

Neurotensin serum levels and skin gene expression are increased in atopic dermatitis

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Summary

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Conflicts of interest

None declared.

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Background Neurotensin (NT) participates in immune responses, but the mechanisms are not known. We have previously shown that NT augments the ability of corticotropin-releasing hormone (CRH) to increase mast-cell-dependent vascular permeability in rodents. We also showed that NT stimulates human mastcell release of vascular endothelial growth factor, and that CRH is increased in the serum of patients with atopic dermatitis (AD), an inflammatory skin condition involving mast cells.

Objectives To measure serum levels of NT, and lesional skin expression of NT and the main NT receptor (NTR-1) in AD, and to compare it with skin expression in chronic urticaria (CU) and urticaria pigmentosa (UP).

Methods Serum NT was measured with a Milliplex microbead array. Skin NT and NTR-1 gene expression was determined with quantitative polymerase chain reaction. Immunohistochemistry was performed using a mouse monoclonal antibody for NT, and a rabbit polyclonal antibody for NTR-1. Mast cells were counterstained with Leder dye.

Results Neurotensin is significantly elevated in the serum of patients with AD compared with healthy controls ($P = 0.0001$). NT gene expression is also significantly increased in lesional skin of patients with AD compared with controls ($P = 0.0194$). Moreover, immunohistochemistry of AD lesions shows NT > NTR-1 staining of perivascular cells, many of which are identified as mast cells after staining with Leder dye. There was no statistically significant difference in NT and NTR-1 lesional skin gene expression in patients with either CU or UP. **Conclusions** These results suggest that interactions between NT and mast cells may occur and contribute to AD pathogenesis.

What's already known about this topic?

- Atopic dermatitis worsens with stress, and its pathogenesis involves mast cells and neuropeptides.

What does this study add?

- Neurotensin is increased in the serum and skin under stress. It activates mast cells and stimulates release of inflammatory molecules.
- Neurotensin is increased in lesional skin of patients with atopic dermatitis but not patients with chronic urticaria or urticaria pigmentosa.

Neurotensin (NT) is a vasoactive peptide originally isolated from the brain,¹ and has been implicated in mast-cell activation through specific NT receptors (NTRs).² NT is increased in the skin following acute stress; it stimulates skin mast cells and increases vascular permeability in rodents.³

Mast-cell activation leads to the release of multiple mediators with potent vasodilatory, inflammatory and nociceptive properties through which they participate in innate and acquired immunity,⁴ as well as in inflammation,⁵ especially in the skin.⁶

Atopic dermatitis (AD) is a skin disease resulting in chronic inflammation and severe pruritus.⁷ AD lesions contain increased mast cells close to nerve endings,⁸ which contain neuropeptides such as substance P,⁹ and worsen with stress.

We investigated serum levels of NT, and NT and NTR-1 expression in lesional skin of patients with AD and compared them with levels in patients with chronic urticaria (CU), urticaria pigmentosa (UP) and in healthy controls.

Materials and methods

Serum and full-depth punch skin biopsies (3 mm³) were obtained out of allergy season from lesional skin of patients with AD, CU and UP, and from controls who had not received any local or systemic treatment for 15 days prior to sample collection, and were free of major systemic medical conditions. Patients with contact dermatitis were excluded by history. Two patients with AD had asthma and one had allergic rhinitis. Total serum IgE was within the normal range in all but two patients with AD. Patients were selected from the Attikon and A. Sygros Hospitals of the Athens University Medical School (Athens, Greece). The institutional review boards of both hospitals approved this protocol. All participants gave their written informed consent according to the Declaration of Helsinki principles. Skin biopsies were immediately placed in RNAlater[®] solution (Ambion Inc., Austin, TX, U.S.A.) for quantitative polymerase chain reaction (PCR), or in freezing medium (Triangle Biomedical Sciences, Durham, NC, U.S.A.) for immunohistochemistry, and were stored along with the serum at -80 °C. Control skin samples from abdominal reductions were obtained from the Agios Savas Oncology Clinic (Athens, Greece), while control serum samples were obtained from Agios Savas and the Ano Toumba IKA Polyclinic (Thessaloniki, Greece).

