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Substance P and Vasoactive Intestinal Peptide in Bullous and Inflammatory Skin Disease

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Immunoreactivity (IR) of substance P (SP) and Vasoactive Intestinal Peptide (VIP) were determined by radioimmunoassay in serum of 56 patients with inflammatory skin diseases, in blister fluid of 40 patients with spontaneous blisters and 31 subjects with induced skin blisters. Serum concentrations of SP-IR and VIP-IR were mostly low or non-detectable. Spontaneous blisters contained high amounts of SP-IR, particularly in bullous pemphigoid and in some inflammatory dermatoses, while VIP-IR levels usually were low. Suction blisters from inflamed but not from normal skin often contained SP-IR but more seldom VIP-IR. *Key words: Peptides; Substance P; Vasoactive Intestinal Peptide; Blister fluid; Radioimmunoassay.* (Received May 15, 1985.)

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The neuropeptides Substance P (SP) and Vasoactive Intestinal Peptide (VIP) are found concentrated in the central as well as peripheral nervous system. Their close connection to peripheral nerves implies their presence in most mammalian organs and structures.

In the skin, low concentrations of SP and VIP were demonstrated by radioimmunoassay (RIA) in the cat and pig (1–3). Immunocytochemically, both neuropeptides were observed in subepithelial nerves, SP in free nerve endings, VIP deeper in connection with small blood vessels and sweat glands (4). In human digital skin, SP-IR (immunoreactivity) nerve endings have been demonstrated in the dermal papillae and in the epidermis, in some Meissner's corpuscles and in close connection to sweat ducts and blood vessels (5, 6).

Although SP, VIP and other neuropeptides are present and demonstrable in human skin their function is far from clear. Intradermal injection of SP and VIP exert vasodilatation and increased permeability; therefore, primary effects are erythema and sometimes edema (7, 8). SP has been attributed a mediating role of the axon reflex flare in man (7, 9) and both neuropeptides may contribute to inflammatory and itching reactions in the skin (10, 11). In the present investigation we have studied patients with disseminated inflammatory skin disease for blood levels of SP-IR and VIP-IR. The tissue levels of both these neuropeptides in the skin were assayed by examining fluid from spontaneous and suction blisters by specific RIA. High performance liquid chromatography (HPLC) was performed on some pools of fluid from spontaneous blisters to characterize SP-IR and VIP-IR.

MATERIAL AND METHODS

Patients

Blood samples from 56 patients with various inflammatory skin diseases were collected in tubes containing sodium EDTA 1 mg/ml and Trasylol (500 KIU/ml), immediately cooled with ice and centrifuged at 4°C. Patients with extensive dermatitis were selected; exceptions were the cases of generalized pruritus without primary lesions and those with bullous pemphigoid in which the skin area involved usually was limited. The material is presented in Table I.

Table I. *Patients examined for serumlevels of SP and VIP*

Diagnosis	Nr. of patients	Age range	Mean age	No. of females	No. of males
Bullous pemphigoid	9	59–89	76	4	5
Eczema	13	17–83	44	8	5
Psoriasis	5	51–78	64	2	3
UV dermatitis	5	28–81	46	3	2
Pruritus	7	27–91	66	1	6
Toxicoderma	9	29–83	61	7	2
Acute urticaria	4	21–82	50	2	2
Chronic urticaria	4	32–67	50	3	1

Collection of blister fluid

Blister fluid from 40 other patients with various bullous diseases was collected with a syringe, cooled with ice and rapidly frozen to −20°C. The material included 14 patients with bullous pemphigoid, six with eczematous disease, six with phototoxic and two with photoallergic dermatitis, two with burns, and four with skin infection. When only small amounts of blister fluid could be collected the analysis was limited to SP.

Suction blisters were induced on forearm skin (ventral proximal area) by a vacuum pressure of 4.5 mWS in 31 subjects. In each patient eight 4 mm blisters were provoked within 60–90 min. Blister fluid was immediately collected and treated as above. There were six patients with bullous pemphigoid, six with endogenous eczema, seven with psoriasis, one with dermatitis herpetiformis, two with acute urticaria, and six subjects with normal skin apart from localized leg ulcer, pyoderma etc.

Except for controls and patients with bullous pemphigoid the suction blisters were induced on inflamed skin only.

In five patients (two with bullous pemphigoid and three with leg ulcers) suction blisters were induced in duplicate, one set being harvested immediately, the other after 12–72 h.

The frozen samples of serum and blister fluid were stored at −20°C until extraction for determination of SP and VIP in serum. The blister fluid was analysed unextracted.

