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Serum neurotensin (NT) is increased in psoriasis and NT induces vascular endothelial growth factor release from human mast cells

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Summary

Background—Psoriasis involves skin inflammation that often worsens with stress, but the mechanism of this effect remains obscure. We have shown that corticotrophin- releasing hormone (CRH) is increased in the serum of patients with psoriasis. A peptide, neurotensin (NT), can trigger skin histamine release and augment the ability of CRH to increase skin vascular permeability.

Objectives—To investigate the serum level of NT, and the expression of genes for NT and NT receptor-1 (NTR-1) in lesional and nonlesional skin of patients with psoriasis, compared with normal controls. Also, to study the effect of NT on human mast cell release of vascular endothelial growth factor (VEGF), which is increased in psoriasis skin.

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Conflicts of interest

None declared.

Methods—Serum was obtained from patients with psoriasis ($n = 56$) and controls ($n = 33$); NT levels were measured with the Milliplex microbead assay. Biopsies were obtained from the lesional and nonlesional skin of patients with chronic plaque psoriasis ($n = 40$), who had not received any treatment for at least 15 days and were free of any systemic inflammatory diseases. Control skin samples were obtained from healthy subjects ($n = 30$). Expression of genes for NT and NTR-1 in the skin was evaluated by quantitative reverse transcriptase–polymerase chain reaction. LAD2 human mast cells were stimulated by NT ($1 \mu\text{mol L}^{-1}$) for 24 h and VEGF was measured by enzyme-linked immunosorbent assay.

Results—Serum NT was increased in patients with psoriasis, while expression of genes for NT and NTR-1 in lesional skin was decreased compared with controls. NT induced VEGF release from mast cells and was augmented by interleukin-33.

Conclusion—NT may play a role in psoriasis pathogenesis and its worsening by stress, at least in part through activation of skin mast cells.

Psoriasis is a chronic inflammatory skin disorder characterized by keratinocyte hyperproliferation and increased epidermal vascularization, associated with high skin vascular endothelial growth factor (VEGF).¹ Neuropeptides have been implicated in psoriasis,² but their mechanism of action is not well understood.

Neurotensin (NT), is a vasoactive peptide,³ which increases histamine release from rodent skin in a mast cell-dependent manner.⁴ NT also augments the effect of corticotrophin-releasing hormone (CRH), secreted under stress, to increase mast cell-dependent skin vascular permeability.⁵

We therefore investigated NT serum levels, as well as the expression of genes encoding NT and NT receptor-1 (NTR-1) in the skin of patients with psoriasis and controls. We also studied the effect of NT on VEGF release in human cultured mast cells.

Materials and methods

Subjects

Serum was collected from patients, who had not received any medication for 15 days prior to biopsy and were free from any systemic allergic or inflammatory diseases, and from controls, at least 2 h after food; samples were stored at -80°C . Punch skin biopsies 3 mm^3 were obtained from nonexposed skin (back and gluteal). Samples of unaffected skin were obtained from sites at least 15 cm away from the lesional skin. All biopsies were immediately placed in RNAlater solution (Ambion, Inc., Austin, TX, U.S.A.) and stored at -80°C . There were fewer skin biopsy samples than serum samples because it was more difficult to obtain consent. The medical ethics committees of Attikon and A. Sygros Hospitals approved the protocol. All the human samples had no identifiers except for age and sex. There was no statistical difference in the mean age between patients with psoriasis and controls (Table 1).

Quantitative reverse transcriptase–polymerase chain reaction

Total skin RNA was extracted using the Qiagen Fibrous Tissue mini kit (Qiagen, Valencia, CA, U.S.A.) and cDNA synthesis was performed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, U.S.A.). Real-time quantitative polymerase chain reaction (PCR) was carried out in a 7300 Sequence Detector, according to TaqMan Gene Expression Assay instructions (Applied Biosystems, Foster City, CA, U.S.A.) using Taqman MGB probes: Hs00175048_m1 for *NTS*, Hs00901551_m1 for *NTSR1* and 4310884E-1102047 for *GAPDH*. Thermal cycling proceeded at 50°C for 2 min, 95°C for 10 min, 95°C for 15 s,

for 40 cycles, and 60 °C for 1 min. Negative controls included samples with water instead of template. Human pituitary cDNA was used as a positive control for *NTS*, while cDNA from LAD2 human mast cells was used as a positive control for *NTSRI*. Experiments were performed in triplicate for each data point. Results were normalized against the endogenous gene, *GAPDH*, and were expressed relative to the mean of the control for each gene.

Culture of human mast cells

LAD2 cells (kindly supplied by Dr A.S. Kirshenbaum, National Institutes of Health, Bethesda, MD, U.S.A.), were cultured in StemPro[®]-34 SFM medium (Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 100 U mL⁻¹ penicillin/streptomycin and 100 ng mL⁻¹ recombinant human stem cell factor (rhSCF) kindly supplied by Swedish Orphan Biovitrum AB (Stockholm, Sweden).

Vascular endothelial growth factor release assay

LAD2 cells were treated for 24 h either with NT alone (1 µmol L⁻¹; Sigma Aldrich, St Louis, MO, U.S.A.) or together with interleukin (IL)-33 (10 ng mL⁻¹; R&D Systems, Minneapolis, MN, U.S.A.). VEGF release was measured using an enzyme-linked immunosorbent assay (ELISA; R&D Systems) in the supernatant fluid of control and stimulated LAD2 cells.

