Expression of selected neuropeptides in pathogenesis of bullous pemphigoid and dermatitis herpetiformis

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Bullous pemphigoid (BP) and dermatitis herpetiformis (DH) are chronic subepidermal bullous diseases, which progress together with an itch and an inflammatory reaction. These symptoms may be the cause of a phenomenon described in the literature as a neurogenic skin inflammation. Neuropeptides are one of the mediators which take part in this process. The aim of our study was to indicate the expression of selected neuropeptides – CRF (corticotropin releasing factor), CGRP (calcitonin gene-related peptide), NKB (neurokinin B), SP (substance P) and the receptor for endothelin B (ETRB) – in the skin of patients suffering from BP or DH. A significantly increased expression of CRF was found in the specimen collected from the skin lesions of patients with BP and DH as well as a significantly increased expression of receptor for endothelin B in the patients with DH by the immunohistochemical method. The results obtained give evidence of a possible participation of CRF and receptor for endothelin B in the pathogenesis of the itch in the dermatitis herpetiformis as well as CRF in bullous pemphigoid.

Key words: bullous pemphigoid (BP), dermatitis herpetiformis (DH), neuropeptides.

Introduction

Pruritus is one of the main symptoms of many skin diseases as well as an important skin manifestation of systemic dermatoses [1]. The phenomenon of pruritus is one of the basic subjective symptoms both in bullous pemphigoid (BP) and in dermatitis herpetiformis (DH). Despite different clinical picture, the common feature observed in the majority of patients is the itch.

Bullous pemphigoid is a blistering disease, characterized by inflammatory infiltrate in the dermis, presence of IgG and C3 deposits along the basement membrane zone and circulating IgG autoantibodies. Autoantibodies binding to autoantigens (glycoproteins: 230 kD – BPAG1 and 180 kD – BPAG2) localized in the basement membrane of the epidermis activate a series of immunological and enzymatic phenomena leading to destruction of basement membrane components and anchoring fibers and blister formation [2, 3].

The formation of the infiltrates is preceded by early accumulation of leukocytes, depending on activity of adhesion molecules, especially selectins and integrins. The binding of autoantibodies leads to activation of keratinocytes, releasing interleukin 6 and interleukin 8, as well as activation of C5 component of the complement [4, 5]. Matrix metalloproteinases released by inflammatory cells and keratinocytes are finally responsible for blister formation [5-7].

Dermatitis herpetiformis is characterized by skin and intestinal lesions. Skin lesions include polymorphic eruption accompanied by severe pruritus. Intestinal lesions are characterized by atrophy of intestinal villi result-

ing from the immunological process. Diagnosis of DH is established based on results of the direct immunofluorescence test (DIF) revealing granular deposits of IgA in the papillae and the presence of circulating IgA antibodies directed against endomysium and/or tissue and epidermal transglutaminase (*t*TG, *e*TG). Skin lesions in DH are histologically characterized by neutrophilic infiltrate leading to destruction of basement membrane zone (BMZ) proteins, anchoring fibers and blister formation [8].

In the recent years, the skin has been noted as an active neuro-, endocryno- and immunological organ, which has its own systems of quick response to different kinds of stimuli: mechanical, thermal, chemical to mention but a few [9]. One of the main factors influencing the state of the skin is broadly understood stress, under the influence of which the polypeptide compounds (i.e. neuropeptides) are being released from the nerve endings in the skin [10].

Neuropeptide synthesis in the nervous system occurs in the dorsal root ganglion of the spinal cord, from where they are transported antidromically to the nerve endings in the skin. The neuropeptides are mainly released from the afferent C-type unmyelinated fibers as well as A delta-type myelinated fibers [11]. A part of neuropeptides such as SOM (somatostatin), NPY (neuropeptide Y) and VIP (vasoactive intestinal peptide) can also be released by autonomic nerve fibers [12].

Not only can the cells of the nerve system synthesize neuropeptides, but also a variety of other cells existing permanently or temporarily in the skin have such function: Langerhans cells, fibroblasts, mast cells or keratinocytes to mention but a few [13, 14].

After the release of neuropeptides, a set of pro-inflammatory reactions occur such as the broadening and increase of transmission of vessels, followed by transmission of plasma proteins to the surrounding tissues and the influx of leukocytes. Such processes are widely described in the literature and are referred to as neurogenic skin inflammation [13].

The inflammation created in the skin under the influence of nociceptive stimuli or broadly understood stress constitutes the foundation of a certain group of chronic dermatoses [10]. Additionally, it was observed that the most frequent symptom found in the majority of the above mentioned inflammatory diseases is the skin itch. It was stated that the mediators as well as neuropeptides, which are released locally or systemically in the skin in response to stress, increase the production of factors stimulating the itch by strengthening the neurogenic inflammation and lowering the threshold of releasing the itch [15].

