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ORIGINAL PAPER

Normal and PPP-affected palmoplantar sweat gland express neuroendocrine markers chromogranins and synaptophysin differently

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Abstract Earlier findings indicate the acrosyringium as the target for the inflammation in the chronic and intensely inflammatory skin disease palmoplantar pustulosis (PPP). The sweat gland apparatus seems to be an immune-competent structure that probably contributes to the defence of the skin. Furthermore, the sweat gland and duct may be a hitherto unrecognized neuroendocrine organ because it expresses cholineacetyl-transferase and acetyl-cholinesterase, nicotinic receptors, beta-adrenergic and angiotensin receptors. The aim of this study was to obtain further information about neuroendocrine properties of the sweat gland apparatus by examining the expression of common neuroendocrine markers synaptophysin and chromogranins A and B in healthy palmar skin and in PPP skin. Synaptophysin and chromogranins were expressed in the sweat glands and ducts with some variation in the pattern and intensity of the expression. In PPP skin the expression differed, being higher and lower, depending on the part of the sweat duct. Chromogranins were further expressed in the epidermis, endothelium and inflammatory cells, but its intensity was weaker in epidermis than in the sweat gland apparatus. In most cases, chromogranins in epidermis in involved PPP were weakly expressed compared to healthy controls. The presence of synaptophysin and chromogranins in palmoplantar skin may have marked neuroendocrine effects, and the palmoplantar skin is likely to have important neuroimmuno-endocrine properties. Moreover, the altered chromogranin expression in PPP skin might influence both the neuroendocrine and neuroimmunologic properties of palmoplantar skin in these patients. These results indicate important neuroendocrine properties of the palmoplantar skin.

Keywords Eccrine sweat gland apparatus · Neuroendocrine organ · Palmoplantar pustulosis · Immunohistochemistry · Chromogranin

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Introduction

The eccrine sweat duct in palmar and plantar skin is the target for the intense inflammation in the psoriasis variant palmoplantar pustulosis (PPP) [5]. This disease is most common in smoking women and is characterized by sterile pustules, erythema and scaling [2]. The reason why palmoplantar sweat ducts but not those in hairy skin are inflamed in PPP is not known. Palmoplantar skin is rich in eccrine sweat glands, which react in response to heat and mental stimuli, and many of the patients (46%) consider stress as an obvious worsening factor with precipitation of new pustules [2]. Knowledge about the properties of the palmoplantar sweat glands and ducts is limited. PPP is associated with increased prevalence of autoimmune thyroid disease, [2] abnormal calcium homeostasis, [7] coeliac disease/gluten intolerance [2] and diabetes type 2, [7] but it is not yet known whether the skin disorder and the co-existing diseases have a common pathogenetic background.

The eccrine sweat duct differs in several respects from the inter-appendageal epidermis. Thus, no Langerhans cells are present but the duct cells are HLA-DR positive and express IL-1, IL-8 and TNF-alpha [17, 24]. In palmoplantar skin the gland and duct also display a strong expression of

cholineacetyl-transferase and acetylcholinesterase (the acetyl choline synthesizing and degrading enzymes) and nicotinic receptors [5], beta-adrenergic (unpubl.) and angiotensin receptors [32]. Parathyroid hormone-related protein is also strongly expressed [7] indicating that the gland and duct may be a hitherto unrecognized neuroendocrine organ. Because palmoplantar skin has a high density of sweat glands (620/cm^2 compared to 65/cm^2 in skin from the trunk) the normal sweat gland apparatus in palmoplantar skin may have important neuroendocrine effects.

Chromogranins (Cg) and secretogranins (Sg) are members of the granin family of proteins, are established neuroendocrine markers and are expressed in neuroendocrine and nervous tissue [10, 33]. They share common physiochemical properties such as calcium binding properties, acidic isoelectric points due to high contents of acidic amino acid residues, and multiple pairs of basic amino acids, which are potential cleavage sites for enzymes such as the proconvertases [33]. However, the Sgs do not have the N-terminally located disulfide-bonded loop as the Cgs [10, 33]. Cgs are processed to smaller peptides, which often possess biological activity. This processing varies in different neuroendocrine tissue as shown in pancreatic islet cells [22]. All granins are stored in secretory granules and are co-released with hormones and neuropeptides. Like the Cgs, the Sgs also exhibit multiple biological functions, of which some are generated by cleavage products from the granins. The most important peptide generated from SgII is secretoneurin (SgII 154–186) [13, 16]. Secretoneurin has been found in both neuronal and neuroendocrine tissue [13, 16].