Radioimmunoassay of SP and VIP

The concentrations of SP and VIP immunoreactants in plasma and blister fluid were assayed by RIA procedures specifically modified and optimized to the small sample quantities available for each assay. All samples were assayed in serial dilutions.

Immunoreactive SP was quantitated using a rabbit antiserum (SP-8, a generous givt from Dr K. Nilsson, Sweden) in a final dilution of 1:125000. ^{125}I-Tyr^{8}-SP was used as tracer after HPLC purification. The smallest amount detectable was 3 pmol/L (4 pg/ml) after concentration of the samples. The antiserum does not cross-react with bombesin, gastrin releasing peptide, the tachykinins: eledoisin and substance K. The intra- and interassay variations were 4 and 8.3% (n=20), respectively.

Table II. *SP-IR in spontaneous blisters (pmol/l)*

Diagnosis	No.	Mean	Range
Bullous pemphigoid	14	106	<20–315
Eczema	6	168	<20–420
Burn	2	78	25–130
Phototoxic reaction	6	30	<30–45
Photoallergic	2	32	<20–45
Photodermatitis	8	30	<20–45
Infection	4	52	20–145
Others	4	39	<20–44

Table III. *VIP-IR in spontaneous blisters (pmol/l)*

Diagnosis	No.	Mean	Range
Bullous pemphigoid	12	12	<6–50
Photodermatitis	4	16	10–16
Burn	2	265	31>500
Tinea pedis	1	79	
Others	2	19	11–25

For RIA of VIP we used a rabbit antiserum (No. 7852, MILAB, Malmö, Sweden), final dilution 1 : 40 000. The antiserum recognizes the N-terminal 15 amino acid sequence of VIP. It does not cross-react with cholecystokinin, secretin, gastric inhibitory peptide or glucagon. VIP was radio-labelled using a modification of the chloramine T method (12). The assay could detect (with 95 % confidence) a minimum of 4 pmol/L (13 pg/ml). The intra- and interassay variations were 4.0 and 8.5 % (n=20), respectively.

High performance liquid chromatography (HPLC)

The HPLC system consisted of a Waters model 204 liquid chromatograph equipped with a model U6K injector and an absorption detector 441, a Model 6000 A pump and automatic gradiant controller and a gradient former pump M45. Reverse column μ BondaPak C18 (Waters) was used. The sample was eluted with acetonitril (CH_3CN)/0.08 % trifluoroacetic acid (V/V), pH 2.5. The eluting conditions used for SP were a linear gradient of CH_3CN from 28 to 40 % for 60 min, then a linear gradient of CH_3CN from 40 to 58 % for 10 min. For VIP the sequence was a linear gradient of CH_3CN from 20 to 32.5 % for 25 min, then 10 min of isocratic elution at 32.5 % acetonitril, followed by a linear gradient from 32.5 to 40 % acetonitril for 10 min (12).

Fractions of 0.5 ml were collected (flow rate 1.0 ml/min) and lyophilized. The dry residues were dissolved in 0.1 ml 0.05 M phosphate buffer, pH 7.4, containing 0.25 % human serum albumin and 0.05 % sodium azide, and assayed for SP and VIP immunoreactivity.

RESULTS

Serum

In all cases the concentration of SP-IR was below the level of detection. The same held true for the VIP-IR assay with one exception: a case of atopic dermatitis with VIP-IR > 200 pmol/l.

Spontaneous blisters

The results of the SP-IR assay are given in Table II. High SP-IR values, >20 pmol/l, were found in 11/14 patients with bullous pemphigoid, all six with eczematous disease, 3/8 with photodermatitis, 1/2 with burn, and 1/4 with skin infection (tinea pedis).

Table IV. *SP-IR in suction blisters (pmol/l)*

Diagnosis	No.	Mean	Range
Bullous pemphigoid	6	<20	<20
Eczema	5	33	<20–67
Acute urticaria	2	<20	<20
Psoriasis	7	86	<20–349
Controls	7	<20	<20

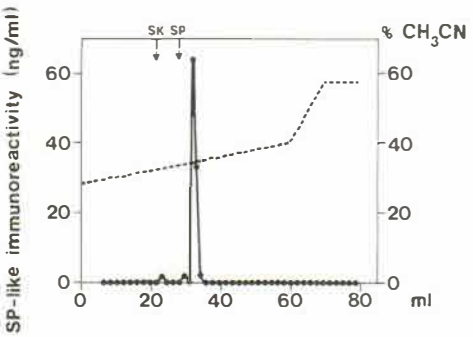

Fig. 1. Reverse phase high performance liquid chromatography of SP-immunoreactive material in extracts of the bullous fluid from patients with pemphigoid. The elution position of synthetic SP and substance K is indicated by arrows.