Neuropeptides released in the skin can be divided into three groups. The first group comprises the opioid neuropeptides (met-enkephalins Met-E and leu-enkephalins Leu-E), which are products coming from enkephalin A (PEA). The second group is non-opioid neuropeptides including: SP (substance P), NKA (neurokinin A), CGRP (calcitonin gene-related peptide), VIP (vasoactive intestinal peptide), NPY (neuropeptide Y), SOM (somatostatin), ANP (atrial natriuretic peptide), GPR (gastrin releasing peptide), CCK (cholecystokinin) and bradykinin. The last group are neurotrophins, in which NGF, NT-3, NT-4, NG-5, BDNF (brain-derived neurotrophic factor) can be found. The CRF (corticotropin releasing factor) is a factor of a wider spectrum of functioning, which connects many systems. Therefore, it is classified in a different way than others ("hypothalamic" neuropeptides) [15-20].

There is still little evidence of the expression and the possible function of neuropeptides in blister diseases. Nevertheless, recent studies have established biochemical properties of neuropeptides and their influence on inflammation of the skin. Therefore, recently available data have created the scientific basis for research focused on establishing the role of these proteins in the pathogenesis of subepidermal bullous diseases. Thus, the goal of our study was to evaluate the contribution of selected neuropeptides in pathogenesis of BP and DH, through examination of their expression and distribution in the healthy skin, as well as in the lesions collected from patients with BP and DH in the active stage of the disease.

Material and methods

Twenty-seven (15 women, 12 men, mean age 68.5 years, range: 58-84 years) BP patients as well as 13 untreated patients with DH (5 women, 8 men, mean age 44.8 years; range: 18-58 years) were qualified to the researched group. The specimen of the perilesional skin as well as the specimen from the skin lesions was collected from both DH and BP patients. The research material was obtained during the active disease process before treatment in the patients.

The diagnosis of pemphigoid was established on the basis of the clinical picture, histopathological examination and an Indirect Immunofluorescence test (IIF) and Direct Immunofluorescence test (DIF). During the IIF examination the antibodies IgG against the antigens of basement membrane were found in all BP patients. The DH patients were qualified to the examination on the basis of clinical symptoms, histopathological examination, DIF, IIF confirming the diagnosis. In the DIF, granular deposits of IgA in the tops of dermal papillae were found in all DH patients and in the IIF circulatory antibodies IgAEmA (esophagus monkey IgAEmA, Medizinische Labordiagnostika) in all the patients (titer range 1 : 10 – 1 : 320, median 1 : 40) were observed.

The received research results were compared to an expression of the examined proteins in the skin of healthy people. The control group consisted of 10 healthy individuals (5 women and 5 men, age between 19 and 49 years, mean age: 42 years).

The research was conducted with the consent of the Bioethical Committee of Medical University of Lodz (RNN/145/09/KB – 17.02.2009). The research results were compiled by means of statistical methods – Student's t-test preceded by the assessment of distribution and variance and in justified cases, the Mann-Whitney U test was done. The expected value and standard deviations were provided. The expression in the control group was assessed by the semi-quantitative method. The expression results of the examined neuropeptides in the group of DH and BP patients were compared to the expression received in the skin specimens of the people from the control group. The research is a preliminary report and the examined and the control groups will be enlarged.

Tissue specimens

Formalin-fixed, paraffin-embedded biopsy specimens of lesions from the buttock or trunk skin were taken. Additional biopsy specimens were taken from buttock skin from healthy volunteers, age and sex matched with the patient group.

Immunohistochemistry

Paraffin-embedded sections (3-4 μm) were used for routine H + E staining and for immunohistochemistry in DAKO EnVision detection system using the immunoperoxidase method.

Paraffin sections were mounted onto SuperFrost slides, deparaffinized, then treated in a microwave oven in a solution of citrate buffer and transferred to distilled water. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in distilled water for 30 minutes, and then sections were rinsed with Tris-buffered saline (TBS, DakoCytomation, Denmark) and incubated with rabbit anti-human Neurokinin B Receptor antibody (Abcam, Cambridge, UK; ab13397; dilution: 1 : 200), rabbit anti-human Endothelin B Receptor (Abcam, Cambridge, UK, ab65972; dilution 1 : 250), rabbit anti-human CGRP (Abcam, Cambridge, UK, ab43873, dilution 1 : 400), mouse anti-human Corticotropin Releasing Factor (Abcam, Cambridge, UK, ab35748, dilution 1 : 200) and mouse anti-human Substance P (Abcam, Cambridge, UK, ab14184, dilution 1 : 800). Afterwards, an appropriate EnVision+System-HRP (DakoCytomation, Denmark) were used. Visualisation was performed by incubating the sections in a solution of 3,3'-diaminobenzidine (DakoCytomation, Denmark). After washing, the sections were counter-stained with hematoxylin and coverslipped. For each antibody and for each sample, a negative control was processed. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results.