Synaptophysin (Syn) is a glycoprotein located in presynaptic vesicle membranes of neurons and is also found in the chromaffin cells in the adrenal medulla [18]. Syn has also been observed in all cell types of the endocrine pancreas [14]. Both granins and Syn are established markers for neuroendocrine tissue and are commonly used as immunohistochemical markers [1, 34]. In skin, granins and synaptophysin have been found in Merkel cells [9].

In recent years the interplay between the nervous system and skin has received increased attention. An extensive review on the neuronal control of skin function with emphasis on the skin as a neuroimmunoendocrine organ was recently published by Roosterman et al. [25]. Based on the indications that the eccrine sweat apparatus is a neuroendocrine organ, the aim of this study was to obtain further information about neuroendocrine properties of the palmoplantar skin and in particular the sweat gland apparatus in normal skin and in PPP skin. To do this, the expression of CgA, CgB, SgII and Syn was studied with immunohistochemistry on palmoplantar skin from healthy persons and PPP patients.

Materials and methods

Patients

A total of 25 patients (23 women, 18–75 years old; 2 men, 39 and 59 years old) with typical PPP on the palms and/or soles, participated in the study. Most of the patients were smokers. None received treatment with beta-blockers or lithium. Most used only emollients at the time of the examination.

Control group

The control group consisted of 25 healthy subjects (22 women, 24–65 years old; 3 men, 23–37 years old). Of these 6 women and 1 man were smokers.

The study was approved by the Ethics Committee at the University of Uppsala. The Declaration of Helsinki protocols was followed and patients gave their written, informed consent.

Biopsies

Biopsy specimens (3 mm) were taken from involved skin in the hypothenar or thenar region in the PPP patients, after injection of xylocaine-adrenaline, and snap-frozen and kept at −70°C. In the healthy controls biopsy specimens were taken from the hypothenar region. For comparison biopsies were also taken from the gluteal skin from one healthy subject and from the scalp skin of four healthy volunteers.

Antibodies

Five antibodies against defined sequences of chromogranins and one antibody against a sequence of synaptophysin were used (Table 1). The production of antibodies has been described elsewhere [21, 22, 30].

Immunohistochemistry

After cryosectioning (6 μm thick), tissue specimens were fixed in 100% ice-cold acetone (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) for 10 min. Sections were first blocked in 0.6% H_2O_2 (Acros Organics, Morris Plains, NJ, USA) in phosphate-buffered saline 15 min and then in 10% normal goat serum (Vector Laboratories, Burlingame, CA, USA) for 20 min before incubation overnight at 4°C with the primary antibodies (Table 1). The immunoreaction was visualized with Vectastain ABC Elite Kit (Vector Laboratories) and 3-amino-9-ethylcarbazole (Sigma Chemical Co., St Louis, MO, USA) as chromogen. The sections were counterstained with Mayer's haematoxylin (Histolab Products AB, Gothenburg, Sweden).

Table 1 Antibodies against defined sequences of chromogranins (Cg) and synaptophysin (Sy 84), dilutions and number of patients with palmoplantar pustulosis (PPP) and healthy controls

Antibodies used (rabbit polyclonal)	Antigen (amino acid sequence number)	Dilutions	Number of patients analysed	Number of healthy controls analysed
CgA 4A	116–130	1/2,000	22	14
CgA 10A	176–195	1/2,500	24	13
CgB 1A	1–16	1/2,000	22	13
CgB 4A	244–255	1/1,100	19	10
SNCTA = SgII	172–186	1/6,000	21	12
Sy84	258–272	1/5,000	22	14

Control staining

The control staining was performed in three ways:

a. Omission of the primary antisera.
b. Replacement of the first layer of antibody by 10% rabbit serum (non-immune serum).
c. Pre-incubation (60 h at 4°C) of primary antiserum (SgII) with relevant peptide (1 nmol/µl peptide in 50 µl diluted antibody solution) before incubation of sections (Fig. 1m, n).

