Systemic sclerosis is characterized by vascular dysfunction. Itch is sometimes present in early stages of the disease. This prompted us to study the innervation of the skin by immunocytochemistry. Antibodies to neuropeptide Y and vasoactive intestinal peptide were used for autonomic nerves. Sensory innervation was studied using antibodies to substance P and calcitonin gene-related peptide. Protein gene product 9.5 was used as a general neuronal marker.

Skin biopsies from affected (lower arm) and non-affected (upper back) sites on 10 patients with systemic sclerosis and from corresponding sites on 10 sex- and age-matched healthy controls were studied. Regional variations were found in the occurrence of peptidergic nerve fibers. In the patients the density of nerve fibers (measured semiquantitatively) stained by the pan-neuronal marker was lower in affected than in unaffected skin (p<0.05). There were no significant differences in peptidergic innervation between patients and controls. However, there was a tendency to higher density of neuropeptide Y-positive nerve fibers in the forearm skin in 6 of 10 patients, as compared to only 1 of 10 healthy controls. Key words: substance P; calcitonin gene-related peptide; neuropeptide Y.

(Accepted October 9, 1995.)


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In view of the vasoregulatory actions of certain neuropeptides, their presence in sensory neurons and proinflammatory actions, we have examined skin biopsies from SSc patients for several neuropeptides. Overall nerve density was examined using a pan-neuronal marker, protein gene product 9.5 (PGP 9.5)(7).

MATERIAL AND METHODS

Ten patients (8 women and 2 men) with SSc and 10 age- and sex-matched healthy controls participated in the study. Six patients had limited cutaneous systemic sclerosis (lSSc) and 4 patients the diffuse form of the disease (dSSc)(14). Their median age was 56 years (range 29–80) and the median duration of disease was 3 years (range 1–20 years). Full-thickness skin specimens were obtained by 3-mm punch biopsies from involved skin (forearm) and from uninvolved skin (upper back) in patients, and from corresponding sites in controls. All specimens were fixed by immersion overnight in ice-chilled 4% buffered formaldehyde, pH 7.2. They were then rinsed repeatedly in chilled sucrose-enriched buffer and frozen on dry ice.

Cryostat sections (10-μm thickness) were processed for indirect immunofluorescence using antibodies against the neuropeptides – SP, CGRP, VIP and NPY – and against the general neuronal marker PGP 9.5. Details of the antibodies are given in Table I. Control sections were exposed to primary antiserum that had been preabsorbed with an excess amount of the antigen (0.1–100 μg synthetic peptide/ml diluted antiserum). Additionally, each of the peptide antisera was tested for cross-reactions with the other neuropeptides examined (10–100 μg/ml diluted antiserum). Three nonserial sections from each specimen were used for each antiserum. The density of immunoreactive fibers in each specimen was assessed visually, and the results were graded according to the number of nerve fibers per visual field seen at ×175 magnification as follows: 0, absence of nerve fibers; 1, 1–3 nerve fibers; 2, 4–5 nerve fibers; and 3, >5 nerve fibers. The observer was unaware of the identity of the individual (patient or control) and of the site of the biopsy. The difference from one section to another in the same subject was invariably small (not exceeding one score). Statistical analysis of the data was performed using the Wilcoxon signed rank test and the Wilcoxon rank sum test, as appropriate.

RESULTS

PGP-IR nerve fibers occurred mostly as free subepidermal and epidermal nerve fibers (Fig. 1a) but also around blood vessels and sweat glands. SP- and CGRP-IR nerve fibers occurred mostly as free subepidermal nerve endings (Fig. 1b). NPY- and VIP-IR nerve fibers predominated in the deeper parts of the dermis around blood vessels and sweat glands, respectively (Fig. 1c, d). Regional difference in the density of innervation (between the lower arm and the back) was found for PGP-containing fibers among patients and for NPY and VIP among controls (Figs. 2, 3).

There were no differences between patients and controls in the nerve fiber density at each of the different skin regions examined.

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Table I. Details of the antibodies used

<table>
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<th>Antigen</th>
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<th>Raised in</th>
<th>Working dilution</th>
<th>Source</th>
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<td>SP</td>
<td>SP8</td>
<td>Protein-conjugated synthetic bovine SP</td>
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<td>8427</td>
<td>Protein-conjugated synthetic rat αCGRP</td>
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<td>VIP</td>
<td>7852</td>
<td>Unconjugated pure natural porcine VIP</td>
<td>Rabbit</td>
<td>1:640</td>
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<td>NPY</td>
<td>8404</td>
<td>Protein-conjugated synthetic porcine NPY</td>
<td>Rabbit</td>
<td>1:320</td>
<td>Euro Diagnostica</td>
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<tr>
<td>PGP 9.5</td>
<td>—</td>
<td>Unconjugated human brain PGP 9.5</td>
<td>Rabbit</td>
<td>1:200</td>
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</table>

Fig 1. Skin biopsies from patients (forearm) immunostained for PGP 9.5 (a), CGRP (b), VIP (c) and NPY (d). PGP-IR nerve fibers (arrow) are situated in the epidermal (a), CGRP-IR free nerve fibers (exemplified by arrow) are localized subepithelially (b), VIP-IR nerve fibers (arrow) are situated around a hair follicle (c), NPY-IR nerve fibers (arrow) innervate a blood vessel (d). Magnification: a and c × 270; b and d × 200.

NPY nerve fibers were scant, being present in 6 of 10 patient biopsies from the forearm (as compared to only one of 10 controls) and in 6 of 10 patient biopsies from the back (as compared to 3 of 10 controls)(ns). Among patients there was no difference in the density of nerve fibers containing any of the peptides examined or PGP, either between the limited and diffuse SSc subgroups or between long (5–20 years) and short (1–5 years) disease duration subgroups.

DISCUSSION
The regional variation in the occurrence of neuropeptides in the skin both in SSc patients and the controls emphasizes the
importance of taking biopsies from standardized sites when comparing groups of patients and controls. We found no significant differences between patients and controls in the regional occurrence and distribution of peptidergic nerve fibers. Bunker et al. (15) found a reduction of CGRP-IR nerve fibers in the skin of patients with primary Raynaud’s phenomenon and in patients with SSc in biopsies taken from the dorsum of the proximal phalanges. This discrepancy between our results and those of Bunker et al. may be due to the regional variation and to the richer nerve supply in the digital skin, where differences might be easier to quantify.

We found NPY-IR nerve fibers in the involved skin of 6 of 10 patients, but in the corresponding regions in only one of 10 controls. The absence of a significant difference might be explained by the small number of patients studied and by the sparse occurrence of NPY-positive nerve fibers in human skin.

Increased density of NPY-positive nerve fibers in the skin of patients with SSc has not been reported previously, though plasma NPY concentrations have been shown to be higher in SSc patients than in healthy subjects (16). NPY, a potent vasoconstrictor, often coexisting and coacting with norepi-

nephrine (11–13), has been implicated in the maintenance of systemic and peripheral vascular tone. Increased density of NPY nerve fibers in the skin of SSc patients may be associated with the vasoconstriction seen in these patients. If so, it might contribute to the future development of such new therapeutic vasodilating drugs as an NPY antagonist.

ACKNOWLEDGEMENTS

The study was supported by grants from the Medical Faculty, Lund University and the Edvard Wehander Foundation. The statistical analysis was performed by Björn Edman, PhD. Joanna Wallengren was recipient of a scholarship from the Swedish Society of Medicine.

REFERENCES