Methodology of semiquantitative research

In each specimen, staining intensity of CRF (in keratinocytes, sebaceous glands, sweat glands and inflammatory cells), endothelin B receptor (in keratinocytes, endothelial cells and smooth muscle cells) was recorded semiquantitatively by two independent observers in 4-6 adjacent high power fields and graded 0 (staining not detectable), 1 (weak immunostaining), 2 (moderate immunostaining intensity) or 3 (strong staining). The mean grade was calculated by averaging grades assigned by two authors and approximating the arithmetical mean to the nearest unity.

Morphometry

Histological morphometry was performed by means of an image analysis system consisting of a PC computer equipped with a Pentagram graphical tablet, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and color TV camera Panasonic (Japan) coupled to a Carl Zeiss microscope (Germany). This system was programmed (MultiScan 8.08 software, produced by Computer Scanning Systems, Poland) to calculate the number of objects (semiautomatic function). The colored microscopic images were saved serially in the memory of a computer, and then quantitative examinations were been carried out.

The immunoexpression of substance P, neurokinin B and CGRP was determined by counting the immunopositive nerve endings in epidermis and dermis (semiautomatic function) in a sequence of 4-6 consecutive computer images of 400× high power fields – 0.0047 mm^2 each. The results were expressed as a mean number of these objects per high power field.

Statistical analysis

All values were expressed as the mean ± SD (standard deviation). The differences between groups were tested using Student t-test for independent samples preceded by evaluation of normality and homogeneity of variances with Levene's test. Additionally, the Mann-Whitney U test was used where appropriate. Results were considered statistically significant if $p < 0.05$.

Results

The results of conducted research are presented in Tables I and II. In Table I one can find data concerning the expression of tested neuropeptides and receptors in the specimens collected from the skin lesion and its perilesional skin of BP patients and the skin of healthy people who constitute the control group. Table II presents the results of the expression of the same proteins as Table I but from DH patients as well as the results from people from the control group. In both tables statistical data are additionally presented which compare the intensity of the expression of test-

Table I. Expression of the provided neuropeptides in BP patients from the specimens from skin lesions and perilesional skin. The comparison was conducted between the expressions

	Substance P	Neurokinin B	CGRP	CRF	Endothelin B receptor
Pemphigoid skin lesion (n = 27)	1.32 ±0.56	0.08 ±0.07	0.21 ±0.15	1.58 ±0.64	1.12 ±0.59
Pemphigoid perilesional skin (n = 27)	1.58 ±0.66	0.07 ±0.08	0.24 ±0.18	1.04 ±0.48	0.86 ±0.39
Control (n = 3)	1.22 ±0.2	0.07 ±0.1	0.33 ±0.33	0.36 ±0.15	0.58 ±0.32
Skin lesion vs. perilesional skin	p = 0.12 (NS)	p = 0.62 (NS)	p = 0.5 (NS)	p < 0.001	p = 0.06 (NS)

NS – non significant

Table II. Expression of the provided neuropeptides in DH patients from the specimens from skin lesions and perilesional skin. The comparison was conducted between the expressions

	Substance P	Neurokinin B	CGRP	CRF	Endothelin B receptor
Duhring's disease skin lesion (n = 13)	1.4 ±0.47	0.10 ±0.1	0.32 ±0.26	1.36 ±0.89	1.24 ±0.81
Duhring's disease perilesional skin (n = 13)	1.52 ±0.67	0.07 ±0.05	0.21 ±0.15	0.71 ±0.61	0.66 ±0.43
Control (n = 3)	1.2 ±0.2	0.07 ±0.1	0.33 ±0.33	0.36 ±0.15	0.58 ±0.32
Skin lesion vs. perilesional skin	p = 0.59 (NS)	p = 0.21 (NS)	p = 0.19 (NS)	p < 0.05	p < 0.04

NS – non significant

ed factors in the skin lesion as well as the skin collected from the perilesional skin.

Substance P

Substance P (Fig. 1) was found in nerve endings localized in the epidermis and the dermis. The expression in the skin lesions was moderate, in BP patients – 1.32 ±0.56 and DH patients – 1.4 ±0.47. The expression in skin lesions was similar to the perilesional skin.

