. Applying phosphoramidon significantly increased the flare size due to increased CGRP levels in the skin (61). Microdialysis has been extensively used to study the regulation of the vascular tone in health and disease, including during sub-clinical inflammation in the elderly and in early atherosclerosis (62–64).

Bradykinin provokes vasodilatation and an increase in endothelial permeability. The vascular response to the activation of bradykinin B1 and B2 receptors has been explored in the normal and the UVB-irradiated skin of healthy volunteers (65). In normal skin, both B1 and B2 receptor activation dose-dependently

evoked pain, vasodilatation and protein extravasation. In UVB-irradiated skin, B1 and B2 receptor activation enhanced pain sensation, whereas local vasodilatation was increased only following B1 receptor stimulation. UVB irradiation did not enhance B1 and B2 receptor-induced protein extravasation, suggesting a differential sensitisation of these neuronal responses (65).

Immediate hypersensitivity

Mast cells play a central role in inflammatory processes via the release of stored inflammatory mediators such as histamine and tryptase, newly synthesised mediators such as prostaglandins and leukotrienes and a variety of other inflammatory and pro-inflammatory compounds (66). Elevated blood flow, redness (flare), and plasma extravasation, which lead to oedema (wheal), are the key clinical signs of mast-cell activation. The exact roles of histamine and the other inflammatory mediators in each of these clinical manifestations have been studied in detail. Injecting sensitised subjects with allergens such as grass pollen, house dust mites, or bee venom induces notable histamine release (67–71), and this release was highly correlated with skin reactions in most of the studies. Allergen-induced PGD2 synthesis was demonstrated by some investigators (72, 73) but not by others (71). Similarly, Church et al. (71) failed to detect cysteinyl leukotrienes upon atopic allergen provocation in volunteers, whereas leukotriene C4 release in the skin was demonstrated by others (74, 75). The conflicting results may be due to the low molar amounts of the latter compounds compared with that of released histamine. Immediate hypersensitivity has been an area of great interest for investigation using skin microdialysis. The first such papers were published in 1992 (76, 77), and the number of reports in PubMed in which microdialysis was used to obtain data regarding histamine now exceeds 80. A detailed description of the use of microdialysis in studies of allergic inflammation is beyond the scope of this review (66, 78). However, some illustrative examples should be mentioned. The ability to use microdialysis to simultaneously study mediator release and skin reactions allowed investigators to study the mechanisms of action of anti-allergic drugs. It has been debated for years whether antihistamines impaired the release of inflammatory mediators or simply blocked the effect of the released mediators. While H-1 antihistamines reduced wheal and flare reactions, they did not reduce the level of allergen- or codeine-induced histamine release or that of PGD2 synthesis in the underlying skin during experimental studies (73, 79, 80). The ability of microdialysis to differentiate between the effects on the vasculature and those of the release of inflammatory mediators has also been applied in studies of glucocorticosteroids (81), beta-2 agonists (72, 82), muscle relaxants (83, 84), or heparin (85).

Another related area of interest is the pathophysiology of inflammatory reactions, in particular the effect of neuropeptides on mast cell secretion. Histamine release has been associated with substance P, vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide, and somatostatin (52, 86–88). Weidner et al. (88) performed a pivotal study of the mechanism of action of substance P and CGRP that showed the competitive edge obtained using microdialysis (Fig. 3). Whereas numerous agents induce skin reactions that are indicative of mast-cell activation, experimental studies using bradykinin (80, 89), endothelin-1 (90), cholinergic agonist (49), platelet-activator factor (91, 92), and capsaicin (87, 93) have detected little if any histamine release.

Inflammatory pain

There has been great interest in the use of microdialysis to study dermal pain, hyperalgesia, and itch (41). Skin inflammation sensitises peripheral nerve endings, which may lead to hyperalgesia and spontaneous pain. In addition, the release of sensory neuropeptides may potentiate inflammation through an increase in blood flow, swelling, and the release of mediators, as reviewed in the previous section. Substance P has been considered a pivotal neuropeptide for mediating inflammatory pain. However, studies have failed to demonstrate substance P release in capsaicin-induced neurogenic inflammation (56, 93). These data are supported by studies showing little if any mast cell degranulation following retrograde nerve stimulation or capsaicin treatment (56, 87, 93, 94). Strong electrical stimulation has been shown to cause the release of minute amounts of neuropeptides, including substance P (95). CGRP is another important neuropeptide that is released from sensory

nerves, which plays a significant role in neurogenic inflammation. A significant increase in the CGRP level was found in histamine-induced and capsaicin-induced flare reactions (56, 96). Likewise, CGRP may be released upon electrical stimulation, although conflicting results have been observed, likely due to variations in the stimulus conditions (95, 96). Thus, different types of inflammation or nerve stimulation may induce differential biological responses. Eberle et al. (97) showed an increase in the proinflammatory cytokine TNF- α in the skin after mechanical but not electrical stimulation despite comparable skin reactions.