All control samples were processed identically to those from patients and had no identifiers except for age and sex. There

was no statistical difference in the mean age between controls and patients. The SCORing Atopic Dermatitis (SCORAD) index was calculated using the SCORAD Index application by Link-Wave Inc. (<http://linkwave.com/>). The mean score for male patients was 25.70 ± 8.49 and for female patients 25.75 ± 6.01 . The mean SCORAD score of those patients ($n = 5$, out of season) who had either asthma ($n = 2$), allergic rhinitis ($n = 1$) or high plasma IgE ($n = 2$) was 24.18 ± 5.63 . There was no statistically significant difference among the SCORAD scores of any subgroups.

Quantitative polymerase chain reaction and neurotensin measurement

Quantitative PCR was performed using the following TaqMan probes: Hs00175048_m1 for NTS (NT gene), Hs00901551_m1 for NTSR1 (NTR-1 gene) and 4310884E-1102047 for GAPDH (glyceraldehyde 3-phosphate dehydrogenase gene; Applied Biosystems, Foster City, CA, U.S.A.), as described previously.¹⁰ Results were normalized against the endogenous gene, GAPDH, and were expressed relative to the mean of the control for each gene.

NT serum levels were measured using a Milliplex array, which was performed blind by Millipore (Millipore, St Charles, MI, U.S.A.).

Immunohistochemistry

Paraffin sections (4 µm) were cut, and immunohistochemistry was performed using an indirect biotin streptavidin detection system (Ventana Medical Systems, Roche, Basel, Switzerland) with the following primary antibodies: rabbit polyclonal for NTR-1 (ab117592, 1 : 500 dilution; Abcam Inc., Cambridge, MA, U.S.A.); mouse monoclonal for NT (E5) (sc-377503, 1 : 50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and rabbit polyclonal for c-kit (CD117, 1 : 200 dilution; Dako Cytomation, Carpinteria, CA, U.S.A.).

Statistics

Patient serum levels and skin gene expression results were compared with controls using the Mann–Whitney nonparametric U-test. Significance of comparisons is denoted by $P < 0.05$.

Results

Serum levels of NT were significantly increased at 151.2 ± 50.5 pg mL⁻¹ in patients with AD ($n = 19$; six male, mean age 28 ± 10 years and 13 female, mean age 41 ± 23 years) compared with 81.9 ± 41.2 pg mL⁻¹ in controls ($n = 25$; 15 male, mean age 49 ± 15 years and 10 female, mean age 43 ± 12 years; $P = 0.0001$) (Fig. 1a). There was no apparent correlation between NT levels and clinical status.

Skin NT gene expression was significantly increased in affected skin samples from patients with AD (2.2 ± 2.5 ; $n = 16$; 10 male, mean age 28 ± 10 years and six female, mean age 40 ± 24 years) compared with controls (1 ± 1.2 ; $n = 25$; six male, mean age 35 ± 16 years and 19 female, mean age 52 ± 17 years; $P = 0.0194$) (Fig. 1b). There was no significant difference in skin NTR-1 gene expression between affected skin samples from patients with AD (1.6 ± 1.6) and controls (1 ± 0.8) (Fig. 1c).

The presence of NT and NTR-1 proteins in lesional skin was confirmed by immunohistochemistry. Control tissue showed no positivity for either NT or NTR-1 (Fig. 2a, d). Semiquantitative analysis of the cases studied ($n = 5$) revealed higher expression of NT (Fig. 2b, c) than NTR-1 (Fig. 1e, f), ranging from 26% to 50% of the dermal infiltrate. Mast cells (Fig. 2g, solid arrow) were characteristically the most positive cells, located mainly in the papillary dermis, as evidenced by dark brown staining.