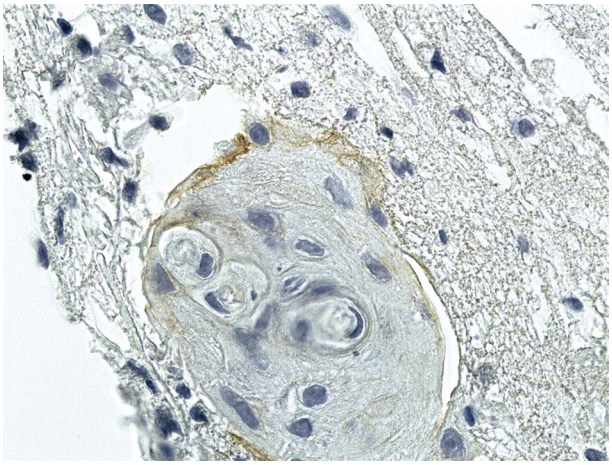

Fig. 1. Skin lesions. Bullous pemphigoid. Immunohistochemistry. Expression of SP in nerve endings localized in the epidermis and dermis. Original magnification 400×

Neurokinin B

Neurokinin B (Fig. 2) was found in nerve endings localized in the epidermis and the dermis. The expression in the skin lesions was moderate, in BP patients – 0.08 ±0.07 and DH patients – 0.10 ±0.1. The expression in skin lesions was similar to the perilesional skin.

Calcitonin gene-related peptide

The CGRP (Fig. 3) was found in nerve endings localized in the epidermis and the dermis. The expression in the skin lesions was weak, in BP patients – 0.21 ±0.15 and DH patients – 0.32 ±0.26. The expression in skin lesions was similar to the perilesional skin.

Corticotropin releasing factor

On the basis of the conducted research, the presence of CRF (Fig. 4) was found in keratinocytes, inflammation infiltration cells as well as the cells of sebaceous and apocrine glands.

On the basis of received results, a significantly larger expression of CRF was found in biopsies taken from the skin lesions than those from the perilesional skin

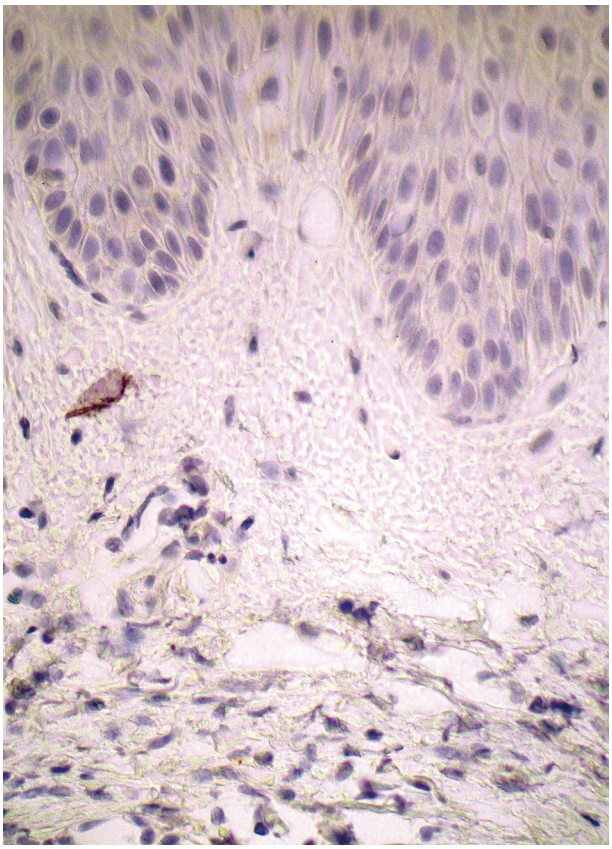

Fig. 2. Skin lesions. Bullous pemphigoid. Immunohistochemistry. Expression of neurokinin B in nerve endings localized in the epidermis and dermis. Original magnification 400×

in BP patients (1.58 ±0.64 vs. 1.04 ±0.48, $p < 0.001$) and DH patients (1.36 ±0.89 vs. 0.71 ±0.61, $p < 0.05$). The lowest expression of CRF was found in the biopsies coming from the skin of healthy people constituting the control group (0.36 ±0.15) (Fig. 5).

Endothelin B receptor

Endothelin B receptor (Fig. 6) however, was present in keratinocytes, the endothelium cells and the vascular smooth muscle cells. The phenomenon of the increased expression was also observed for endothelin B receptor in the specimens coming from lesions from DH patients 1.24 ±0.81. The expression was significantly larger than in the biopsies coming from the perilesional skin (0.66 ±0.43, $p < 0.04$). It seems that there is no difference in endothelin B receptor expression in specimens collected from the skin seemingly unchanged in DH patients and the control group (0.58 ±0.32) (Fig. 7). Such dependence was not found in biopsies collected from BP patients in skin lesion (1.12 ±0.59) and perilesional skin (0.86 ±0.39).

