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Cutaneous Innervation in Chronic Renal Failure Patients

An Immunohistochemical Study

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Most chronic renal failure patients suffer from generalized pruritus. An involvement of cutaneous nerve terminals in the pathogenesis of uremic pruritus has been suggested. Skin specimens from 24 uremic patients and 10 healthy subjects were processed with an indirect immunofluorescence method to investigate the presence and distribution of a number of neuronal markers and neuropeptides. No difference was found between the two groups in the distribution pattern of the positive nerve fibres. However, a reduction in the total number of skin nerve terminals in the uremic patients was detected. No correlation could be found between the immunohistochemical findings and the clinical features. Our results suggest that the skin innervation is altered in most chronic renal failure patients, possibly as a consequence of neuropathy. ***Key words: Uremia; Nerve fibres; Neuropeptides; Neuron-specific enolase; Pruritus.***

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Generalized pruritus is one of the most common and troublesome symptoms of end-stage renal failure. It is reported to affect, at least occasionally, the majority (78%) of patients undergoing maintenance hemodialysis (1).

The etiology of uremic pruritus is still unknown. A number of possible explanations have been proposed, such as xerosis (2), secondary hyperparathyroidism (2,3), imbalance in divalent ions (4,5,6), dermal mast cell proliferation and histamine release (7). These factors have recently been extensively reviewed (8).

A neurogenic mediation has also been suggested (9); indeed, polyneuropathy is a common complication of long-standing chronic renal failure (10,11). Dysfunctions of the peripheral nerve endings involved in the transmission of itch sensations might offer some explanation for uremic pruritus.

We therefore decided to investigate, by means of immunohistochemistry, the cutaneous innervation in patients suffering from chronic renal failure. We have used antisera to neuron-specific enolase (NSE) and neurofilaments (NF) to evaluate the distribution pattern of cutaneous nerves and antisera to neuropeptides which are normally present in human skin.

MATERIALS AND METHODS

Patients

Skin biopsies were taken from the normal-appearing skin of the volar aspect of the forearms of 24 patients (19 males, 5 females, aged 42–85, mean age 61.8 years) with chronic renal failure. Eight patients were not dialysed, 5 were short-term dialysed and 11 were long-term dialysed.

Eleven patients (mostly non-dialysed subjects) had no history of itch. The other patients experienced pruritus (ranging from slight to severe) during the course of the disease, intermittently or at the time of biopsy. Peripheral neuropathy was clinically evaluated in all subjects. Clinical details of the patients are given in Table I.

For control purposes, specimens from the volar aspect of the forearm of 10 age-matched healthy volunteers were obtained.

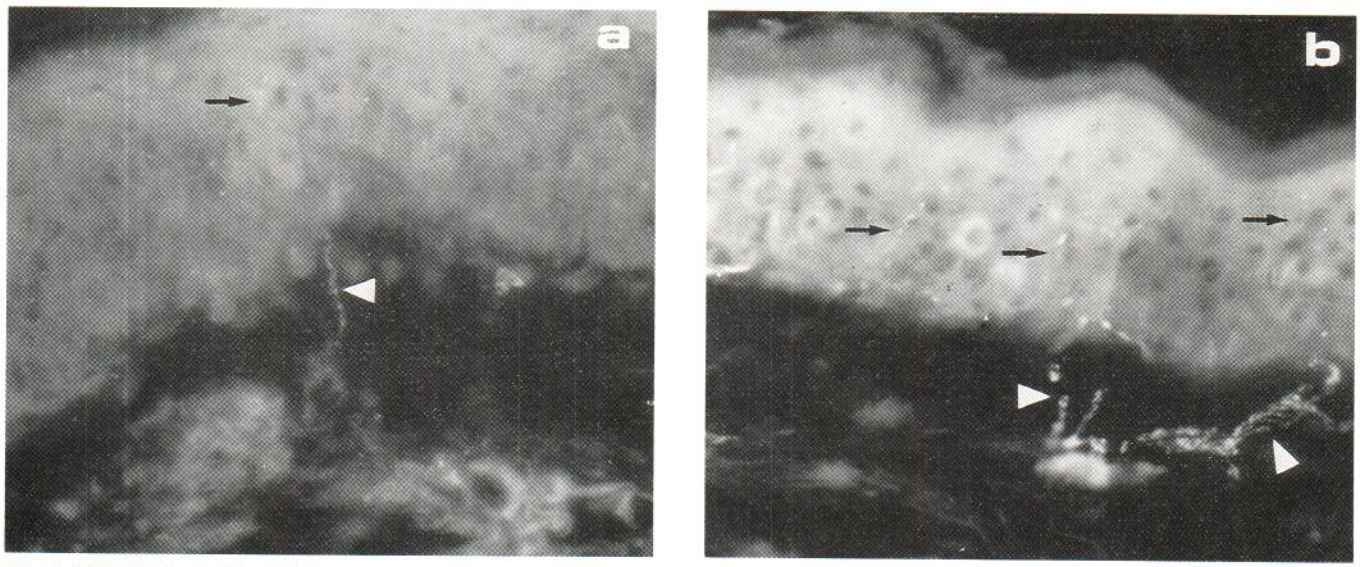

Fig. 1. NSE-IR intraepidermal *(arrows)* and dermal *(arrowheads)* nerve terminals in two representative fields, from uremic patients (*a*) and healthy controls (*b*). A decreased number of nerve fibres in uremic patients vis-à-vis controls is clearly seen (×400).