Evaluation of immunohistochemistry

Two to four sections of each specimen were examined under a ×40 objective in a Leica DMLB microscope with a DC 200 digital camera. All evaluations were carried out on coded sections in a semi-quantitative fashion. The staining intensity was evaluated in the sweat gland and duct as well as in the different layers of epidermis (stratum granulosum, spinosum and basale) and classified as absent, weak, medium or strong. The numbers of unstained and stained ducts in stratum corneum, the vital epidermis, papillary dermis, reticular dermis and coils were counted. All visible ducts were counted as one duct each. The proportion of unstained, weakly, moderately and strongly stained ducts was calculated. In the vital epidermis the staining intensity was estimated in the different strata as follows: unstained = 0, weak = 1, medium = 2 and strong = 3. The staining intensity of the pustule and of the endothelial cells and inflammatory cells in the papillary and reticular dermis were estimated in the same way.

Statistics

Statistical differences between normal skin and PPP skin were evaluated by the Mann–Whitney *U* test. Differences in staining intensity between the various antibodies (Tables 2 and 3) were not evaluated statistically because the staining with the different antibodies was not performed at the same time.

Results

The results for the sweat ducts and glands in healthy subjects and PPP patients are summarized in Table 2. The expression in epidermis, endothelium and inflammatory cells is shown in Table 3. The results in normal palmar as well as in PPP skin are also shown in Fig. 1.

Expression of chromogranins and synaptophysin in palmar skin in healthy subjects

Figure 1a–g illustrates examples of the expression of the Cgs and Fig. 1f shows the Syn expression in palmar skin from healthy persons.

