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# Vasoactive Intestinal Polypeptide and Substance P in the Pathogenesis of Atopic Dermatitis

ALBERTO GIANNETTI, FABRIZIO FANTINI, AUGUSTO CIMITAN and CARLO PINCELLI

*Institute of Dermatology, University of Modena, Modena, Italy*

**Neurogenic components are probably involved in the pathogenesis of atopic dermatitis (AD) and several neuropeptides have been implicated in the mechanisms underlying this disease. The aim of the present study was to evaluate by radio-immunoassay (RIA), the vasoactive intestinal polypeptide (VIP) and substance P (SP) content in whole-skin homogenates of AD lesions. RIA was performed using an antiserum, AH78, recognizing the carboxy-terminal fragment VIP (22–28) and a polyclonal antiserum directed against SP. VIP levels were markedly increased in lesional AD skin (5.62±1.25 pmol/g tissue) vis-à-vis controls (0.43±0.08 pmol/g tissue), whereas SP levels were significantly lower in lesional skin (0.25±0.03 pmol/g tissue) than in normal skin (0.97±0.24 pmol/g tissue). The results confirm that VIP and SP are relevant to the pathogenesis of AD and their imbalance might reflect diverse roles of these NP in the modulation of AD lesion.** ***Key words: Neuropeptides; Vasoactive Intestinal Polypeptide; Substance P; Radio-immunoassay; Atopic Dermatitis.***

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Prof. Alberto Giannetti, M.D., Institute of Dermatology, University of Modena, Via del Pozzo 71. 41100 Modena, Italy.

Atopic dermatitis (AD) is known to be exacerbated by psychic stress, scratching and sweating (1, 2). This suggests a possible involvement of a neurogenic component in the pathogenesis of the disease. NP are thought to be the main mediators of neurogenic inflammation, which may be involved in the pathogenesis of certain inflammatory dermatoses (3). Therefore, NP may also play a role in the mechanisms underlying AD lesions. Indeed, reduced flare and wheal reactions to intradermal injections of several neuropeptides (NP) have been observed in AD (4), while marked changes in the expression of certain NP have been found by immunohistochemistry in skin of patients with AD (5).

Vasoactive intestinal polypeptide (VIP) and substance P (SP) are widely distributed neuropeptides in the central and peripheral nervous system (6, 7). Immunohistochemical studies have established that both peptides are present in normal human skin (8, 9). VIP and SP are known to have important physiological functions, including vasodilation (10), mast cell degranulation (11) and immunomodulation (12).

The aim of the present study was to evaluate, by means of radio-immunoassay (RIA), the VIP and SP content in lesional skin of patients with AD.

## MATERIALS AND METHODS

For VIP, 13 AD patients (8 females and 5 males) ranging in age between 9 and 38 were studied. For SP, we examined 16 AD patients (12 females and 4 males, age range 11–27). Diagnosis was made according to Hanifin & Rajka's criteria (1). All patients recruited to the study presented chronic lesions involving either elbows of popliteal flexures. Patients had not received any treatment for at least 8 weeks prior to the study. Six-mm punch biopsies were taken from lichenified skin of elbow flexures. Mean weight of the specimens was 60.1 mg, including subcutaneous fat tissue. Specimens were immediately frozen and stored at –80°C until further processing. For control purposes, 26 skin specimens (13 for VIP and 13 for SP) from the same area of 11 age- and sex-matched healthy volunteers were obtained. Specimens were homogenized in 10 ml/g of 0.1 M acetic acid at 95°C and maintained at this temperature for 10 min. Extracts were cooled and centrifuged at 12,000 g for 20 min. The supernatants were removed and stored at –20°C prior to assay.

VIP (22–28) was iodinated by the chloramine T method (13) and purified by microcolumn Sep-Pak C18 (Waters Ass., Milford, Mass. USA) reverse-phase chromatography. The labelled peptides were stable for 2–3 months at –20°C. The VIP (22–28) antiserum used in this experiment, AH78, was prepared as previously described (14). Concerning RIA for SP, extracts were incubated with $^{125}$I-SP labelled with Bolton and Hunter reagent (specific activity ~ 200 Ci/ml, Amersham International, Bucks, England) and with a specific rabbit polyclonal antiserum to SP (Amersham). RIA was carried out in 0.15 M sodium phosphate buffer (pH 7.4). The antibody was used at a final dilution of 1 : 6000 in an assay volume of 300 µl. In a typical assay, the $IC_{50}$ for VIP (22–28) was about 60 fmol/assay tube. Extracts were incubated for 18–24 h at 4°C with AH78 antiserum and $^{125}$I-VIP (22–28). The reaction was terminated by adding 1.0 ml of charcoal suspension, and the bound peptide was separated by centrifugation and estimated essentially as described by Ghazarossian et al. (13). The detection limit was 3 fmol/tube. The inter- and intra-assay coefficients of variation were 7% and 5% respectively. All samples were assayed in triplicate. The results in each group studied are expressed as the mean ± standard error of the mean (SEM), and Student's *t*-test has been used for statistical comparison of the means.