Table I. *Clinical data*

	Sex/age (yrs)	Diagnosis	Dialysis months	Pruritus	Neuropathy
1.*	M/50	Obstructive uropathy	–	–	–
2.*	M/42	Obstructive uropathy	–	–	–
3.*	M/58	Renal vasculitis	–	–	–
4.*	M/55	Diabetic nephropathy	–	–	–
5.*	M/66	Chronic glomerulonephritis	–	–	–
6.*	M/65	Polycystic kidney disease	–	+	–
7.*	M/68	Nephroangiosclerosis	–	–	–
8.*	M/70	Rheumatoid arthritis	–	–	+
9.#	M/54	Diabetic nephropathy	6	+++	–
10.#	M/60	Nephroangiosclerosis	3	+	–
11.#	F/79	Not identified	18	+++	NI
12.#	M/85	Nephroangiosclerosis	24	+++	NI
13.#	F/58	Chronic glomerulonephritis	21	+++	–
14.§	M/74	Chronic pyelonephritis	171	++	+
15.§	M/62	Chronic glomerulonephritis	145	–	+
16.§	M/46	Diabetic nephropathy	84	–	++
17.§	M/69	Chronic glomerulonephritis	214	–	++
18.§	M/50	Chronic glomerulonephritis	118	–	–
19.§	F/75	Analgesic nephropathy	109	+++	+
20.§	F/70	Chronic pyelonephritis	133	++	+
21.§	F/53	Chronic glomerulonephritis	174	++	–
22.§	M/52	Chronic glomerulonephritis	252	++	–
23.§	M/62	Nephroangiosclerosis	84	+++	–
24.§	M/62	Diabetic nephropathy	62	+++	–

* Chronic renal failure patients (glomerular filtration rate = 5–30 ml/min).
\# Short-term dialysed patients (dialytic age < 24 months).
§ Long-term dialysed patients (dialytic age > 24 months).

Pruritus: –, absent; +, slight; ++, moderate/intermittent; +++, severe.
Neuropathy: –, absent; +, moderate; ++, severe; NI, not investigated.

Methods

Punch biopsies (4 mm) were taken under local anesthesia (Mepivacain 2%, Pierrel, Italy) and immediately immersed in a 4% paraformaldehyde solution containing 0.3% picric acid in Sørensen's buffer for 2 h at +4°C. After overnight rinsing in Sørensen's buffer with 10% sucrose, the specimens were frozen and stored at −80°C until immunostained. Consecutive frozen sections (14 μm) were cut at right angles to the surface, taken up on gelatin-coated glass slides and allowed to air-dry for 60 min at room temperature. A biotin-streptavidin-fluorescein technique was then applied, as previously described (12). Briefly, sections (2 for each primary antibody) were first incubated overnight at 4°C with a panel of antisera (Table II). After washing in phosphate-buffered saline (PBS; pH 7.2) (3 × 5 min changes), they were incubated for 30 min at 37°C with biotin-labelled secondary antisera (Vector, California, USA). After washing again in PBS, sections were finally incubated for 30 min at room temperature with fluorescein-isothiocyanate-labelled streptavidin (Amersham International plc., Bucks, England). All antisera were diluted in PBS containing 1% bovine serum albumin and 0.3% Triton X-100. Negative controls, obtained by replacing the primary antisera with non-immune sera, were run in parallel. The sections were examined 'blind' by two independent observers using a Zeiss fluorescence miscroscope. Ten consecutive fields for each section were thoroughly evaluated.

We considered as positive fibres those exhibiting the typical varicose appearance. Immunoreactive fibres were assessed semi-quantitatively (subjectively) as a number of fibres per field at ×250. The results were expressed as absent (no positive fibres), moderate (1 to 5 positive fibres), and numerous (more than five positive fibres) both in epidermis and dermis.