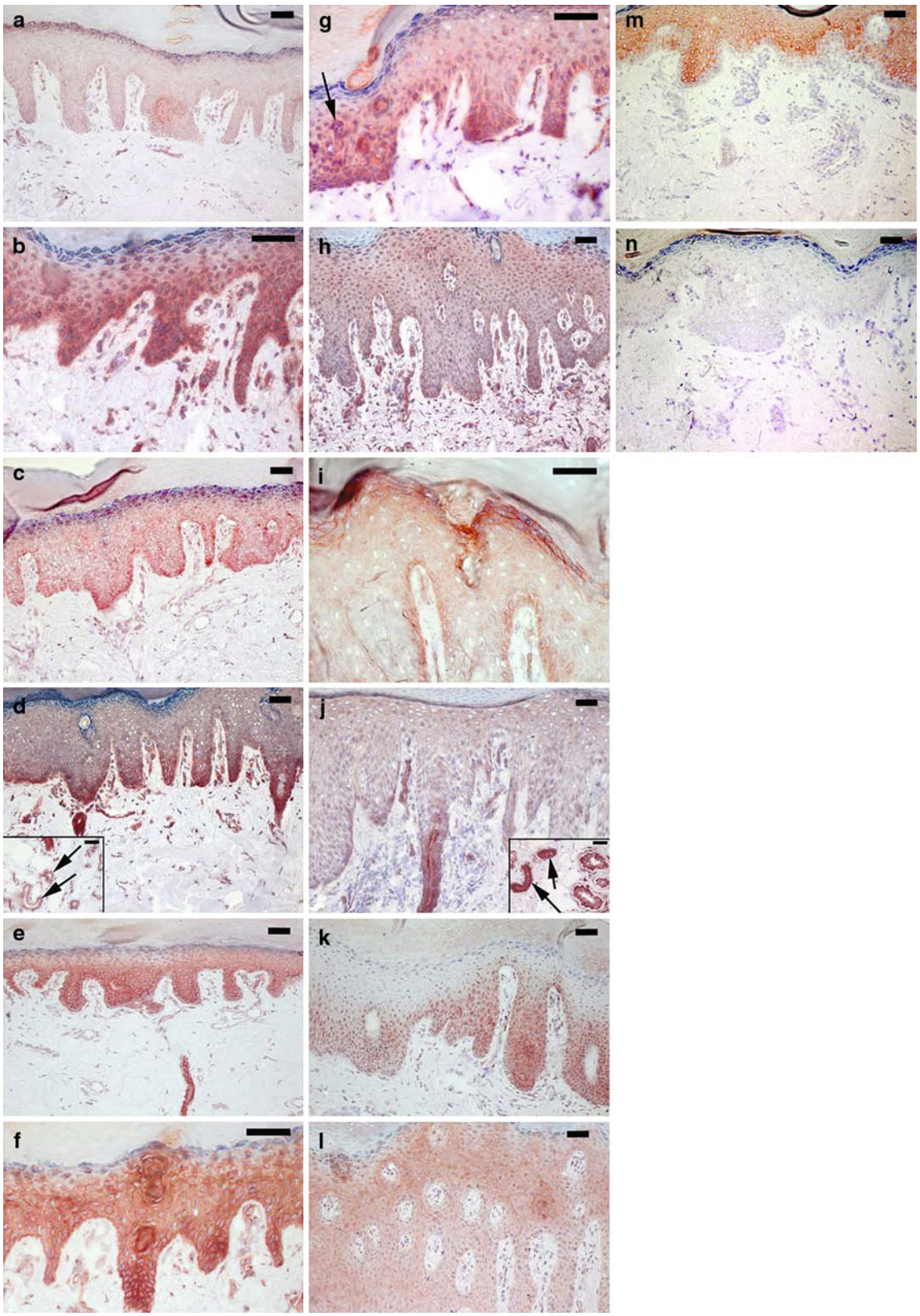
The eccrine sweat gland apparatus

Sweat ducts in stratum corneum expressed CgA 116–130 (Fig. 1a), SgII 172–186 and Syn (Fig. 1f).

A high proportion of the intraepidermal ducts showed a moderate-strong expression of CgA 176–195, SgII 172–186 and of Syn (Fig. 1f), whereas a high proportion of CgB 244–255 was unstained or had weak expression (Fig. 1d).

Most ducts in papillary dermis showed strong staining with all the antibodies (Fig. 1d). In reticular ducts the strongest expression was observed with CgA 176–195 and SgII 172–186 and Syn. However, staining of the ducts was obtained with all of the six antibodies, although with variable intensity and in variable proportions of the ducts.

All coils expressed CgA, CgB, SgII and Syn with the highest proportion of strongly stained coils with Cg A 116–130, SgII 172–186 and Syn. The staining pattern varied from total to partial with staining of only a few cells. The most common pattern was a strong homogenous staining (55% of SgII 172–186) but partial staining or in a few



◀ **Fig. 1** Expression of chromogranins (Cgs) and synaptophysin (Syn) in palmar skin from healthy persons (**a**–**f**) and from patients with palmoplantar pustulosis (PPP) (**g**–**l**). **m** Immunoreactivity with the secretogranin II (SgII) antibody and **n** visualizes the results after a pre-incubation of the SgII antibody with a relevant peptide before incubation of the section (1 nmol/μl peptide in 50 μl diluted antibody). Positive staining is *brown* and nuclei are stained *blue*. **a** and **g** CgA 116–130; a stronger expression is observed in the sweat ducts in epidermis and stratum corneum in both healthy persons and patients than in the surrounding epidermis and stratum corneum, respectively. *Arrow* indicates positive inflammatory cell in epidermis in PPP patient epidermis. The expression in epidermis in the PPP skin seems to be stronger than in the healthy skin in this image, but our data showed no significant differences. **b** and **h** CgA 176–195; example of a weaker expression in stratum spinosum and basale in a PPP patient compared with that in healthy subjects. (In epidermis the sweat ducts had a weaker expression in PPP patients compared with controls.) **c** and **i** CgB 1–16; keratinocytes in the basal and granular layers of epidermis had a stronger expression in healthy controls compared with PPP patients, although these differences were not significant. Strong staining is seen in the acrosyringium in the PPP skin. **d** and **j** CgB 244–255; stratum spinosum and basale showed a weaker expression in patients. The inserted images show sweat ducts and coils in reticular dermis. *Arrows* indicate the ducts in the reticular dermis, which had a strong expression of CgB 244–255 in PPP patients. **e** and **k** SgII 172–186; present the weaker staining of stratum granulosum and spinosum in the PPP patients. **f** and **l** Syn; demonstrates the strong epidermal expression with an even stronger expression in the epidermal sweat duct in healthy persons. Syn showed a stronger expression than the Cgs in all layers of the epidermis. All *scale bars* are 50 μm

specimens only staining of basal cells was also observed. Unstained coils were uncommon.

The epidermis

All the epidermal layers expressed all the chromogranins usually with a mild–moderate intensity (Table 2). The strongest staining was present in stratum basale (CgA 176–195 (Fig. 1b)), stratum spinosum (SgII 172–186) (Fig. 1e) and with Syn the expression was strong in all three layers (Fig. 1f). However, the acrosyringium displayed a stronger staining than the surrounding epidermis (Fig. 1f).