Skin NT and NTR-1 gene expression was not significantly increased in affected skin samples from patients with CU (0.8 ± 0.4 and 1.6 ± 1.5 , respectively; $n = 5$; one male, age 75 years and four female, mean age 51 ± 17 years) compared with controls (1 ± 1.2 and 1 ± 0.8 , respectively; $n = 25$; six male, mean age 35 ± 16 years and 19 female, mean age 52 ± 17 years) (Fig. 3a, b). Skin NT and NTR-1 gene expression was also not significantly increased in affected skin samples from patients with UP (0.1 ± 0.1 and 0.8 ± 0.6 ; $n = 4$; three male, mean age 22 ± 2 years and

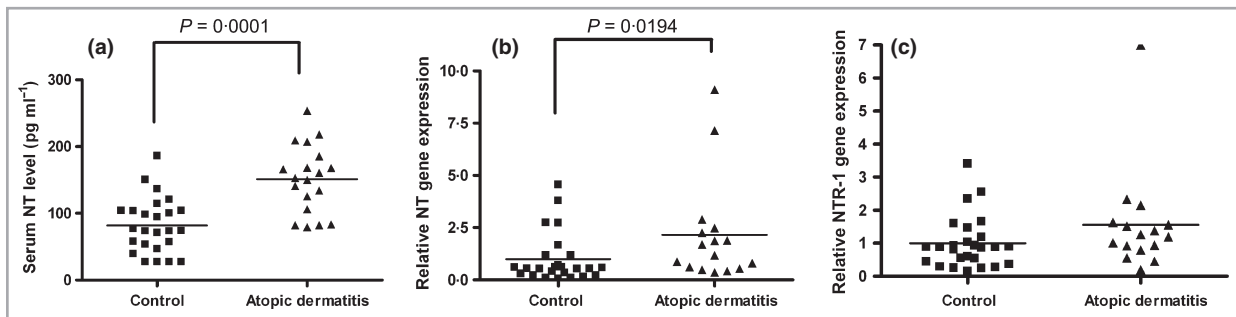


Fig 1. Scattergrams showing serum levels of neurotensin (NT), and NT and NT receptor (NTR)-1 gene expression in skin of patients with atopic dermatitis (AD) and controls. (a) Serum NT (controls, $n = 25$; patients with AD, $n = 19$). Expression of (b) NT gene (controls, $n = 25$; AD, $n = 16$) and (c) NTR-1 gene (controls, $n = 25$; AD, $n = 16$). Horizontal bars indicate the means.