RESULTS

Neuron-specific enolase-immunoreactive (NSE-IR) fibres were found both within the epidermis, mostly as sparse, varicose-free terminals reaching the stratum granulosum, and in the dermis as free or perivscular nerve endings and periadnexal networks. NSE-IR nerve bundles were also seen in the mid and lower dermis. The NSE-positive cutaneous nerve

Table II. *Details of antisera*

Antiserum	Working dilution	Source
Anti-neuron-specific enolase (polyclonal)	1:4	Incstar, USA
Anti-neurofilaments (monoclonal)	1:10	Sorin, Italy
Anti-substance P (monoclonal)	1:200	Seralab, England
Anti-calcitonin gene-related peptide (polyclonal)	1:800	Byk-Gulden, Sweden
Anti-neuropeptide Y (polyclonal)	1:800	Peninsula, England
Anti-vasoactive intestinal peptide (polyclonal)	1:800	Peninsula, England
Anti-somatostatin (polyclonal)	1:200	Peninsula, England

Table III. *Semiquantitative evlauation of the number of NSE-IR fibres in the skin of uremic patients and controls*

	Epidermis		Dermis	
	Uremics	Controls	Uremics	Controls
NSE-IR				
Absent	21/24	2/10	16/24	0/10
Moderate	2/24	6/10	7/24	5/10
Numerous	1/24	2/10	1/24	5/10

structures showed the same distribution pattern in the chronic renal failure patients as in the healthy controls. However, the number of NSE-positive fibres in most of the uremic patients was markedly reduced in both epidermis and dermis, as compared with healthy controls (Fig. 1, Table III). Only in 3 patients (nos. 4, 13, 15 in Table I) could NSE-IR intra-epidermal nerve terminals be detected.

Neurofilament (NF) immunoreactivity (IR) was observed in the reticular dermis, mainly in nerve bundles. To a lesser extent, isolated positive nerve endings were detected. No intra-epidermal fibres were observed. There were no major differences in teh NF-IR between the two groups.

Neuropeptide (NP)-IR was expressed in the skin of healthy controls. In particular, substance P (SP) and calcitonin gene-related peptide (CGRP)-IR fibres were found mostly around blood vessels or as apparent free nerve endings in the papillary dermis. Both vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY)-IR fibres were seen supplying sweat glands and blood vessels in the mid and lower dermis. Scattered somatostatin-IR fibres were observed in the superficial dermis. In 20 out of 24 uremic patients, no staining was observed with any of the NP antisera. In patient no. 16 (Table I), isolated VIP-IR nerve terminals were seen in a periglandular location, while in patients 1, 9 and 22 (Table I), scant VIP, NPY, CGRP and SP-IR fibres were found with the same distribution pattern as in healthy subjects.

No immunofluorescent structures were observed in control sections.

DISCUSSION

Immunocytochemistry is considered to be a reliable technique for detecting the histological localization in the skin of nerve structures containing peptides (13). We therefore used this technique to study the cutaneous innervation of patients in various stages of uremic disease, in order to establish whether uremic pruritus could be related to morphological or neurochemical alterations.

We evaluated the distribution of two structural neuronal proteins (NSE and NF) and of a number of peptidergic neurotransmitters which have recently been demonstrated in specific cutaneous nerve fibres (13, 14). In particular, NSE is reported as an accurate marker for both sensory and autonomic cutaneous innervation, while NF-IR is believed to be found only in sensory nerves (15).

We could not find any difference between the two groups in the histological distribution of NSE-stained fibres. Johansson and co-workers found NSE-IR fibres sprouting throughout the epidermis in all uremic patients they investigated, whereas in the normal controls, they observed NSE-IR epidermal fibres only in the stratum basale (16, 17). It was then hypothesized that the distal sprouting could be the morphological basis for uremic pruritus. In contrast, in our study, only 3 uremic patients showed intra-epidermal NSE-IR nerve endings. In addition, in the great majority of healthy subjects, NSE-IR terminals within the epidermis were detected. Indeed, the presence of intra-epidermal nerve fibres in normal human skin is consistent with recent immunohistochemical (15, 18) and histochemical (19) observations.

No significant difference in the distribution pattern of the other markers could be found. It must be mentioned, however, that NF-IR was seen mainly as deep nerve bundles and rarely as superficial, isolated fibres, so that any minor changes in the NF-IR are likely to have passed unnoticed.

The most remarkable finding was a reduction in the total number of the positive fibres in the uremic group, which could reflect either a decreased number or a reduced functional activity (NSE and NP content) of skin nerve structures. Although immunohistochemistry does not allow of an accurate quantification of small variations in intracellular substances, the hypothesis of a decreased number or a decreased functional activity of cutaneous nerve terminals may be consistent with the pathological changes occurring in uremic polyneuropathy. Indeed, uremic neuropathy is associated with distal axonal degeneration and demyelination with a loss of nerve fibres (10, 11). Our findings may, then, reflect disturbances in the peripheral nerve fibres. Interestingly, a reduced number of cutaneous nerve fibres and NP has been demonstrated in leprosy infection, which is characterized by involvement of the peripheral nervous system (20). It should be remembered, however, that in the present study the immunohistochemical findings did not correlate with the clinical data, such as the presence of neuropathy, pruritus, hemodialysis and dialytic age. As far as neuropathy and pruritus are concerned, a possible explanation is that the clinical assessment is often insensitive and unreliable in mild cases. A previous study also reported a lack of correlation between immunohistochemical and clinical data (17).

In conclusion, abnormalities of the cutaneous innervation, which may be related to neuropathy, do occur in chronic renal failure patients. Whether these alterations can account for uremic pruritus remains to be clarified.

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