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This paper clearly suggests that the mast cells of the skin may not be the only source of, and target of, histamine and histamine-related effects, respectively.

Histaminergic nerves demonstrated in the skin. A new direct mode of neurogenic inflammation?

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Abstract: An intradermal administration of histamine into human skin results in a local erythema, edema and often also the sensations of itch and/or pain. These effects have classically been attributed to the presence of histamine-containing mast cells. However, in the present investigation, we report the observation of histamine-immunoreactive nerves in the skin of Sprague-Dawley rats using a new and highly sensitive immunohistochemical approach. These data suggest a more direct route of cutaneous histamine effects, mediated exclusively by the peripheral nervous system. The findings could also give a new basis for explaining histamine-related issues, such as itch.

Key words: histamine – nerve fibres – neurogenic inflammation – mast cells – skin – rat

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Introduction

Histamine is one of the classic substances of pharmacology and physiology. It is involved in a variety of biological effects with clinical relevance: e.g. allergic hypersensitivity, gastric secretion, asthma, itch, and many types of dermatoses. The general physiological functions of histamine were first studied by the famous Sir Henry Dale whose criteria for classifying inflammatory mediators, such as histamine, have become widely known and used for characterizing neurotransmittors (1). The effects of histamine in skin were studied by another English legend in the medical field, Sir Thomas Lewis, whose descriptions of the so-called "triple response" (local reddening, wheal and flare) and of hyperalgesia have recently again been discussed in the context of certain major scientific endeavors (2–5).

The major bodily location of histamine in mammals is the mast cell, in its characteristic granulae (6). But histamine has also been found in basophils, in some endocrine (chromaffin) cells of the intestinal mucosa (7) and more recently in some nerve cell populations of the central nervous system (5, 8, 9). These latter morphological findings come from the use of modern immunohistochemical techniques. There are also some reports of histamine located in other cell types and tissues: epidermis of skin (10) and capillary endothelium (11), but they have been considered as methodologically 'weak' or physiologically non-important.

The effect of histamine in the peripheral tissues is on the smooth muscle, causing relaxation or contraction of, for example, capillary vessels and bronchioli (3). Histamine also causes a leakage of blood plasma from the post-capillary venulae resulting in an edema. The release of histamine from mast cells can be triggered by both immunological (binding of antibody) and non-immunological factors (e.g. physical stimulation, endogenous mediators such as neuropeptides and drugs) (12). The adverse physiological effects of an uncontrolled and extensive histamine release can be limited by effective anti-histaminergic drugs of which several types are commercially available at present (3, 5).

All these investigations have relied upon the notion that histamine, to the largest extent, is a mast cell-derived mediator. However, in the present re-

port we describe histamine-immunoreactive nerves in the skin of Sprague-Dawley rats.

Material and methods

Adult male Sprague-Dawley rats (Alab, Sweden) were killed by an overdose of pentobarbital (Mebumal, NordVacc, Sweden) in the peritoneum. This study was approved by the local Committee of Ethics. Samples of rat skin (hairy and non-hairy) were obtained from the nose and abdomen. They were immersion-fixed in 4% carbodiimide (1-ethyl-3-3-dimethylaminopropyl-carbodiimide; Sigma Chem. Comp., USA) dissolved in phosphate buffer (0.1 M, pH 7.4). The tissues were then sectioned on a cryostat (Microm, Heidelberg, Germany) to 14-μm thick sections and prepared for indirect immunohistochemistry. A rabbit polyclonal anti-histamine antibody (Milab AB, Sweden) in a titer of 1:2000 was used as the primary antiserum and a tetramethylrhodamine-isothiocyanate isomer R (TRITC)-conjugated goat anti-rabbit antibody (Boehringer-Mannheim, USA) as the secondary antiserum. Control of the antiserum specificity was done by preabsorption with histamine dihydrochloride (10^{-4} M; Sigma Chem. Comp., USA). In this context, Milab AB has shown that the antiserum does not cross-react with noradrenalin, serotonin, vasoactive intestinal polypeptide, glucagon or histidine. Other sections were incubated with the secondary antiserum only. All sections were examined and photographed in a Nikon Microphot-FXA fluorescence microscope by two independent observers. For a more extensive description of the method, see Johansson et al. (13).