In addition to neuropeptides, several other mediators are likely key players in inflammatory pain. Neurotrophins are important for the induction and maintenance of hyperalgesia. Increased levels of neurotrophins in inflamed human skin were found using dermal microdialysis, indicating those that may contribute to peripheral sensitisation (98). PGE2 is an inflammatory mediator that facilitates nociceptive signals. A significant increase in the level of PGE2 was found following intradermal injection of sodium dodecyl sulphate (99). Similarly, whereas histamine plays a key role in the immediate phase of inflammation that is elicited by topical 5-aminolaevulinic acid photodynamic therapy (100), PGE2 and NO are released during the prolonged erythema phase (101). The involvement of PGD2 and NO in this phase was confirmed by blockade using topical indometacin, a non-selective COX inhibitor and by injection of the NOS inhibitor, NG-nitro-L-arginine methyl ester, which significantly reduced the erythematous reaction (101).

Muscle relaxants are known to cause pain upon administration, but the pathophysiology of this phenomenon is largely unknown. Koppert et al. (83) perfused dermal microdialysis catheters with 8 different muscle relaxants

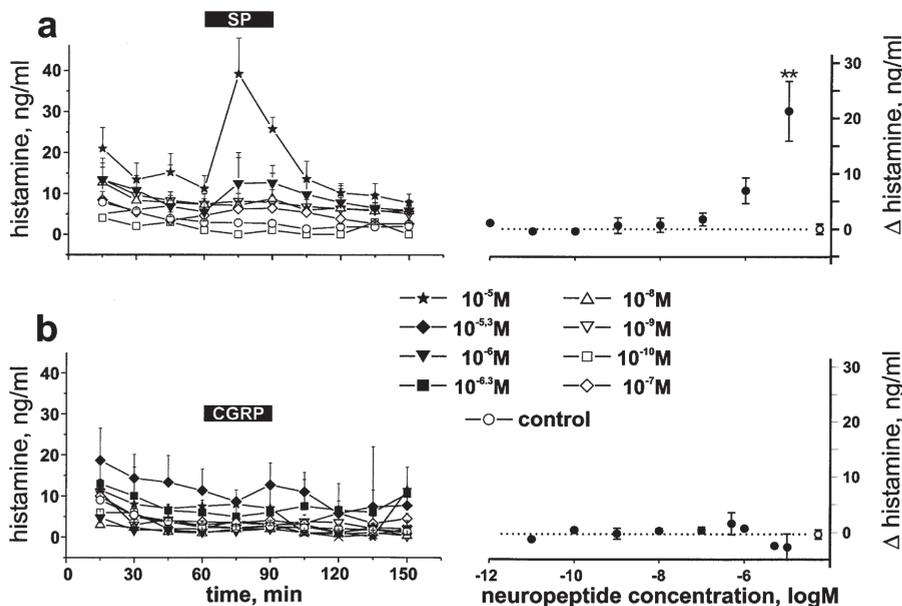


Fig. 3. Only SP, but not CGRP, induced histamine release in human skin. The time course of histamine concentration in the dialysate following application of SP (a) or CGRP (b) via the membrane (black bar) is shown. In the right panels mean increase in histamine concentration during the stimulation period is depicted. Control values (stimulation with Ringer's solution) are indicated by a dotted horizontal line (mean \pm SEM). Only SP in a concentration of 10⁻⁵ M provoked a significant release of histamine (** p < 0.01; ANOVA, Scheffé post hoc test). Figure and legend reprinted by permission from Macmillan Publishers Ltd: Journal of Investigative Dermatology (88), copyright (2000).

and studied the dose-response curves of the vascular and sensory effects. Succinylcholine and the isoquinolines differed from aminosteroids with regard to mediator release and pain reactions. Dose-response retrodialysis of rocuronium and vecuronium also demonstrated a dissociation of the inflammatory mediator release profile and the pain reactions (84).