The endothelium

Expression of all the screened chromogranins and of Syn was present in both papillary and reticular dermis endothelium although with variable intensity of the staining. As shown in Table 2 the strongest staining was observed with CgA 116–130, CgA 176–195 and Syn.

The inflammatory cells

The few inflammatory cells in the papillary dermis and around the coils were stained, with the strongest expression of CgA 116–130. On the other hand SgII was not expressed in inflammatory cells, which contrasts to the strong expression in the ducts and coils as well as in epidermis. Syn was weakly expressed in the inflammatory cells.

Expression of chromogranins and synaptophysin in palmar skin in patients with palmoplantar pustulosis

As shown in Tables 2 and 3 staining intensity differed significantly (despite large SDs) between involved PPP skin and normal palmar skin. Figure 1g–k illustrates example of the expression of the Cgs and Fig. 1l shows the Syn expression in palmar skin from PPP patients.

The eccrine sweat gland apparatus

With CgA 176–195 fewer epidermal ducts were strongly stained in PPP and a fewer papillary ducts expressed CgB 1–16 in PPP. On the other hand, in PPP patients more ducts in stratum corneum expressed CgB 1–16, and more ducts in reticular dermis expressed CgB 244–255 (see inserted picture in Fig. 1j).

The epidermis

In involved PPP, the epidermis displayed weaker expression of SgII and Syn (stratum granulosum) (Fig. 1k, l), CgB 244–255, SgII 172–186 and Syn (stratum spinosum) (Fig. 1j, k, l) and of CgB 244–255 (stratum basale) (Fig. 1j).

The endothelial cells

In endothelial cells the expression of Cg and Syn displayed similar staining intensity in normal and PPP skin.

The inflammatory cells

The inflammatory cells within the pustules (neutrophils and eosinophils) were unstained.

Inflammatory cells were numerous in involved PPP skin, in particular in the papillary dermis with the strongest expression of CgA 116–130. As shown in Fig. 1g, some of these cells (arrow) which also infiltrated epidermis were intensely stained (hitherto not identified). In contrast to the strong expression of CgA 116–130 (and modest expression of CgA 176–195 and CgB 244–255), the inflammatory cells in involved skin did not express SgII.

Comparison of the expression in hairy skin versus palmar skin

One scalp skin specimen showed strong SgII 172–186 expression in the eccrine sweat gland and duct, while another scalp skin specimen expressed no or minimal SgII

Table 2 Expression of chromogranins and synaptophysin in sweat gland apparatus in palmar skin from healthy controls and patients with palmoplantar pustulos (PPP)

	CgA 116–130 Healthy $n = 14$ PPP $n = 22$	CgA 176–195 Healthy $n = 13$ PPP $n = 24$	CgB 1–16 Healthy $n = 13$ PPP $n = 22$	CgB 244–255 Healthy $n = 10$ PPP $n = 19$	SgII 172–186 Healthy $n = 12$ PPP $n = 21$	Synaptophysin Healthy $n = 14$ PPP $n = 22$
Proportion of M + S expressing ducts in						
Str corneum in healthy controls	0.49 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.15	0.96 ± 0.09	0.80 ± 0.37
Str corneum in PPP patients	0.52 ± 0.41	0.17 ± 0.31	▲ 0.45 ± 0.48[b]	0.32 ± 0.46	0.85 ± 0.35	0.67 ± 0.45
Epidermis in healthy controls	0.63 ± 0.37	0.93 ± 0.15	0.58 ± 0.44	0.29 ± 0.32	1.00 ± 0.00	1.00 ± 0.00
Epidermis in PPP patients	0.74 ± 0.37	▼ 0.23 ± 0.39[a]	0.76 ± 0.40	0.41 ± 0.43	1.00 ± 0.00	1.00 ± 0.00
Papillary dermis in healthy controls	0.70 ± 0.45	1.00 ± 0.14	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Papillary dermis in PPP patients	0.57 ± 0.49	0.88 ± 0.32	▼ 0.66 ± 0.39[c]	0.96 ± 0.08	0.94 ± 0.25	0.86 ± 0.35
Reticular dermis in healthy controls	0.78 ± 0.30	0.97 ± 0.09	0.55 ± 0.46	0.73 ± 0.40	0.94 ± 0.13	1.00 ± 0.00
Reticular dermis in PPP patients	0.80 ± 0.32	0.85 ± 0.31	0.55 ± 0.37	▲ 0.86 ± 0.32[d]	0.92 ± 0.26	0.94 ± 0.22
Proportion of M + S expressing						
Coils in healthy controls	0.94 ± 0.19	0.80 ± 0.33	0.62 ± 0.42	0.65 ± 0.46	0.92 ± 0.21	1.00 ± 0.00
Coils in PPP patients	0.84 ± 0.32	0.82 ± 0.35	0.57 ± 0.42	0.90 ± 0.26	1.00 ± 0.02	0.99 ± 0.03