Discussion

Neuropeptides are short-chain amino acids, which in an indirect and direct way can modulate synaptic ac-

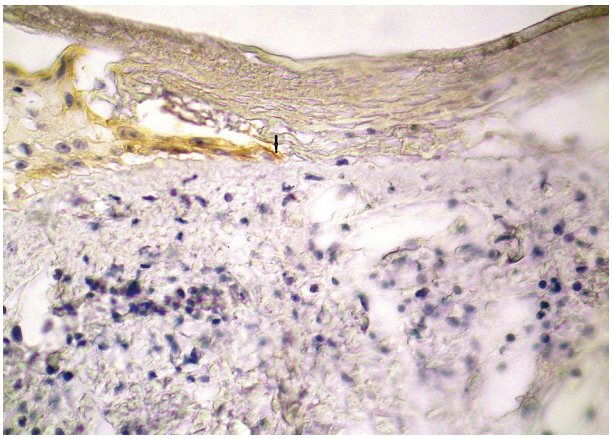

Fig. 3. Skin lesions. Bullous pemphigoid. Immunohistochemistry. Expression of CGRP in nerve endings localized in the epidermis and dermis. Original magnification 400×

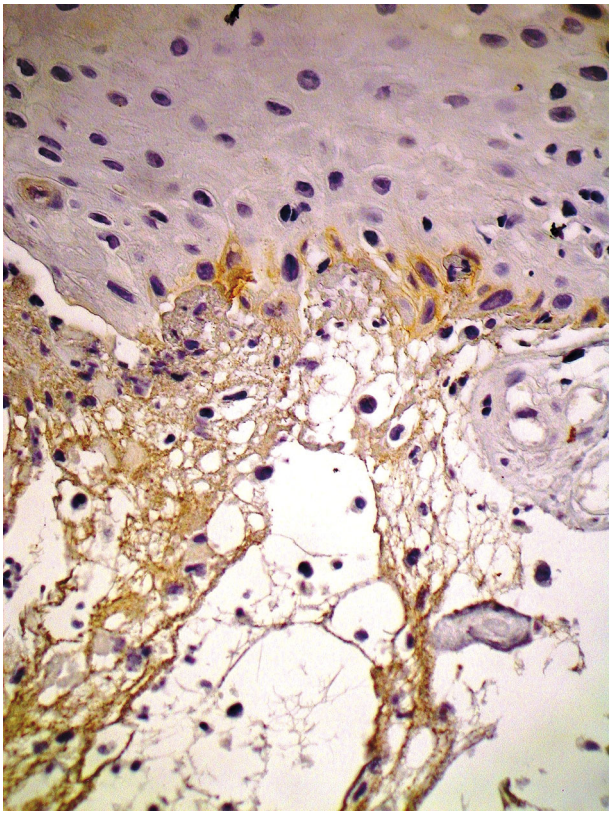

Fig. 4. Skin lesions. Bullous pemphigoid. Immunohistochemistry. Expression of CRF in keratinocytes, inflammation infiltration cells and the cells of sebaceous and apocrine glands. Original magnification 400×

tivity. It was proven that over 50 of them have a possibility to pass the signal not only within the elements of the nervous system, but also between the nervous cells and the cells of the immune system [16]. Therefore the term "neuropeptides", which means the peptide mediators coming from the cells of the nervous system, is not totally appropriate, but is used because of the historic and didactic terms [21]. What is more, neuropeptides often have been observed in a much broad-