The multiplex protein microarray technique has provided the opportunity to measure the amounts of several cytokines that are involved in inflammatory skin reactions in low-volume samples using large-pore microdialysis probes (53, 70). Angst et al. (5) coupled microdialysis with a protein microarray technique to study cytokines involved in inflammatory pain. They studied cytokine release in UVB-inflamed skin and skin exposed to noxious heat and examined the effect of the COX-inhibitor ibuprofen on hyperalgesia and mediator release. Increased levels of IL-1beta, IL-6, IL-8, IL-10, granulocyte colony-stimulating factor, and macrophage inflammatory protein-1beta were observed upon UVB inflammation, whereas noxious heat induced the release of IL-7 and IL-13. Oral ibuprofen produced anti-hyperalgesic effects and led to the dose-dependent reduction of the tissue levels of IL-1beta and IL-6. Noxious heat has been shown to induce mast cell activation in rodent studies (46) but not in human studies (47). In humans, noxious heat causes the release of beta-thromboglobulin and protein extravasation, indicating that platelets play a role in these reactions (102). Please refer to a recent review on the use of microdialysis for the study of pain and itch (41).

Wound and tissue repair

Microdialysis has been used to evaluate metabolic perturbations and vascular changes during wound healing and tissue repair (103). The majority of studies have been performed in patients with burn injuries or chronic wounds (see later). Microdialysis has been used to study the association between uric acid, one of the major antioxidants in the skin, and ageing in the skin of healthy subjects (104). The difference between the uric acid levels of the young group and the aged group were not statistically significant. Hyaluronan, a major component of the cutaneous extracellular-matrix, is likely involved in tissue repair. Increased accumulation of hyaluronan degradation products were observed at 24 h after exposure in an experimental UVB-induced inflammation (105). To date, the potential of microdialysis in assessing local tissue metabolism in wound research remains largely unexplored.

Miscellaneous

Inserting a microdialysis fibre into the skin elicits some inflammatory changes. The vascular perturbation lasts approximately 1–2 h, as measured using laser Doppler

methods (54, 106). Immediately after the insertion, the histamine level is high but it reaches a plateau at approximately 40 min (76). Inserting a microdialysis probe can also provoke changes in cytokine production. The release of IL-6, IL-8, TNF- α , and IL-1b has been shown after probe insertion (53, 70, 107, 108). Cytokine production that is induced by needle trauma may last for up to 48 h after probe insertion (53, 107). It is important to consider these aspects when designing microdialysis studies (5, 70).

CLINICAL STUDIES

Microdialysis has been applied in studies of a number of skin diseases, including psoriasis, atopic dermatitis, and urticaria. Examples of the application of microdialysis to skin within clinical settings are provided below (also presented in Table III).

Psoriasis

Psoriasis is a chronic skin disease resulting from abnormal immune function that is characterised by the presence of scaly psoriatic plaques. The psoriatic plaques contain mast cells, which are increased in abundance in the uppermost dermis of the psoriatic lesion (109). The pathophysiology of mast cells in psoriasis is controversial. Studies have demonstrated that histamine levels are significantly increased in the lesional psoriatic skin compared to the non-lesional skin of patients (110) and is also increased in the lesional skin compared of the skin of healthy controls (109), although conflicting results have been presented (111). Microdialysis samples taken from the non-lesional and lesional skin of patients with psoriasis have indicated perturbations in the release of key cytokines (112). In addition, psoriatic skin exhibited a significantly increased release of histamine to codeine and substance P in the lesional sites, and these responses returned to normal following the administration of the H2 antihistamine, ranitidine (109).

Exposure to capsaicin, even at doses that cause intense pain, did not lead to the release of histamine in normal human skin (87, 93). However, studies of psoriasis patients (113) revealed a significant although modest increase in histamine in response to topical capsaicin and no normalisation after local anaesthesia (110, 113, 114). Therefore, the role of histamine and mast cells in psoriasis remains unclear.

Atopic dermatitis

Atopic dermatitis is a common itchy inflammatory skin disease, but the mediators responsible for the pruritus remain largely unknown. Several substances that are involved in atopic dermatitis (e.g., histamine and PGE2)

have been studied using cutaneous microdialysis. Both the inflammatory and the sensory skin reactions to infused PGE₂ have been studied in atopic dermatitis patients compared with healthy controls (115). PGE₂ dose-dependently provoked intense local vasodilation, weak pruritus, and pain but no protein extravasation when measured using microdialysis. However, no difference was found between the patients and healthy controls for any parameter (115).