The proportion of sweat ducts at various levels and of coils with moderate + strong staining (M + S). (Means ± SD). Arrows indicate increase (▲) or decrease (▼) of the proportion of ducts expressing chromogranins in PPP patients versus healthy controls

PPP compared to controls; [a] $p = 0.009$; [b] $p = 0.042$; [c] $p = 0.046$; [d] $p = 0.035$

Table 3 Expression of chromogranins and synaptophysin in palmar skin from healthy controls and patients with palmoplantar pustulos (PPP)

	CgA 116–130 Healthy $n = 14$ PPP $n = 22$	CgA 176–195 Healthy $n = 13$ PPP $n = 24$	CgB 1–16 Healthy $n = 13$ PPP $n = 22$	CgB 244–255 Healthy $n = 10$ PPP $n = 19$	SgII 172–186 Healthy $n = 12$ PPP $n = 21$	Synaptophysin Healthy $n = 14$ PPP $n = 22$
Epidermis mean stain intensity						
Str granulosum healthy controls	1.33 ± 0.36	1.11 ± 0.35	1.40 ± 0.61	1.04 ± 0.51	1.33 ± 0.43	2.32 ± 0.72
Str granulosum PPP	1.01 ± 0.62	0.93 ± 0.31	1.26 ± 0.47	0.89 ± 0.27	▼0.93 ± 0.47[f]	▼1.50 ± 0.88[h]
Str spinosum healthy controls	1.04 ± 0.29	1.64 ± 0.22	1.15 ± 0.52	1.19 ± 0.61	2.32 ± 0.29	2.54 ± 0.42
Str spinosum PPP	1.00 ± 0.25	▼0.97 ± 0.20[a]	1.27 ± 0.49	▼0.95 ± 0.14[d]	▼1.93 ± 0.54[g]	2.25 ± 0.54
Str basale healthy controls	1.45 ± 0.43	2.46 ± 0.30	1.96 ± 1.35	1.56 ± 0.83	1.21 ± 0.38	2.86 ± 0.31
Str basale PPP	1.30 ± 0.45	▼1.25 ± 0.20[b]	1.60 ± 0.57	▼1.04 ± 0.22[e]	1.14 ± 0.62	2.72 ± 0.47
Endothelium mean stain intensity						
Papillary dermis healthy controls	2.25 ± 0.67	2.06 ± 0.59	1.36 ± 0.80	1.56 ± 0.90	1.83 ± 0.52	2.80 ± 0.32
Papillary dermis PPP	2.18 ± 0.67	▼1.60 ± 0.59[c]	1.36 ± 0.63	1.46 ± 0.50	1.74 ± 0.83	2.66 ± 0.56
Reticular dermis healthy controls	2.27 ± 0.56	2.08 ± 0.67	0.98 ± 0.80	1.50 ± 0.87	1.30 ± 0.46	2.79 ± 0.38
Reticular dermis PPP	2.50 ± 0.69	1.82 ± 0.60	1.24 ± 0.55	1.88 ± 0.56	1.33 ± 0.69	2.71 ± 0.55
Inflammatory cells mean stain intensity						
Papillary dermis healthy controls	2.27 ± 0.81	1.16 ± 0.50	0.71 ± 0.43	1.12 ± 0.58	0.00 ± 0.00	0.25 ± 0.55
Papillary dermis PPP	2.67 ± 0.56	1.18 ± 0.44	1.02 ± 0.63	1.10 ± 0.42	0.12 ± 0.38	0.65 ± 1.06
Reticular dermis healthy controls	2.32 ± 0.84	1.14 ± 0.49	0.44 ± 0.43	1.09 ± 0.62	0.00 ± 0.00	0.36 ± 0.63
Reticular dermis PPP	2.68 ± 0.55	1.15 ± 0.56	0.77 ± 0.85	1.13 ± 0.40	0.07 ± 0.33	0.69 ± 1.05