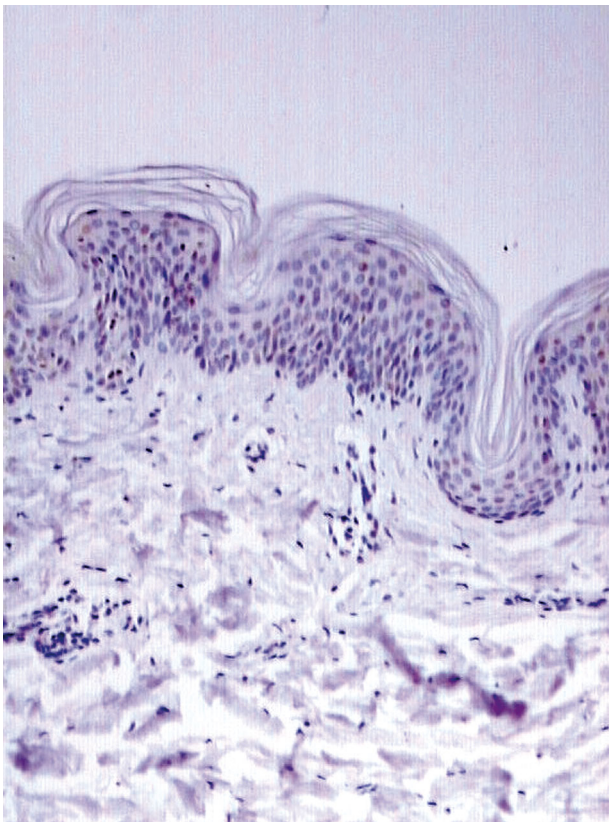

Fig. 5. Healthy skin. Immunohistochemistry. Single expression of CRF in keratinocytes. Original magnification 400×

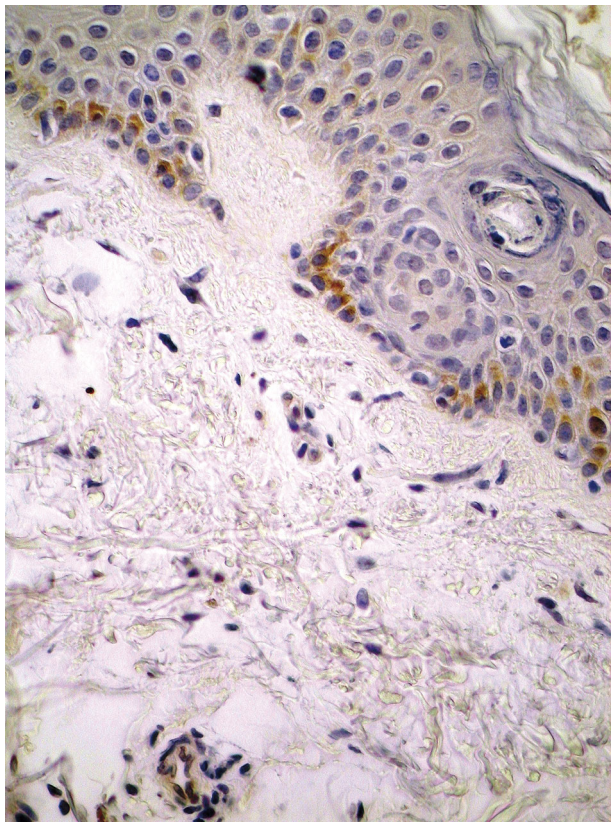

Fig. 6. Skin lesions. Dermatitis herpetiformis. Immunohistochemistry. Expression of endothelin B receptor in keratinocytes, endothelium cells and vascular smooth muscle cells. Original magnification 400×

er context lately. That means as a part of the neuroimmuno-endocrynological system [22, 23].

In the nervous system they fulfill most of the criteria, which allow for perceiving them as neurotransmitters, neuromodulators, neurohormones or hormones. They can perform their regulatory function in many ways: firstly as a part of the autonomous nervous system by working peripherally. The second way is as a brain peptide functioning in the central nervous system or as neurohormones reaching target organs through hypophyseal portal vessels or systemic circulation [16].

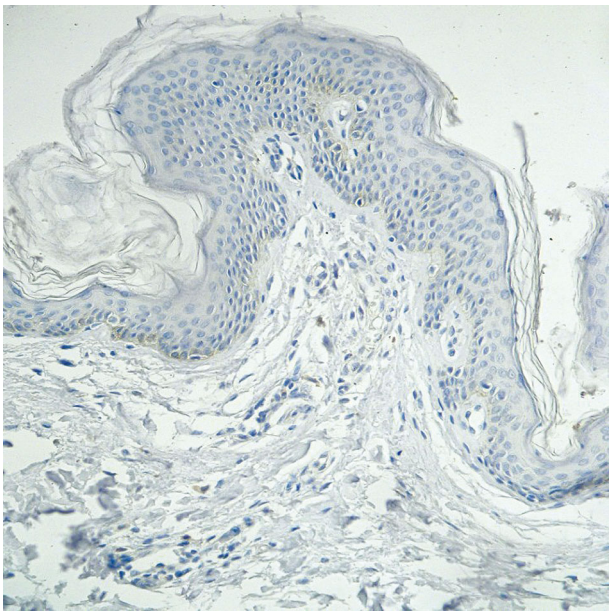

Fig. 7. Healthy skin. Immunohistochemistry. No signal for endothelin receptor B. Original magnification 100×

The proposal of research into the neuropeptide participation in the pathology of pruritus was based on their unstable presence in the skin, especially in the skin lesions observed in patients with allergic changes or with atopic dermatitis (AD) and in which the nerve fibers with an appropriate fluctuation profile for neuropeptides was observed [24].