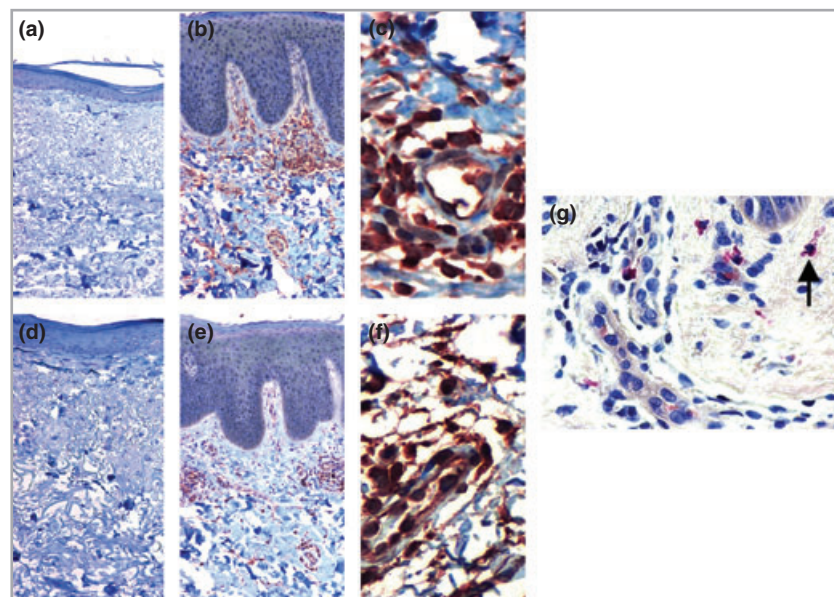


Fig 2. Photomicrographs showing immunohistochemistry of neurotensin (NT), NT receptor (NTR)-1 and mast cells in lesional atopic dermatitis (AD) skin. (a) Healthy skin stained for NT (magnification $5\times$). (b, c) Skin of patient with AD stained for NT (b, $20\times$; c, $40\times$). (d) Healthy skin stained for NTR-1 ($5\times$). (e, f) AD skin stained for NTR-1 (e, $20\times$; f, $40\times$). (g) Mast cells identified by naphthol AS-D chloroacetate esterase (Leder) staining ($20\times$, solid arrow) and confirmed by c-kit (not shown).

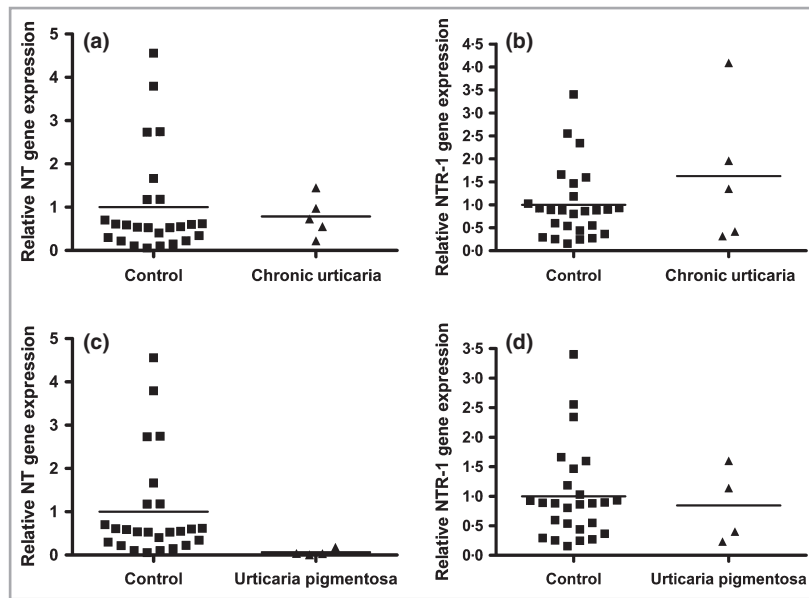


Fig 3. Scattergrams showing neurotensin (NT) and NT receptor (NTR)-1 gene expression in the skin of patients with chronic urticaria (CU) and urticaria pigmentosa (UP), and controls. (a, b) CU: gene expression of (a) NT (controls, $n = 25$; patients with CU, $n = 5$) and (b) NTR-1 (controls, $n = 25$; CU, $n = 5$). (c, d) UP: gene expression of (c) NT (controls, $n = 25$; UP, $n = 4$) and (d) NTR-1 (controls, $n = 25$; UP, $n = 4$). Horizontal bars indicate the means.

one female, age 29 years) compared with controls (1 ± 1.2 and 1 ± 0.8 ; $n = 25$; six male, mean age 35 ± 16 years and 19 female, mean age 52 ± 17 years) (Fig. 3c, d).

Discussion

Levels of NT and expression of its gene, NTS, are increased in the serum and lesional skin, respectively, of patients with AD compared with healthy controls. Immunohistochemistry confirmed the presence of NT peptide in lesional AD skin, but not in healthy skin. A possible explanation may be that NT is not present in skin under normal conditions, but is released in a precursor form from nerve endings and is generated by proteases, as we have shown in rodent skin.³ NT can also be released from mast cells.²

We recently showed that NT is increased also in the serum of patients with psoriasis, and it stimulates release of vascular endothelial growth factor (VEGF) from human mast cells.¹⁰ It is interesting that NT was also elevated in the serum of children with autism,¹¹ which has been associated with AD,¹² and who are born to mothers who had psoriasis during pregnancy,¹³ implying a possible causal connection.