The mean value of staining intensity of epidermis (stratum granulosum, spinosum and basale), and endothelium and inflammatory cells in papillary and reticular dermis, respectively. Unstained = 0, weak staining = 1, moderate staining = 2, strong staining = 3. (Means ± SD) When chromogranin expression differed, there was always a decrease (marked by ▼) in the PPP patients compared with healthy controls

PPP compared with controls; [a] and [b] $p < 0.0001$; [c] $p = 0.049$; [d] $p = 0.057$; [e] $p = 0.029$; [f] $p = 0.014$; [g] $p = 0.034$; [h] $p = 0.012$

in the eccrine sweat apparatus, whereas in the palmar skin of the same healthy control there was strong expression of SgII. In two scalp skin specimens no sweat glands or ducts were found. Staining intensity of epidermis did not differ between gluteal and palmar skin but no eccrine sweat glands were found in the gluteal skin sections.

Sebaceous glands in both the scalp and gluteal skin were unstained or weakly stained.

Discussion

Previous studies with immunohistochemical staining of skin with the established neuroendocrine markers synaptophysin and granins have focused on Merkel cells [9]. The results of this study confirm that the skin and in particular the palmoplantar sweat gland and duct is a neuroimmunoendocrine organ with strong expression of chromogranins and synaptophysin. With regard to the high density of sweat glands the palmoplantar sweat glands and ducts may have marked neuroendocrine effects, locally and possibly also systemically. Chromogranins were also expressed in the epidermis, endothelium and inflammatory cells, although in epidermis with weaker intensity than in the sweat gland apparatus. Taken together, the results illustrate that the palmoplantar skin is likely to have important neuroimmuno-endocrine properties.

The expression of CgA was studied with two region-specific antibodies (CgA 116–130 and CgA 176–195) and CgB with two antibodies (CgB 1–16 and CgB 244–255) and secretogranin (SgII) with an antibody against the secretoneurin sequence 172–186. These antibodies were chosen because they have previously been found suitable for immunohistochemistry in studies of human pancreas [22], and human gastrointestinal tract [20].

All the chromogranins were expressed in the sweat glands and ducts. However, the pattern and intensity of the expression varied. Thus, only SgII 172–186 and Syn, and sometimes CgA 116–130, were expressed in the ducts in stratum corneum. CgA 176–195 and SgII 172–186, and Syn, displayed the strongest staining of the epidermal and dermal ducts. Differences in expression of the various chromogranins in the sweat gland or ducts might mean that the different chromogranins have different functions. This could also indicate selective processing of Cgs and SgII in the different parts of the sweat gland and duct as Portela-Gomes and Stridsberg (2001) found that CgA was selectively processed in the different cells in the pancreas. Possible differences in the function can be illustrated by differences in the staining of inflammatory cells. Thus CgA 116–130 was strongly expressed in inflammatory cells, whereas SgII 172–186 was not expressed in inflammatory cells but was strongly expressed in the coils and ducts.

Biological functions of a number of CgA, CgB and SgII sequences have been reported [33]. Thus, CgA 173–194 (chromacin I and II) and CgA 352–372 (catestatin) have been shown to have antibacterial properties [23, 31]. The eccrine sweat duct has an important role in the defence against infections with expression and excretion of the sweat gland-specific dermcidin [27] and cathelicidin (LL-37) in sweat and eccrine sweat glands and duct cells [19] and also of TNFα, IL1 and IL8. It seems probable that chromacin is a hitherto unknown peptide produced in the sweat gland and duct, which has a role in the defence against infections. CgA 116–130 (chromostatin) and CgA 352–372 (catestatin) have been found to inhibit the release of catecholamine both in an autocrine and paracrine way [4, 15]. Several hormones are expressed in the sweat gland and duct, and chromostatin could be involved in their release. Corticotrophin-releasing hormone and the receptor are expressed in human skin [28], and in psoriasis lesions the expression in sweat glands is significantly increased compared with that healthy skin [12].