Results

Using the modern histamine immunofluorescence technique, nerve fibres were seen in the dermis (Fig. 1A). They were single, seemingly without varicosities and branches. The nerve fibres were often located in close proximity to round, granular mast cells, but without any apparent contact between them. Deeper in the dermis some nerve bundles with immunoreactive nerve fibres could be seen (Fig. 1B). In the dermal vessels we saw thin, threadlike structures passing perpendicularly from the lumen through the endothelium (Fig. 1C). These structures could be very thin nerve fibers, or processes from mast cells, or histamine present in the intercellular spaces. No nerve fibres were seen adjacent to the epidermal appendages, such as sweat glands, hair follicles or vessels. In addition, histamine was, as expected, located to the mast cells. They appeared as typical round to oval cells

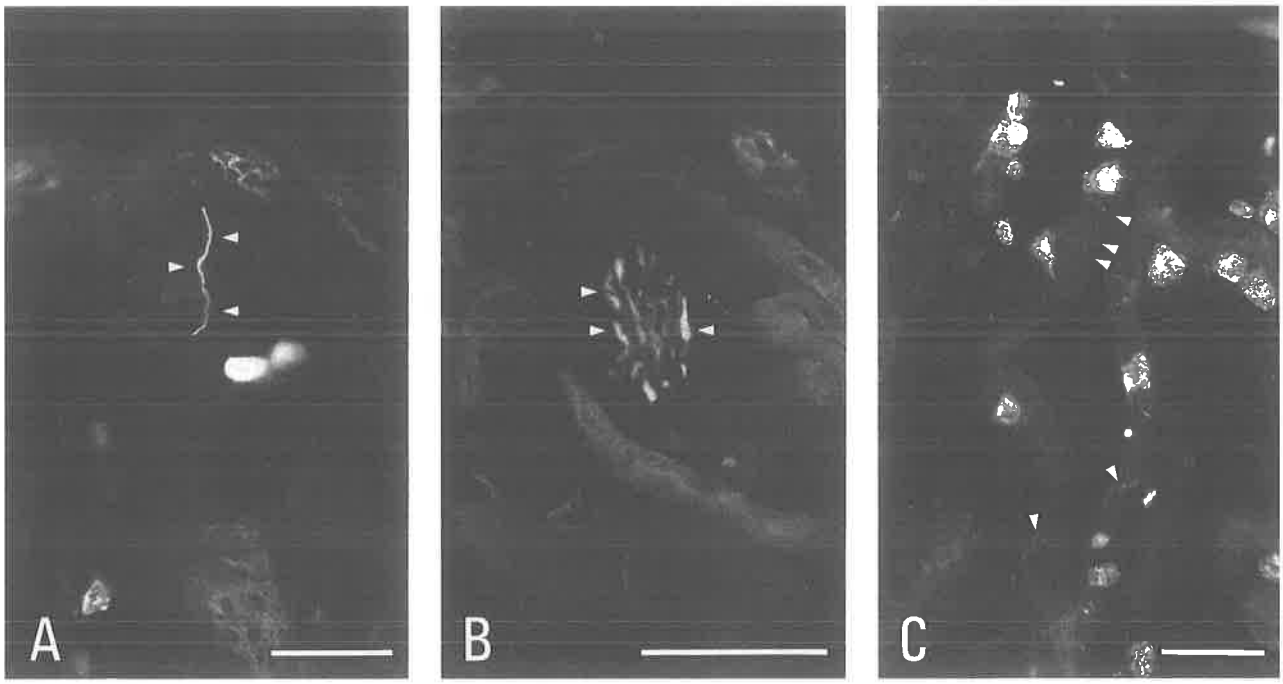

Figure 1. Histamine immunofluorescence micrographs from the rat nose showing in A a single, smooth nerve fibre (arrowheads) in the upper dermis. (B) In the lower dermis a nerve bundle with immunoreactive nerve fibres (arrowheads) is found. (C) In a dermal vessel, thin, threadlike structures (arrowheads) are seen passing perpendicular from the lumen through the endothelium. These structures could be very thin nerve fibres, or processes from mast cells, or histamine present in the intercellular spaces. In addition, histamine was, as expected, located to mast cells (cf. A and C). Bars indicate 50 μm.

with a granular cytoplasm and an unstained nucleus.

Discussion

The existence of histamine in peripheral nerves was proposed already in 1943 by Kwiatkowski and further discussed by von Euler and Werle (cf. 14, 15). Their work was based on biochemical assays without cytological or histological support. They both reported it to exist in the postganglionic sympathetic nerves, preferentially in the cranial region, of several species, including humans. Later, in 1961, these histaminergic nerves were considered to be a methodological artefact and the histamine to come from mast cells close to nerves (16). More recently, peripheral histaminergic nerves have been found in the mucosa of the gut, in addition to the histamine-containing enterochromaffin cells (17).

