Neurogenic Inflammation in Human and Rodent Skin
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The combination of vasodilation and protein extravasation following activation of nociceptors has been termed “neurogenic inflammation.” In contrast to rodents, no neurogenic protein extravasation can be elicited in healthy human skin. Dermal microdialysis has considerably increased our knowledge about neurogenic inflammation in human skin, including the involvement of mast cells.

It has been nearly 100 years since Bayliss (2) described antidromic vasodilation following electric stimulation of centrally cut dorsal roots. Following this first description of efferent functions of afferent nerve fibers, our knowledge about their mechanisms has considerably increased. Various neuropeptides and the receptors responsible for their efferent functions have been identified. Pivotal peptides in the induction of neurogenic inflammation comprise calcitonin gene-related peptide (CGRP) and substance P (SP). However, a variety of neuropeptides, such as neurokinin A, neurokinin B, somatostatin, galanin, and, recently, endomorphins, have been found in primary afferent neurons.

Activation of thin nociceptive nerve endings in the skin generates action potentials that are conducted by their axons to the spinal cord, and after processing of this nociceptive information, finally a sensation of pain or itch may be generated in the central nervous system. However, the action potentials will also retrogradely invade the arborizations of the primary afferent neuron (“axon reflex”) and release neuropeptides from their terminals (15) (Fig. 1).

Neuronal basis of neurogenic inflammation

Chemical, thermal, and electric stimulation have been widely used to elicit neurogenic inflammation. As a result of previous studies, mechanoinensitive, but heat- and chemosensitive, C nociceptors have been found responsible for the neurogenic vasodilation in pig skin (9). Direct evidence of neuropeptide release has been obtained using capsaicin, the pungent constituent of hot chili pepper, as well as by antidromic electric nerve stimulation (6, 7).

It was generally believed that receptive fields of nociceptors in human skin are too small to account for the widespread flare that is elicited, for example, after a histamine stimulation. Therefore, a cascade theory was proposed that was based on the activation of cutaneous mast cells in the axon reflex flare reaction. This concept, which suggests that SP released from nociceptive terminals should activate cutaneous mast cells that in turn should activate chemonociceptors via the release of histamine, is described in detail elsewhere (8).

Neuropeptide-mast cell interaction

Studies of neuropeptide-mast cell interaction have, to a large extent, been performed in peritoneal or dispersed connective tissue mast cells in rodents. Furthermore, many in

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vivo studies have been performed in animals. However, during the last decade it has become obvious that mast cells in different species, and also within separate tissues in individual species, vary considerably regarding their morphology, biochemical composition, secretory function, and pharmacological susceptibility to drugs (1, 4).

Studies in rodents have described large variations in the susceptibility of mast cells to release vasoactive mediators on challenge with neuropeptides in different tissues as well as large variations within identical tissues in different species. In human mast cell subsets, mast cell degranulation by neuropeptides has been observed in skin mast cells only. Mast cells from the lung, adenoids, heart, and intestinal mucosa as well as blood basophils are unresponsive to neuropeptides (1, 4). Thus, of all human mast cells, it is a special feature of skin mast cells to degranulate on neuropeptide challenge.

A multitude of studies have suggested a role for mast cells in the development and maintenance of neurogenic inflammation in the skin (4, 5). The suggestive evidence for mast cell-nerve interactions is based on anatomic and physiological findings. Studies using immunohistochemical methods and electron microscopy have observed mast cells and nerves positioned in close proximity in different tissues, including the skin. Several lines of circumstantial evidence point toward a physiological role for mast cell-derived histamine in neurogenic inflammation. First, the key signs of neurogenic inflammation in rodent skin, plasma extravasation and vasodilation, are significantly reduced by H1 receptor antagonists (antihistamines). Second, neuropeptide-induced cutaneous inflammation is markedly reduced in mast cell-deficient mice. Third, the human reflex vasodilation (flare) to endothelin-1 and platelet-activating factor, neither of which release histamine from human dispersed skin mast cells in vitro, is reduced by antihistamines in human skin. Fourth, the flare reaction of intradermally injected SP directly parallels its capacity to release histamine from mast cells, and this response can be abolished by antihistamines. Fifth, topical capsaicin has been shown to degranulate human skin mast cells in situ. Finally, topical capsaicin inhibited plasma extravasation and reflex vasodilation to thermal provocation in patients with heat and cold urticaria.

However, there are a number of findings showing no role of histamine in neurogenic vasodilation. There is no close morphological relationship between the cutaneous mast cells and the dilating skin vessels, histamine is not released in venous outflow following chemical or electric nerve stimulation, and antihistamines do not reduce the flare response to intradermal capsaicin (4). In addition, there is growing evidence for non-H1 receptor effects of antihistamines (3) that obscure the interpretation of their inhibitory effects on neurogenic inflammation. The hypothetical activation of skin mast cells by endogenously released neurotransmitters has been based on indirect evidence and studies in rodents only. Until recently, no direct analysis of mediator release in human skin had been performed.

**Analysis of neurogenic inflammation**

A few approaches so far have tried to directly measure neuropeptide release in the skin, e.g., ex vivo analysis by superfusion of excised rat skin (6, 7). In most studies, neurogenic vasodilation and protein extravasation have been used to functionally assess nociceptor activation or regeneration. Vasodilation has been measured by laser-Doppler techniques and infrared thermography. Assessment of protein extravasation...
requires more invasive techniques, e.g., extravasation of albumin-bound dyes like Evans blue, which can be photometrically quantified ex vivo. In addition, radiolabeled albumin and in vivo microscopical detection of leaky endothelia have been employed. Noninvasive measurements of protein extravasation, such as assessment of wheal diameter or paw thickness, only yield limited and indirect information about intensity of protein extravasation. In humans, few attempts were made to directly assess protein extravasation. The most common parameter for neurogenic inflammation in humans is the axon reflex erythema. It is easily accessible, and the area can be analyzed with simple equipment. Laser-Doppler imaging techniques have further improved the analysis through simultaneous assessment of area and intensity of the vasodilation. In humans, recent advances in dermal microdialysis have opened new possibilities by allowing in vivo measurement of local mediator concentrations, including protein extravasation.

