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Low density of sympathetic nerve fibers relative to substance P-positive nerve fibers in lesional skin of chronic pruritus and prurigo nodularis

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ABSTRACT

Background: In prurigo nodularis (PN), an increase in the density of dermal substance P-positive (SP+) sensory nerve fibers has been demonstrated. In addition, the density of sympathetic nerve fibers is unchanged.
Objective: We aimed to investigate the density and balance of sensory and sympathetic dermal nerves in pruritus on normally appearing skin in comparison to PN.
Methods: In a parallel investigation in lesional and non-lesional skin routine histological and immunofluorescence staining against SP and tyrosine hydroxylase (TH) were performed.
Results: We found an increased density of dermal SP+ nerve fibers in PN and also in pruritus relative to sympathetic nerve fibers in affected areas compared to the unaffected site. The density of SP+ and TH+ nerves did not correlate with clinical parameters such as itch quality, duration or intensity. Sparse lymphocytic infiltration as found in affected pruritus skin may be a source of nerve growth factor and explain the hyperinnervation.
Conclusion: Similar to the situation in PN, chronic pruritus lesions also demonstrate a preponderance of SP+ sensory nerve fibers relative to sympathetic nerve fibers, which probably acts as a causal pro-inflammatory signal in development of pruritus. These findings suggest new therapeutic approaches in patients with chronic pruritus.

1. Introduction

Chronic pruritus is still a major challenge of dermatological and systemic diseases. Although the pathophysiology of pruritus has been investigated during the past decade [1], much light has not yet been shed on specific forms of pruritus. For example, the role of the skin in chronic pruritus without visible cutaneous lesions is enigmatic. Itch sensation emanates from the activity of specialized unmyelinated sensory C-nerve fibers in the epidermis and upper layers of dermis [1–3]. Light microscopy has demonstrated the existence of increased numbers of dermal nerve fibers containing neuropeptides such as substance P (SP) in highly pruritic dermatoses which is assumed to contribute to induction and maintenance of pruritus [4–6]. Accordingly, in prurigo nodularis (PN) lesions, an increased density of SP-positive (SP+) nerve fibers has been described [6]. 50% of patients with PN have an underlying atopic dermatitis or atopic predisposition [7]. Similarly, in long-standing pruritus of atopic dermatitis, the density of SP+ nerve fibers is increased [8]. In both diseases, density of sympathetic nerve fibers was found to be unaltered [6,9].

SP is an important sensory neuropeptide in the skin and has been found to be constitutively expressed in nerve fibers. It belongs to the family of tachykinins, which mediate multiple efferent effects in the skin [1]. Release of SP from nerve endings induces pruritus and vascular responses (erythema, oedema). Experimental studies revealed that intradermally injected SP releases histamine via binding to neurokinin receptors on mast cells and thereby induces flare, wheal and itch [10,11]. In humans, cutaneous SP injections leads to pruritus in both normal and experimentally-evoked inflamed skin in non-atopic healthy volunteers [10,11]. Moreover, SP is pro-inflammatory as it activates the transcription faction NF-κB and induces the release of interleukin (IL)-1, tumor necrosis factor (TNF), prostaglandin E2 and superoxide anion production from various cell types including mast cells [12–15]. In addition, SP affects the functional state of

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fibroblasts by regulating proliferation and extracellular matrix production [16–18].

In contrast to SP, neurotransmitters of the sympathetic nerve endings have anti-inflammatory effects at high concentrations [19]. Catecholamines are the classical neurotransmitters of the sympathetic nervous system. Increased levels of catecholamines such as norepinephrine or adenosine in the vicinity of the nerve terminal lead to an increase of intracellular cAMP in multiple peripheral target cells. Elevation of cAMP by these signals has been demonstrated to induce inhibition of TNF-alpha or interferon-γ secretion [reviewed in [19]]. In addition, sympathetic nerve terminals bear vesicles with endogenous opioids which can influence the inflammatory process [20]. Taken together, the presence of sympathetic nerve fibers leading to high concentrations of sympathetic neurotransmitters can inhibit pro-inflammatory effects of SP, which might be also relevant in itch.

In the present study, we aimed to investigate the density and balance of dermal SP+ sensory and sympathetic nerve fibers in affected and non-affected skin of patients with chronic pruritus and PN. Since tyrosine hydroxylase (TH) is the key enzyme in the production of catecholamines we selected TH for our investigation.