Cutaneous corticotrophin-releasing hormone (CRH) and receptors are believed to regulate various functions in the skin [29].

Expression of adrenocorticotrophic hormone (ACTH) and alpha-melanocyte stimulating hormone (MSH) has been found in the eccrine sweat apparatus in normal human skin and to a higher extent in psoriasis lesions [12].

Both sequences of CgB were expressed in the coil and duct. One of the antibodies was directed against CgB 1–16. Bovine CgB 1–41 has been reported to inhibit parathyroid hormone secretion from chief cells [26] and may thus influence calcium homeostasis. Our earlier report regarding the strong expression of parathyroid hormone-related protein (PTHrp) in palmoplantar sweat ducts [7] and of CgB 1–16 strengthens our hypothesis that calcium is important for the function of the palmoplantar sweat gland and duct. This is of particular interest with regard to PPP which is associated with abnormal calcium homeostasis with raised serum calcium despite decreased levels of parathyroid hormone [7].

All parts of the sweat gland apparatus strongly expressed SgII 172–186, corresponding to secretoneurin, which has been reported to promote chemotactic attraction of monocytes and eosinophils, to influence proliferation of endothelial cells [3], to activate endothelial cells for neutrophil transmigration [11] and to act as an angiogenic cytokine with a potency comparable to that of vascular endothelial growth factor [3]. All these properties are important in the inflammatory processes in psoriasis and PPP [2]. The strong Syn immunoreactivity of the sweat glands and ducts further illustrates the neuroendocrine properties. As shown in Table 2, the sweat ducts in involved PPP skin displayed both stronger and weaker staining depending on the chromogranin studied, and also depending on the part of the sweat duct. With SgII 172–186 and Syn expression was found not to differ.

The inflammation in the sweat duct is the primary change in PPP [6], but it is followed by a psoriasis-like pattern with acanthosis, para- and hyper-keratosis in the

inter-appendageal epidermis. The predominating difference in expression of chromogranins in epidermis in involved PPP was a weaker expression as compared with healthy controls. The results thus indicate that not only is the inflammation in PPP associated with significant changes in the expression of the chromogranins but also that these changes may differ in the sweat ducts and epidermis. We have previously reported that the nerves surrounding the sweat glands in involved PPP skin seem to be abnormal with a fragmented appearance [8]. It is not known whether this is an effect of the inflammation but it may influence the expression of the chromogranins.

The strong expression of several chromogranins in endothelial cells and even more in inflammatory cells in involved skin, particularly CgA 116–130, might indicate that chromogranins may influence inflammation.

Although the expression of secretoneuerin in epidermis in involved skin was significantly weaker than in normal skin, the strong expression in the sweat duct and coil was similar to that in normal skin. In involved PPP skin large numbers of neutrophils and eosinophils migrate outwards in the sweat duct and form the pustule in the lowest part of stratum corneum [8] and there is a pronounced proliferation of the endothelium. In view of the properties of secretoneurin (see above), its strong expression in the sweat duct and the intensity of the inflammation in PPP, it may be speculated that secretoneurin might facilitate/strengthen the inflammation, as the inflammatory component in the PPP skin might be influenced more by the expression of secretoneurin than if you do not have either an inflammatory component or the genetic background for PPP.

The conclusion of this study is that normal palmoplantar skin with its high density of sweat glands and ducts which express all the chromogranins studied might be of particular importance in skin neuroimmuno-endocrinology. Although we do not know the relevance of the changes in chromogranin expression in PPP skin compared with normal skin, we hypothesize that the changes might influence both the neuroendocrine and neuroimmunologic properties of palmoplantar skin, especially the sweat gland apparatus in these patients.

The presence of synaptophysin and chromogranins in palmoplantar skin may have marked neuroendocrine effects, and the palmoplantar skin is likely to have important neuroimmuno-endocrine properties. Moreover, the altered chromogranin expression in PPP skin might influence both the neuroendocrine and neuroimmunologic properties of palmoplantar skin in these patients. We conclude that normal palmoplantar skin, with its high density of sweat glands and ducts which express all the chromogranins studied, might be of particular importance in skin neuroimmuno-endocrinology. Further studies of the relevance of the changes of chromogranin expression in PPP skin compared with normal skin may be helpful for the understanding of the neuroendocrine and neuroimmunologic properties of palmoplantar skin, especially the sweat gland apparatus in these patients.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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