The results of the VIP-IR assay are given in Table III. A high VIP-IR value, >25 pmol/l, was found in the patient with skin infection (tinea pedis) and a very high value in one of the patients with a burn blister, but low values in most pemphigoid blisters.

Suction blisters

Low values for SP-IR were found in all six pemphigoid patients and in the six controls (Table IV). Increased values were observed in 2/5 eczema patients and one with dermatitis herpetiformis (without spontaneous blisters). Increased SP-IR value was found in 3/7 psoriatics (erythroderma), two of them very high.

Table V shows the results of VIP-IR assay. A high value was found in 1/6 eczema patients and the one with dermatitis herpetiformis, a very high value in 2/7 controls.

The SP-IR assay on blisters harvested immediately after suction and 12–72 h later was negtive in all five cases.

Fig. 1 illustrates the HPLC separation of SP-like peptides in pooled fluid of blisters from patients with bullous pemphigoid. The blister content of immunoreactive SP-material eluted as one major component with a larger elution volume than synthetic SP and substance K indicating a more hydrophobic fragment of SP. VIP-like immunoreactivity as studied in one patient with bullous pemphigoid separated into one dominating peak and two minor peaks. The major peak had a very short elution time, indicating that this fragment is less hydrophobic and has a molecular weight lower than synthetic VIP used as marker (fig. not shown).

DISCUSSION

With one exception the serum levels of SP-IR and VIP-IR were below the level of detection. This negative result does not exclude a pathogenetic role of SP and VIP in the development of dermatitis. The concentration fall from the localized area of release or production to the circulating blood should be immense and the metabolism rapid (13). The

Table V. *VIP-IR in suction blisters (pmol/l)*

Diagnosis	No.	Mean	Range
Bullous pemphigoid	4	<6	<6
Psoriasis	5	10	<6–20
Acute urticaria	2	<6	<6
Eczema	5	13	<6–40
Controls	6	31	<6–136

exceptional finding of an increased serum VIP-IR in a patient with atopic dermatitis is outweighed by the negative findings in the other five patients with the same diagnosis.

Instead, high concentrations of SP-IR and VIP-IR were demonstrated in spontaneous bullae from several patients (Table II–III). In the bullous fluid of pemphigoid SP-IR levels were impressive and it is tempting to speculate over a pathogenetic role for SP. Dermal mast cells are activated and degranulated early in the pathogenesis of bullous pemphigoid leading to eosinophilic chemotaxis, release of "eosinophilic major basic protein" and consequent blistering (14), as well as liberation of histamine (15) and diamine oxidase (16) in blister fluid. Apparently, SP exerts its action by way of mast cell degranulation (11, 17).

In bullous pemphigoid 7/14 patients were on systemic treatment with prednisolone, 1/7 also with azathioprine and two other patients were treated with dapsone when blister fluid was collected. Obviously, the therapy did not suppress the release of SP-IR. Nor did the SP-IR levels seem to correlate with the clinical or laboratory activity of the disease.

Suction blisters in pemphigoid did not contain SP-IR which seems to speak against its pathogenetic importance. Such blisters develop on the same level—lamina lucida—as genuine bullae (18, 19). Nor did SP-IR emerge in suction blisters in pemphigoid when harvesting was delayed. It should be pointed out, however, that suction blisters were in no case induced on inflamed skin. The reason for this was that for comparison we chose to induce all blisters on the forearm.

Alternatively, the high SP-IR in pemphigoid bullae may be secondary to the blistering process, a result of damage to subepithelial or epidermal structures. This would explain the absence of VIP-IR in the bullous fluid (Table III)—because of the deeper location of VIP nerves—and the finding of SP-IR in other spontaneous blistering (Table II) when superficial structures might be damaged.

In suction blisters, VIP-IR rather than SP-IR was demonstrated in some instances. This might be explained by an influence of the induction process on VIP-containing structures in particular. In several cases of suction blisters in inflamed skin SP-IR was demonstrated. This may be explained by a suggested connection between SP and leukocyte-mediated inflammations (20).

In the present SP-RIA the antiserum is directed against the middle part of the SP molecule. This minimizes cross-reactivity with other members of the tachykinin family. Efforts to ascertain the structure of the main SP-IR and VIP-IR components are in progress.

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