The role of mast cells, and of histamine in particular, in atopic dermatitis is unclear. A series of clinical trials have failed to show that non-sedative antihistamines lead to a clinically meaningful reduction of itch in atopic dermatitis patients. Protein extravasation that was induced by histamine and the mast activator, compound 48/80, was significantly reduced in atopic dermatitis patients (116). Pruritus in atopic dermatitis patients was unchanged after H₁ blockade, suggesting that mast cell mediators other than histamine are most likely involved in pruritus in these patients (116). This hypothesis was further supported by findings that antihistamines completely abolished itch induced by codeine, an opioid that generally causes the release of histamine, in healthy controls but not in atopic dermatitis patients (117). Steinhoff et al. (117) suggested that another mast cell mediator, tryptase, may be involved in itch. Tryptase, a neuronal proteinase-activated receptor-2 activator, which was increased 4-fold in lesional atopic skin, was found to be increasingly expressed, primarily in afferent nerve fibres in skin biopsies, and

it caused enhanced and prolonged itch compared with that of controls upon intradermal injection. Apparently, nerve fibres in atopic dermatitis patients respond with itch sensations to a variety of stimuli that induce pain in healthy controls and in psoriasis patients (118). Thus, the relationship between mast cells and afferent nerves appeared altered in atopic dermatitis patients. This difference may also include the expression of nerve growth factors. However, the serum and eczematous skin levels of nerve growth factor in patients were recently shown to be significantly lower than those of controls (119). Fluctuations in nerve growth factor levels during a day suggest a complex modulation of this neurotrophin that is potentially linked to stress or to an altered neurophysiological mechanism (119).

Among other findings that were obtained using microdialysis in atopic dermatitis patients are high levels of iron and low levels of ascorbic acid, a catalyst and a scavenger for reactive oxygen species, respectively, compared to those of healthy subjects examined using microdialysis (120).

Urticaria

Wheal reactions and itch are key features of the many subtypes of urticaria. As originally described by Anderson et al. (121), histamine was found to be released in cold urticaria during the ice-cube challenge. Increased histamine was also observed in lesional vs. non-lesional skin in mastocytosis (122). A pilot study of nettle-

Table III. Examples of compounds recovered by microdialysis in inflammatory skin conditions and diseases

Compound	Condition/disease	Reference
Ascorbic acid	Atopic dermatitis	(120)
Complement 3a	Burn injuries	(135)
Cortisol	Burn injuries	(134)
Glucose	Burn injuries	(132, 135)
Glycerol	Burn injuries	(132, 135)
Granulocyte-macrophage colony-stimulating factor	Psoriasis	(112)
Histamine	Atopic dermatitis	(117)
	Cold urticaria	(121, 124, 126)
	Cutaneous mastocytosis	(122)
	Psoriasis	(109–111, 113)
Interleukin-1 β	Psoriasis	(112)
Interleukin-6	Psoriasis	(112)
	Cold urticaria	(126)
Interleukin-8	Psoriasis	(112)
	Cold urticaria	(126)
Iron	Atopic dermatitis	(120)
Lactate	Burn injuries	(132, 135)
	Complex regional pain syndrome	(127)
Nerve growth factor	Atopic dermatitis	(119)
Noradrenaline	Complex regional pain syndrome	(131)
Pyruvate	Burn injuries	(132)
Serotonin	Burn injuries	(133)
Tryptase	Atopic dermatitis	(117)
Tumour necrosis factor- α	Psoriasis	(112)
	Cold urticaria	(126)
Urea	Burn injury	(132)

induced urticaria showed immediate discomfort and stinging pain but marginal histamine release and no leukotriene release (123). The absence of leukotriene release was directly confirmed in cold urticaria using microdialysis samples and indirectly by the absence of a clinical effect of montelukast, a leukotriene antagonist (124). Recently, Kring Tannert et al. (125) performed an elegant study showing normalisation of histamine release in the skin of patients with cold-test urticaria after successful cold desensitisation but the maintenance of its releasability to codeine treatment. This year, Krause et al. (126) demonstrated that the H-1 antagonist, bilastine, reduced the skin reactions in cold-contact urticaria as well as the releases of histamine, IL-6, and IL-8 in cold-contact urticaria. These data are in contrast to the findings using other non-sedative antihistamines in healthy controls. Thus, experimental studies in healthy volunteers may not mimic the conditions in specific diseases.