On the basis of conducted research it has also been observed that the neuropeptides given through percutaneous injection may induce the phenomenon of pruritus in human skin [24]. The SP may be an example of such neuropeptide. Phenomena such as skin edema, urtica or reddening are ascribed to SP and later on also skin itch can be ascribed so after its percutaneous injection. Pruritic reaction to SP, as the research presented it, is stopped by antihistaminic medicines, which in turn proves that mast cells also take part in the process [24-26]. The SP was the first to be discovered

and therefore the best known protein belonging to the neuropeptide group [21].

Its release occurs under the influence of different kinds of factors such as pain, thermal, skin-irritating stimuli or as a result of UV radiation. It is released by unmyelinated C-fibers and through the cells belonging mostly to the immune system such as mast cells, eosinophiles and macrophages [27].

The main influence of SP on the skin results in negative influence on mast cells, which as a result of a stimulus, go through degranulation causing the release of histamine, tumor necrosis factor α (TNF-α) as well as a number of cytokines: IL-1, IL-6 and IL-8. As a result, the following occurs as well widening of vessels and the increase of cell adhesion molecule expression in the endothelium cells, the migration of immune cells to the peripheral cells and angiogenesis intensification [28]. Those substances are co-responsible for creation of inflammatory reaction in the skin and the development of pruritus. Both in pemphigoid and Duhring's disease there are certain reactions responsible for the development of the disease process. It was proven that in BP keratinocytes stimulated in the effect of binding autoantibodies with an antigen BPAG2 release interleukins 6 and 8 as well as a complement protein C5 [29, 30]. Apart from that in the etiopathogenesis of BP the activation of mast cells and neutrophils takes place as well [29].

In the histopathological picture of the dermatitis herpetiformis we can observe microabscesses which are present in the dermal papilla. They are a result of accumulation of inflammatory infiltration of mainly neutrophils as well as eosinophiles. The accumulation of neutrophils in the skin in the DH is triggered by the accumulation of lymphocytes CD4+ and the interferon γ (INF-γ), TNF-α and IL-2 factors released by them [29]. Our previous research about the role of cell adhesive molecules in the pathogenesis of subepidermal bullous diseases demonstrated their significance both in BP and DH [28, 29, 31]. On top of that, the chemokines, which first were described only as chemotactic factors, are now taken into consideration as substances influencing the immunological processes [29].

Most of neuropeptides making up a group of small peptides that exert their effects by interacting with members of a superfamily of G protein-coupled receptors [32]. Expression of G protein-coupled neurokinin receptors NK-1R, NK-2R, and NK-3R has been found in human and rodent skin [16, 33]. Neurokinins A and B (NKA and NKB) as well as SP activate the above mentioned receptors, through signal transduction pathways involving adenylate cyclase and phospholipase C and A2 [15, 34]. Keratinocytes and endothelial cells express NK-1R to NK-3R, and mast cells, fibroblasts, and Langerhans cells express NK-1R [33]. Activation of these receptors stimulates proliferation of keratinocytes, fibroblasts, as well as endothelial cells and neovascularization [33-35]. The NKA and SP stimulate mast cells release of histamine and TNF-α, as well as keratinocyte and endothelial cell to release proinflammatory cytokines, and expression of adhesion molecules [15, 34, 35].

Additionally, SP and NKB may also influence other cells of the skin, immune system and vessels. Our results revealed expression SP and NKB in nerve endings localized in the epidermis and the dermis. But in our paper there was no significantly increased expression of SP or NKB in skin BP and DH biopsies. This neuropeptide does not probably have a main role in pruritus and skin neuroinflammation in these diseases.

The CGRP is built from 37 amino acids, coded by the same gene as preprocalcitonin [36]. In the skin, CGRP is mainly found in the derma-epidermal connection, but can also be present in the epidermis and in the region of blood vessels. The presence of CGRP was detected mainly in free nerve endings, afferent neurons of smooth muscles and also in keratinocytes, Langerhans cells, melanocytes, mast cells and Merkel cells [13]. The CGRP 1, 2 are the receptors for calcitonin gene-related peptide [25, 35]. Similarly to the case of SP, after the connection with the receptor, the degranulation of mast cells occurs as well as the increase of histamine and TNF-α secretion [13]. As it was proven before, these processes are also connected with pemphigoid and Duhring's disease pathogenesis.

The obtained results confirm the presence of SP, CGRP and neurokinin B in nerve endings in the collected skin biopsies. It is consistent with the observations conducted by other researchers [24]. Even though our research did not demonstrate their significantly higher expression in the biopsies collected from patients with dermatitis herpetiformis and pemphigoid, but its connection between the neurons and its release which is changeable in time could influence the results of the research. Therefore, we cannot unequivocally exclude its participation in the pathogenesis of the pruritus in the analyzed diseases.