In the central nervous system, histaminergic neurons are located to the tuberomamillary nucleus of the hypothalamus, having long cytoplasmatic extensions throughout the brain (9). A functional hypothesis is that this histamine is involved in the regulation of the general activity of the central nervous system (8). Even such divergent phyla as insects have similar extensive histaminergic neurons in their central nervous system (18).

In the peripheral nervous system, histamine has been detected in the small interneurons (SIF-cells) of some sympathetic ganglions of rat, but not in the larger principal cells (19). However, peripheral histaminergic nerve fibers can be found in rat embryo (20), but the presence of histamine in fetal tissues is not restricted to neurons but involves cells of liver and kidney, some cells in adrenal medulla and sympathetic trunk (21, 22). A general histamine-forming capacity has been postulated for embryonal tissue (23). After nerve sectioning, histamine is found in the proximal nerve stump during the regeneration period. But it is not clear whether this really comes from a neuronal or extraneuronal source (24). Our findings suggest that we indeed have histaminergic axons terminating in the skin also in healthy and mature animals. The reason for not detecting this earlier could be a matter of the methods used or a neglect of carefully considering the skin from a morphological point of view.

The transmitters of the nerve fibres terminating in the skin are still not exactly known. However, acetylcholine is probably the major transmitter of the autonomic innervation of skin with sympathetic cholinergic postganglionic innervation of sweat glands and the pilo errector musculature. The deeper dermal vessels are innervated with sympathetic noradrenergic post-ganglionic fibres. Most recently, also amino acid transmitters have been described to be present within human skin (25). In addition to these classical transmitters, many neuropeptides have been discovered both within cutaneous nerves as well as non-neuronal cells (26).

The fact that the innervations in our findings terminate mainly as free nerve fibres in the upper dermis, and not adjacent to any of the dermal appendages, suggests that they probably have sensory functions rather than autonomic. Nociceptive and thermoceptive sensory units are considered as having unmyelinated and/or thin myelinated axons and terminating as free nerve fibres in the upper part of skin, whereas mechanoreceptive units have distinct corpuscular nerve endings (27). A major population of the nociceptive units are chemosensitive, reacting to various inflammatory mediators, but their morphological identity is not known at present. Histamine has been shown to be involved in both experimental and clinical itch and pain states, but most probably not being the only, nor the physiologically most important, nociceptive agent.

From a functional point of view, the histaminergic nerves could instead be autonomic afferents. It has been proposed that some dorsal root dark cells (B-afferents) should fulfil such a function (28). This afferent feedback function should, naturally, be especially important for an organ like the skin, because of its superficial location and multiple functional requirements. However, only future investigations may highlight these functional implications.

The effects of histamine is, apparently, to a major degree related to such epithelial tissues having a rich innervation and vascularization. An intradermal injection of histamine gives a local erythema, edema and often also the sensory experience of itch and/or pain (2). Only the edema and itching reactions are considered to be directly caused by histamine released from mast cells, whereas the surrounding flare is attributed to the release of substance P and/or calcitonin gene-related peptide (CGRP) from the surrounding nerve terminals (29).

Our findings of peripheral histaminergic nerves suggests a completely different action of histamine in skin, namely a direct release from locally present histaminergic nerve endings. The functional role of histamine in skin is probably more of a general homeostatic type rather than only to mediate anaphylaxis. Repair processes, such as wound healing and angioneogenesis, are modulated by histamine; additionally, mast cells are often found in scar tissues and keloids. Its importance in modulating immune cell responses is presently under investiga-

tion by several research groups. Whether histamine also is a trophic factor for peripheral nerves and/or cells remains to be investigated.

The chemical neuroanatomy of the cutaneous innervation shows major similarities with the central nervous one. That they also have functional similarities is becoming more and more obvious. Our findings of histaminergic nerves in skin fits well with the general neurobiology of amines and the contemporary knowledge of their localization, physiology and pharmacology. However, based upon the present results, it can be hypothesized that the release of histamine could be of a more direct and precise nature than is currently assumed. Our data will also provoke a completely new basis for explaining histamine-related issues, such as itch, in clinical as well as experimental dermatology. Whether the concepts of anaphylaxis and neurotransmission really have something in common, as they had for Sir Henry Dale, and in a way also for Sir Thomas Lewis, is still an open question, but a more broad approach to the biology of histamine than the traditional one could be a way to get closer to an answer.

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