The involvement of histamine in dermal neurogenic inflammation as assessed by microdialysis technique

It has been clearly demonstrated that many of the peptides localized in sensory neurons in human skin can degranulate human skin mast cells. Thus SP, vasoactive intestinal polypeptide, somatostatin, pituitary adenylate cyclase-activating polypeptide, and other peptides induce histamine release from human skin slices and dispersed human skin mast cells in vitro (1) and elicit histamine release from human skin in vivo as measured by venous drainage methods and microdialysis techniques following intradermal injection (10). However, until recently it remained unsolved whether endogenous peptides are proficient histamine releasers in vivo.

Current studies have examined the extent of histamine release in the human wheal and flare reaction to various mast cell secretagogues (10). Intradermal injection of allergen,
opiates, and neuropeptides release histamine in microdialysis fibers at the site of injection. Histamine diffusion within the skin is restricted to 1–2 mm, and no histamine is detected in the periphery of the wheal. Microdialysis fibers located in the flare area, i.e., remote from the injection site, demonstrated no histamine release at all. Similar findings have been demonstrated using compound 48/80-induced tryptase release as the marker of mast cell activation (Fig. 2). Capsaicin, which is a potent activator of sensory nerves in the skin, elicited intense pain but no histamine release by either intradermal injection or by prolonged topical application (Fig. 3).

In additional studies, infusion of SP (10^{-8}–10^{-5} M) via microdialysis membranes in human skin induced dose-related protein extravasation and vasodilation with secondary release of histamine only at higher concentrations of SP (10^{-5} M). At SP concentrations of <10^{-5} M, the skin reactions could not be antagonized with H1 blockers, confirming that SP provokes neurogenic inflammation in human skin without the involvement of mast cell-derived histamine (16). Similarly, infusion of CGRP (10^{-8}–10^{-5} M) induced a lasting vasodilation without protein extravasation or histamine release. These results strongly indicate the absence of mast cell activation in neurogenic inflammation in normal human skin. It remains largely unsolved whether such events take place in inflammatory skin conditions.

In conclusion, the cascade theory hypothesizing the involvement of cutaneous mast cells in the axon reflex flare reaction apparently cannot be confirmed in human skin. This leaves us with an apparent mismatch of receptive field sizes of nociceptors and the size of the axon reflex. However, recent studies have indicated that the diameter of the receptive field of primary afferent chemonociceptors in human skin can reach up to 9 cm (14), which may well explain the extent of the axon reflex vasodilation.

The release of neuropeptides in dermal neurogenic inflammation as assessed by microdialysis technique

The apparent lack of mast cell activation in neurogenic inflammation in human skin indicates that the cutaneous vascular responses in neurogenic inflammation are directly elicited by neuropeptides. Release of neuropeptides by capsaicin, as well as by antidromic electric nerve stimulation, has been shown in animals. However, direct evidence for neuropeptide release in human skin is scarce. No release of SP could be detected in human skin by microdialysis technique either following histamine iontophoresis (13) or after intradermal injection of capsaicin in doses causing intense pain (10). This failure is most probably due to low concentrations of SP, the dilution effect inherent to the microdialysis technique.

**FIGURE 4.** Differences between electrically evoked neurogenic inflammation in rat and human skin. Transcutaneous electric stimulation at 4 Hz (30 mA, 0.2 ms) resulted in a significant increase in calcitonin gene-related peptide (CGRP) both in human and rat skin (top). However, only in rat skin was an increase in total protein detected at the site of electric stimulation; no difference in protein extravasation between stimulated and nonstimulated sites was detected in human skin (middle). SP concentration slightly increased in human and rat skin following electric stimulation (bottom).
Concluding remarks

In rodents, protein extravasation and vasodilation are the two main components of neurogenic inflammation. In human skin, exogenously applied SP (10^{-8} M) provokes protein extravasation and vasodilation. At high concentrations (>10^{-6} M), this occurs partly as a result of secondary histamine release from skin mast cells, and it then may be accompanied by an itch sensation and an axon reflex flare. In contrast, there is no evidence for protein extravasation or release of mast cell mediators in healthy human skin induced by the release of endogenous SP, even following strong chemical or electric stimulation (Figs. 3 and 4), suggesting that the endogenous SP levels in the skin do not reach 10^{-8} M even after supramaximal stimulation. Thus there is a clear mismatch of assumed endogenous SP levels in the skin (<10^{-8} M) and the threshold concentration to elicit sensory effects (itch) with SP (10^{-5} M), which provides clear evidence against a role of SP as a peripheral “pain” or “itch transmitter” in healthy human skin.

It remains to be clarified whether the lack of neurogenic protein extravasation in human skin can be attributed to lower SP concentrations in the neurons, magnitude of its release, or tissue sensitivity. Irrespective of the mechanism, this species difference corresponds to the failure of neurokinin-1 antagonists in the treatment of migraine (12), although they potently inhibit electrically evoked protein extravasation in rat dura mater (11). Dermal microdialysis experiments offer an elegant combination of local stimulation and mediator analysis with psychophysics and thus will considerably contribute to further elucidation of afferent and efferent functions of nociceptors.

References


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