## RESULTS

With the procedure used in this study both VIP and SP immunoreactivity (IR) could be detected in each specimen of either AD or normal skin. Therefore, measurements were performed without pooling the specimens.

VIP levels were significantly higher in lesional skin from patients with AD (5.62 ± 1.25 pmol/g tissue) than in normal skin (0.43 ± 0.08 pmol/g tissue). By contrast, the levels of SP were significantly decreased in lesional skin from AD patients vis-á-vis control skin (0.25 ± 0.03 vs. 0.97 ± 0.24 pmol/g tissue).

## DISCUSSION

This paper demonstrates that VIP-IR levels are increased, while SP-IR levels are decreased, in lesional skin from patients with AD. The radio-immunological technique used in this study is a very sensitive one and allows measurements of minute amounts of peptides and detection of even minor differences. These and other NP have already been investigated

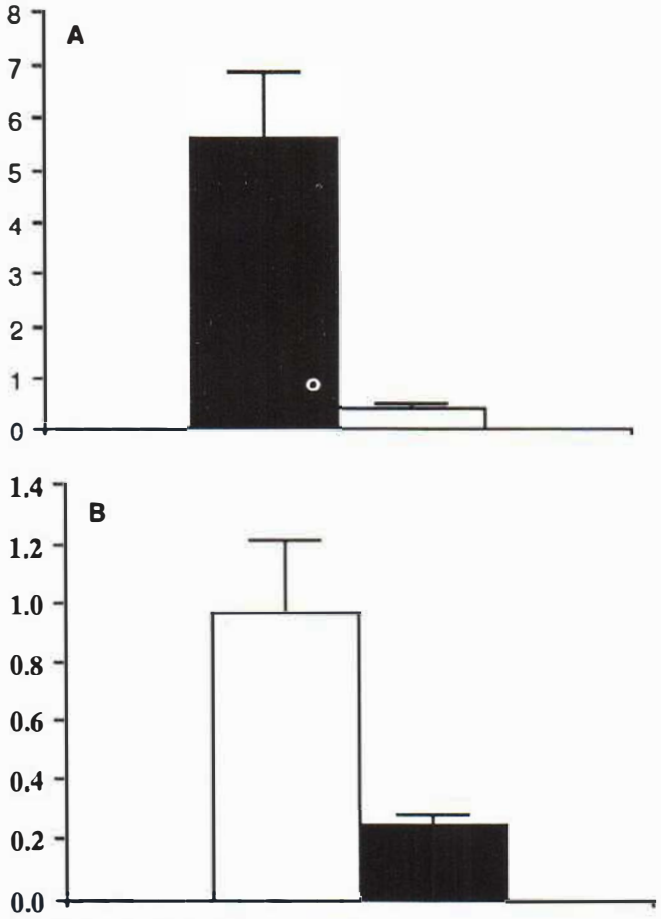


*Fig. 1.* Levels of VIP (*A*) and SP (*B*) in lesional skin of AD patients (■) and control skin (□). Results are expressed as mean pmol/g tissue±SEM. Student's *t*-test was used for comparison of the means ($p<0.01$).

by RIA in both serum and suction blisters of several inflammatory dermatoses. SP and VIP were inconsistenly elevated in suction blister fluid, while only one case of AD showed high VIP serum levels (15). However, it must be pointed out that performing RIA directly on tissue homogenates allows a more reliable measurement of NP-IR levels than does the suction blister method. Indeed, with this latter technique, peptide levels might be artificially changed during the induction phase of the blisters and the actual concentration of NP in the skin is not measured. On the other hand, Anand et al., using RIA on whole-skin homogenates, have recently observed higher VIP-, but unchanged SP-IR levels in lesional AD skin vis-à-vis controls (16). Interestingly, SP has recently been shown to be decreased also in inflammatory skin conditions other than AD. In particular, this peptide was reduced in the oxazolone-induced allergic contact dermatitis in mice (17) and in lesional psoriatic skin (18). Either a diminished synthesis or an increase in peripheral degradation could account for the decrease in cutaneous levels of SP. The increased VIP concentrations and the depletion of SP content in lesional AD skin might reflect a similar change at the central level. Indeed, in rats, following injury to the sciatic nerve, there is a reduction in SP content and an increase in VIP levels (19). It might thus be hypothesized that, when an inflammatory skin process occurs, primary sensory neurons respond by altering the normal NP synthesis.

Changes in SP and VIP-IR levels would seem to suggest that these NP may be involved in the pathogenesis of AD. VIP is known to control sweating (20) and may thus play a role in the mechanisms underlying the abnormal sweating occurring in AD (21). Furthermore, SP and VIP might contribute to the disregulation of cutaneous microcirculation, which is altered in AD (22).

The results of the present study indicate that the imbalance of SP and VIP could reflect different roles of these NP as modulatory agents in skin lesion of AD. It should be noted in this respect that SP and VIP exert diverse activities also in the immune system, the former being more stimulatory, whereas the latter acts as an inhibitory molecule (12). In addition, recent studies strongly indicate that VIP could be now regarded as an anti-inflammatory agent (23). To conclude, this study suggests that SP and VIP could be relevant to the pathogenesis of AD and we hypothesize that these NP may regulate the development of the AD lesion.

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