2. Patients and methods

We investigated non-affected, non-pruritic (healthy) and affected, pruritic skin in 9 patients with chronic pruritus (CP) on clinically normal, non-inflamed skin [21] (Tab. 1; 5 women and 4 men; mean age, 56.6 ± 15.4 years; duration of pruritus, mean 9.53 years) and in 10 patients with PN (Tab. 1; 7 women, 3 men; mean age, 64.4 ± 16.6 years; duration of pruritus, mean 6.4 years). 8 mm skin biopsies were taken from the extremities ($n = 9$) or trunk ($n = 10$) from pruritic and non-pruritic skin. The anatomical localization of the healthy skin was as close as possible to the affected skin. Itch-specific parameters were assessed by a local itch questionnaire (NeuroDerm questionnaire; [22]). Four patients reported on „pure" itching only, while 15 patients reported on itching, burning and/or stinging (Table 1). On the day of skin biopsy, median symptom intensity on the visual analog scale (VAS) was 5.0 ± 2.7 in patients with CP and 9.0 ± 2.8 in patients with PN (total ($n = 19$), median VAS 8.0 ± 2.5). Patients were informed about the study and gave written consent. The study was approved by the Ethics Committee of the University Münster, Germany.

The biopsy was divided into two parts. One part was subjected to routine histology including paraffin embedding and HE staining. For evaluating nerve fibers, immunofluorescence staining was performed in the second part of the biopsy. The determination of innervation has been described previously [23]. Briefly, biopsies were fixed for 1 day in 3.75% formalin in PBS followed by another day in 20% saccharose in PBS and then frozen in Tissue Tek. Six cryosections (5–9 μm thick) of each formaldehyde/sucrose-fixed tissue samples were cut and mounted on microscope slides (SuperFrost Plus; Thermo Scientific, Germany). Sections were pre-incubated with blocking solution containing 10% BSA, 10% FCS and either 10% goat serum (TH staining) or 10% rabbit serum (SP staining) in PBS, followed by application of the primary antibody against tyrosine hydroxylase (TH^+, the key enzyme for catecholamine production in sympathetic nerve endings, 1:1000, AB152, Chemicon, Temecula, CA, USA) and against substance P (SP, one key neurotransmitter of sensory nerve fibers, 1:50, sc-9758, Santa Cruz, CA, USA). Alexa 546 conjugated secondary antibodies (for TH: against rabbit IgG, 1:500; for SP: against goat IgG, 1:500; both from Invitrogen GmbH, Karlsruhe, Germany) were used to achieve immunofluorescent staining of sympathetic and sensory SP+ nerve fibers (Fig. 1). The numbers of TH^+ sympathetic and SP^+ sensory nerve fibers per square millimeter were determined by averaging the number of stained nerve fibers (typical bead chain structure with at least four separated vesicles along the axon, minimum length 50 μm, determined by a micrometer eyepiece) in 17 randomly selected high power viewing fields (400×). To determine the relation between sensory and sympathetic nerves, a ratio of SP+ and Th+ nerves was calculated. Images were taken with a Zeiss Axiovert 40 fluorescence microscope.

To exclude non-specific binding of the secondary antibody control sections were subjected the same staining procedure but without primary antibody. In none of these negative controls staining was detectable.

3. Statistical analyses

Statistical analysis was carried out using SPSS for Microsoft Windows (SPSS, Inc. Chicago, IL). To detect differences between groups, Wilcoxon signed rank test for paired data was used.

Table 1
Demographic and clinical parameters of patients.

Patient no., gender, age (M: male, F: female)	Localization of biopsy	Origin of pruritus	IgE level (<100: normal value; >100: elevated)	VAS range	Duration (years)	Quality
Prurigo nodularis						
1, F, 80 years	Trunk	Dermatosis (bullous pemphigoid)	45.8	10	2	Itching
2, M, 77 years	Trunk	Hepatitis C	18.4	10	9	Itching
3, M, 42 years	Leg	Drug-induced (Opioids)	714.0	8–10	4	Itching
4, F, 50 years	Arm	multifactorial	226.0	6	8	Itching, stinging
5, F, 46 years	Trunk	multifactorial	22.2	3	5	Itching, stinging, biting
6, M, 58 years	Arm	multifactorial	1035.0	10	3	Itching, stinging
7, F, 60 years	Trunk	multifactorial	2322.0	8–10	6	Itching, stinging
8, F, 56 years	Arm	unknown	27.6	8	6	Itching, stinging
9, F, 84 years	Trunk	unknown	48.7	10	20	Itching, burning
10, F, 86 years	Tunk	unknown	83.0	2–4	1	Itching, burning
Pruritus						
11, F, 28 years	Trunk	Hepatitis B	8960.0	8–9	10	Itching, burning
12, F, 79 years	Leg	Diabetes mellitus	35.5	5	1.5	Itching, stinging
13, M, 68 years	Arm	Drug-induced (Carbamazepine)	27.10	3–8	3	Itching, burning
14, F, 55 years	Arm	Brachioradial	<2.0	5–10	28	Itching, stinging
15, F, 56 years	Arm	Brachioradial	46.5	10	8	Itching, stinging, burning
16, F, 65 years	Trunk	Brachioradial	14.8	2–4	20	Itching, burning
17, F, 65 years	Arm	Brachioradial	4.38	3–9	8	Itching
18, M, 43 years	Trunk	unknown	<2.0	6–7	7	Itching, stinging
19, M, 44 years	Trunk	unknown	181.0	7	3	Itching, stinging