Even though there are no universal peripheral mediators for pruritus, it is believed that specific substances characteristic of the diseases do exist. It was proven that a part of the mediators produced by the skin cells may also activate and sensitize pruritic nerve endings. Neuropeptides belong to one of the groups. In this context the pruritus is probably not only linked to the mast cells. Epidermis in itself is nerved by the sensory nerve endings, which are anatomically connected with keratinocytes and Langerhans cells. The last research proved that during stimulation the keratinocytes are capable of releasing pruritic and anti-pruritic mediators such as endorphins, neuropeptides, proteases and cytokines. As it was proved in the research, the keratinocytes which are the source of many cytokines and metalloproteinases also have an undeniable role in the pathogenesis of BP

and DH [7]. The mentioned metalloproteinases and cytokines in these disease entities are responsible for creating the inflammatory infiltrations and forming blisters. Keratinocytes in DH and BP are probably also the source of neuropeptide secretion and therefore can participate in the phenomenon of pruritus. Just like CRF, the SP lowers the electric potential in keratinocytes similarly to the situation of damaging the skin barrier. Therefore, ion channels which are dependent on the change of potential, the ATP and TRPV1 (transient receptor potential vanilloid type 1) can be directly connected with the pruritus transmission through activation of keratinocytes in the dry skin [24].

Corticotropin-releasing factor (corticotropin-releasing hormone, CRH, CRF) is a 41-amino acid peptide derived from a 191-amino acid preprohormone. The CRH is secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress, hypothalamic-pituitary-adrenal axis. The CRF has been implicated at the central level in the functional regulation of behavioral, autonomic, endocrine, reproductive, cardiovascular, gastro-intestinal, metabolic and immune systemic activities [37, 38]. In the skin, similar activities for the CRF peptides family have been reported, occurring mostly through para or autocrine mechanisms supporting a local function [38, 39]. The CRH can also have a role in the regulation of cell differentiation and proliferation [40]. Nevertheless, the skin is the key barrier against environmental stressors. In the skin, CRF is a part of a system organized in a similar way to the HPA [15]. Therefore, skin melanocytes treated with CRF respond with ACTH-mediated increases in cortisol production. The CRF and the corresponding CRH-Rs are expressed in the skin in species and cell type associated manner. The CRH-R1 is expressed in the epidermis, dermis, as well as subcutis with CRHR1α and it is expressed in hair follicle keratinocytes and papilla fibroblasts, sebaceous and eccrine glands, muscle and dermal blood vessels. In our study, we have confirmed the expression of CRF in keratinocytes, inflammation infiltration cells as well as the cells of sebaceous and apocrine glands, which confirms the presence of the receptor for CRF in the structures described above. They also modulate expression of cell surface adhesion molecules and expression of cytokines [39, 41]. Mechanisms of the increase in expression of adhesion molecules and cytokines are crucial for BP and DH. These data define CRF as a pleiotropic cytokine which may regulate proliferation, differentiation, and immune interactions in the skin. The inflammation of skin in both BP and DH is probably also caused by the increase in expression of CRF, which was proved by our study. In the skin lesions of patients suffering from BP and DH, the increase in CRF expression was observed. The expression was statistically higher in the skin lesions than in the perilesional skin [39].

The endothelin group of molecules consists of three polypeptides, ET-1, ET-2 and ET-3, of 21 amino acids that bind to two highly homologous G-coupled protein receptors, endothelin receptor A (ETRA) and endothelin receptor B (ETRB or EDNRB) [42]. The ETRA is highly specific to ET-1 and ET-2, while it binds ET-3 with low affinity. On the other hand, ETRB is a nonselective receptor, which binds ET-1, ET-2, and ET-3 with similar affinity [43].

The ET-1 has been reported to cause pruritus when it is injected intradermally into human skin, but such a detailed study of this pruritogenic action has not yet been conducted [44]. The peptide can be found in human skin and its synthesis and release has been presented in endothelial cells and keratinocytes. The ET-1 induced TNF-α, and IL-6 production by mast cells and induces skin inflammation. The ET-1 is upregulated in response to hypoxia, stress and inflammatory cytokines [45].

In this paper we have suggested the possible role of CRF in pathogenesis of neuroinflammation in DH and BP and as well as endothelin receptor B in DH. Our data may suggest that at least some skin pathologies regarding the blister formation could be linked to neuropeptide family members. Nevertheless, further studies are needed to elucidate the role of neuropeptides in pathogenesis of bullous diseases.

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