Inflammatory pain

Complex regional pain syndrome (CPRS) is characterised by hyperalgesia, spontaneous pain, oedema, increased skin temperature, reddening, and trophic changes, which indicate a localised inflammatory process. Metabolic disturbances with an increased level of skin lactate have been shown using microdialysis in CPRS patients (127). Dermal microdialysis in combination with electrical C-fibre stimulation or infusion of substance P has been employed to induce direct or indirect neurogenic inflammation. Substance P, but not electrical stimulation, led to protein extravasation, whereas axon reflex vasodilation was significantly enhanced even on the patients' unaffected limbs (128, 129). These findings support the hypothesis that facilitated neurogenic inflammation is a predisposing factor for CPRS (130). An abnormal response to sympathetic activation and deactivation may be present in CPRS. Thus, dermal microdialysis was used to monitor noradrenaline levels in the skin during whole body cooling and heating (131). The noradrenaline responses of patients and controls did not differ, indicating the preserved function of the cutaneous sympathetic postganglionic fibres in CPRS.

Wounds and tissue repair

The minimal invasiveness of microdialysis offers great opportunities for wound research (103). Samuelsson et al. (132) performed a series of studies of burn injuries, e.g., a continuous assessment of skin metabolic changes during fluid resuscitation and during up to 4 days post-burn in patients with major burn injuries. The level of skin glucose continued to increase throughout the study period in the patients, and the controls had significantly lower skin glucose levels compared with the burn pa-

tients. In contrast, the level of lactate was significantly higher than that of the controls in both the injured and uninjured skin of the patients. The skin lactate/pyruvate ratio and glycerol level were significantly increased in burn patients. Microdialysis revealed local metabolic processes that are not fully appreciated when examined using venous blood sampling. The same group showed increased skin levels of serotonin in both the uninjured and burned skin of patients compared with controls. The findings of significantly elevated tissue serotonin concentrations, compared to the levels in the blood and urine, suggested that serotonin is a key mediator in local vascular control and the development of oedema (133).

Assessing the adrenal function of critically ill patients is problematic, and there is evidence to suggest that measuring tissue glucocorticoid activity may be more useful than estimating plasma cortisol concentrations in patients with burn injuries. Cohen et al. (134) measured the interstitial cortisol using microdialysis in subcutaneous tissue. The interstitial cortisol concentrations in burned and non-burned skin from patients with severe thermal injury were significantly elevated compared with those of healthy volunteers. There was no correlation between the tissue and plasma cortisol levels.

The role of complement 3a in burn injuries and following plastic surgery has also been studied. The concentration of complement 3a is continuously elevated in deep second-degree burn wounds, and this increase was likely related to the occurrence of significantly more thrombotic blood vessels in the deep dermal tissue of elderly patients (135). Complement 3a was identified as an early marker of reperfusion injury in patients who underwent lower leg reconstruction with free myocutaneous latissimus dorsi muscle (136). Given these results, complement 3a may be a highly sensitive early indicator of ischaemia-reperfusion damage.

Cystic fibrosis

Microdialysis has also been used to study the pathophysiological aspects of cystic fibrosis in patients who have sparse levels of vasoactive intestinal peptide (VIP) in their skin (137). VIP may play a role in reflex cutaneous vasodilation during body heating and therefore, the NO-dependent contribution to active vasodilation may be enhanced in the skin of patients with cystic fibrosis. Comparing the blood flow responses in the skin of patients and healthy controls using microdialysis indicated that the preservation of cutaneous vasodilation in patients with cystic fibrosis is not associated with enhanced NO-dependent vasodilation (137).

CONCLUSION

Skin microdialysis is a unique technique that has been extensively applied in studies of inflammatory reac-

tions in animals and humans. Microdialysis allows the simultaneous assessment of the temporal variation in the content of inflammatory mediators in the skin as well as monitoring vascular and sensory functions. Together with other objective techniques, such as laser Doppler imaging, microdialysis offers the opportunity to simultaneously monitor different physiological or pathological responses over time. Despite extensive use of microdialysis in the studies of inflammation in the skin, comparative studies with histology, plasma levels, skin blisters and other models are sparse. However, studies in burn injuries have shown exclusively metabolic perturbations of cortisol, serotonin, and histamine at the skin level without any notable changes in plasma levels (46, 133, 134). Studies in psoriasis have shown microdialysis sensitive to dermal changes in mediator levels with minimal perturbations in corresponding plasma levels (109, 110). Sjögren & Anderson compared cytokine findings in the skin by microdialysis and biopsies and found moderate to excellent agreement (138). Still, methodology studies may well be required to improve the translational value of microdialysis. In addition, technical challenges remain to be resolved due to the complexity of the analytical aspects, among others. Despite these challenges, microdialysis offers a relatively safe method to study the chronology of physiological, pathophysiological and pharmacological inflammatory mechanisms in intact skin in both animals and humans.

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