Statistical analysis

Results of serum levels of NT and expression of genes for NT and NTR-1 in the skin of patients with psoriasis and controls, as well as experimental and control VEGF release results were compared using the Mann–Whitney nonparametric *U*-test. Lesional and nonlesional skin gene expression results from patients with psoriasis were compared with those of controls using the Kruskal–Wallis test, followed by Dunnett's test for multiple comparisons, while the Wilcoxon signed rank test was used to compare lesional and nonlesional psoriasis skin gene expression from the same patients. Data from cultures are expressed as the mean ± SD. Significance is denoted by $P < 0.05$.

Results

Serum neurotensin levels

There was a statistically significant increase of serum NT levels (Fig. 1a) in patients with psoriasis (411.9 ± 280 pg mL⁻¹) compared with controls (95.4 ± 53.6 pg mL⁻¹).

Expression of genes for NT and NTR-1 in the skin

Relative expression of the gene for NT in the skin was lower ($P = 0.0363$, Fig. 1b) as was expression of the gene for NTR-1 ($P = 0.0007$, Fig. 1c) in affected skin samples from patients with psoriasis compared with controls. In contrast, there was no statistically significant difference in NT mRNA expression ($n = 15$, Fig. 1d), but there was a statistically significant increase in NTR-1 mRNA expression ($n = 15$, Fig. 1e) in samples obtained from unaffected compared with affected psoriasis skin.

Neurotensin effect on human mast cells

In view of the fact that VEGF expression is increased in psoriasis skin, we studied VEGF release from LAD2 human cultured mast cells. NT (10 µmol L⁻¹) significantly increased VEGF release (results not shown). NT (1 µmol L⁻¹) had no effect alone, but induced significant VEGF release when added with IL-33 (10 ng mL⁻¹) (Fig. 2).

Discussion

This is the first report to our knowledge of increased serum levels of NT in any autoimmune or inflammatory disease. We also show decreased expression of genes for NT and NTR-1 in psoriasis skin. Similarly, we previously reported that CRH is increased in the serum, while there is decreased *CRHR1* gene expression in the lesional skin of patients with psoriasis.⁶ Expression of the gene for NTR-1 was increased in nonlesional psoriasis skin. The reason for this finding is not clear, but suggests nonlesional skin may not as yet have been maximally affected by the circulating NT.

We also show that NT induces mast cell VEGF release. Mast cells express NTR⁷ stimulation which results in histamine release⁴ and NT is rapidly degraded by mast cell proteases⁸ implying tight regulation.⁴ We previously showed that NT has a synergistic action with CRH, increasing skin vascular permeability in rodents through mast cell activation.⁵ This finding is relevant in view of evidence showing increased number of skin mast cells in psoriasis.⁹ NT may be involved in the pathogenesis of psoriasis by acting together with CRH secreted under stress in the skin,¹⁰ which has its own equivalent of the hypothalamic–pituitary–adrenal axis.¹¹ The stimulatory effects of NT and IL-33 on VEGF release may be in addition to a similar action by local skin substance P (SP). We have shown that SP stimulates human mast cells to release VEGF¹² and that this action is augmented by IL-33.¹² However, contrary to our findings, there is increased expression of SP and its receptor neurokinin-1 in lesional psoriasis skin,¹³ but plasma concentrations of SP in patients with psoriasis did not differ from controls.¹⁴

Mast cells are now considered critical in allergy and innate immunity in the skin.¹⁵ The present findings suggest that NT may participate in the pathogenesis of psoriasis, especially when worsened by stress, through mast cell involvement in a ‘brain–skin’ connection.¹⁶ NT and mast cell blockers may provide novel treatment approaches.

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What's already known about this topic?

- Psoriasis is worsened by stress.
- Expression of the neuropeptide substance P and vascular endothelial growth factor (VEGF) in lesional psoriatic skin is increased, but levels are not high in the blood.

What does this study add?

- The vasoactive peptide neurotensin (NT) is increased in serum of patients with psoriasis and maximally stimulates lesional skin receptors leading to their downregulation.
- NT induces VEGF release from mast cells, an action that may explain the effect of NT on psoriatic skin.

Fig. 1.

Scattergrams of serum neurotensin (NT) levels, as well as expression of genes for NT and NT receptor-1 (NTR-1) in the skin of patients with psoriasis and controls. (a) Serum NT levels were determined using the Milliplex microbead assay and the measurements were performed by Millipore (St Charles, MI, U.S.A.). Gene expression of (b) NT and (c) NTR-1 in skin samples from patients with psoriasis and controls. Gene expression of (d) NT and (e) NTR-1 in lesional and nonlesional psoriasis skin. Horizontal lines represent the means.

Fig. 2.

Effect of neurotensin (NT) on vascular endothelial growth 2 factor (VEGF) release from human cultured mast cells. LAD2 mast cells were stimulated with either NT ($1 \mu\text{mol L}^{-1}$) alone or together with interleukin (IL)-33 (10 ng mL^{-1}) for 24 h. VEGF release was measured by enzyme-linked immunosorbent assay ($n = 3$, $*P < 0.05$).

Table 1

Age of patients with psoriasis and controls according to sex and sample type

Group	Age (years), mean $\pm$ SD
Psoriasis (serum samples, $n = 56$)	
Female ($n = 26$)	40 ± 14
Male ($n = 30$)	48 ± 19
Psoriasis (skin samples, $n = 40$)	
Female ($n = 19$)	42 ± 15
Male ($n = 21$)	48 ± 18
Controls (serum samples, $n = 33$)	
Female ($n = 19$)	49 ± 19
Male ($n = 14$)	53 ± 16
Controls (skin samples, $n = 30$)	
Female ($n = 22$)	51 ± 18
Male ($n = 8$)	40 ± 16