NT can stimulate VEGF release from human mast cells¹⁰ and augment the ability of corticotropin-releasing hormone (CRH) to increase skin vascular permeability in rodents.³ Interestingly, NT increases expression of CRH receptor 1 in human cultured mast cells.¹⁴ Moreover, CRH is also increased in the serum of patients with AD.¹⁵ These findings imply that NT–CRH interactions may be important for the effect of stress on dermatoses.

In spite of the fact that CU and UP are clearly associated with mast cells, neither NT nor NTR-1 gene expression was increased in the lesional skin of these patients compared with controls. This implies that NT and NTR-1 are probably involved only in skin inflammation.

Mast cells are now considered critical in allergy and innate immunity in the skin.⁶ Interactions between NT, CRH and mast cells may be involved in skin inflammation,¹⁶ which worsens with stress, and may provide new targets for therapy.

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References

- Carraway R, Leeman SE. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J Biol Chem* 1973; **248**:6854–61.
- Cochrane DE, Carraway RE, Harrington K *et al.* HMC-1 human mast cells synthesize neurotensin (NT) precursor, secrete bioactive NT-like peptide(s) and express NT receptor NTS1. *Inflamm Res* 2011; **60**:1139–51.
- Donelan J, Boucher W, Papadopoulou N *et al.* Corticotropin-releasing hormone induces skin vascular permeability through a neurotensin-dependent process. *Proc Natl Acad Sci USA* 2006; **103**:7759–64.
- Galli SJ, Grimbaldston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat Rev Immunol* 2008; **8**:478–86.
- Theoharides TC, Alysandratos KD, Angelidou A *et al.* Mast cells and inflammation. *Biochim Biophys Acta* 2010; **1822**:21–33.
- Metz M, Maurer M. Mast cells – key effector cells in immune responses. *Trends Immunol* 2007; **28**:234–41.
- Bieber T. Atopic dermatitis. *N Engl J Med* 2008; **358**:1483–94.
- Jarvikallio A, Harvima IT, Naukkarinen A. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. *Arch Dermatol Res* 2003; **295**:2–7.
- Pincelli C, Fantini F, Massimi P *et al.* Neuropeptides in skin from patients with atopic dermatitis: an immunohistochemical study. *Br J Dermatol* 1990; **122**:745–50.

- 10 Vasiadi M, Therianou A, Alysandratos KD *et al.* Serum neurotensin (NT) is increased in psoriasis and NT induces VEGF release from human mast cells. *Br J Dermatol* 2012; **166**:1349–52.
- 11 Angelidou A, Francis K, Vasiadi M *et al.* Neurotensin is increased in serum of young children with autistic disorder. *J Neuroinflammation* 2010; **7**:48.
- 12 Yaghmaie P, Koudelka CW, Simpson EL. Mental health comorbidity in patients with atopic dermatitis. *J Allergy Clin Immunol* 2013; **131**:428–33.
- 13 Angelidou A, Asadi S, Alysandratos KD *et al.* Perinatal stress, brain inflammation and risk of autism – review and proposal. *BMC Pediatr* 2012; **12**:89.
- 14 Alysandratos KD, Asadi S, Angelidou A *et al.* Neurotensin and CRH interactions augment human mast cell activation. *PLoS ONE* 2012; **7**:e48934.
- 15 Vasiadi M, Therianou A, Sideri K *et al.* Increased serum CRH levels with decreased skin CRH-R1 gene expression in psoriasis and atopic dermatitis. *J Allergy Clin Immunol* 2012; **129**:1410–13.
- 16 Paus R, Theoharides TC, Arck PC. Neuroimmunoendocrine circuitry of the ‘brain-skin connection’. *Trends Immunol* 2006; **27**:32–9.