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Occurrence and Distribution of Neuropeptides in the Human Skin

An Immunocytochemical and Immunochemical Study on Normal Skin and Blister Fluid from Inflamed Skin

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The content and distribution of substance P (SP), somatostatin, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) in human skin were investigated. Radioimmunoassay was performed on pooled tissue samples from several regions (fingers, toes, axillas and thighs) and on tissue fluid from spontaneous blisters on inflamed skin. Immunocytochemical localization showed all peptides examined except somatostatin to be present in nerve fibers. Nerve fibers storing SP and CGRP, which were found to coexist, were mostly present as free nerve endings in the superficial part of dermis and in epidermis. SP/CGRP fibers were most abundant in fingers and toes. VIP fibers and NPY fibers were localized in the deeper parts of dermis around blood vessels and acini of sweat glands. Also fibers containing these neuropeptides were most common in fingertips and toes. VIP occurred in relatively high amounts also in skin from axilla whereas NPY in this region was below detection limit. Immunoreactive somatostatin was found in low concentrations in tissue extracts and was not present in amounts sufficient for reliable immunostaining. Fluid from spontaneous blisters on inflamed skin contained detectable amounts of all neuropeptides. *Key words: Calcitonin gene-related peptide (CGRP); Substance P (SP); Vasoactive intestinal peptide (VIP); Somatostatin; Neuropeptide Y (NPY); Immunocytochemistry; Radioimmunoassay.* (Received July 4, 1986.)

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The skin receives a rich supply of nerve fibers (1, 2). Many of these fibers are thought to be peripheral ramifications of primary sensory neurons. Substance P (SP) was the first neuropeptide to be found associated with these neurons (3). In the skin SP immunoreactive nerve fibers are found both along blood vessels and as free nerve endings just beneath and occasionally penetrating into the epithelium (4). A rich supply of SP immunoreactive nerve fibers has been demonstrated in human digital skin either as free nerve endings or within Meissner's corpuscles (5).

The content and distribution of several regulatory peptides in the skin of cats and pigs have been investigated (6). Significant amounts of SP, vasoactive intestinal peptide (VIP) and somatostatin were found in the skin, the amounts varying between different regions. SP was the most abundant peptide, somatostatin on the other hand was present in small amounts but with a similar distribution pattern. Only SP and VIP were present in sufficient concentration for reliable immunostaining. These two peptides were confined to nerve fibers (6). In a single report somatostatin-like material has been demonstrated immunocytochemically in epithelial and connective tissue elements in lesions from patients with urticaria pigmentosa (7).

VIP has been found to be distributed preferentially in deeper parts of the dermis in nerve

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fibers around bloodvessels and around acini of sweat glands (4, 8). There is in addition a report on the presence of immunoreactive VIP in Merkel cells (9). Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide recently demonstrated in primary sensory neurons (10) and found to coexist with SP in such neurons (11) including nerve fibers in the guinea pig skin (12). Neuropeptide Y (NPY) is known to coexist with norepinephrine in peripheral adrenergic neurons and to affect various adrenergically mediated functions (13).

When injected intradermally VIP, SP and somatostatin evoke triple response with redness, flare and wheal (14 and own unpublished observations). When injected intradermally CGRP causes vasodilatation (15, 16).

In the present study we investigated the occurrence and distribution of SP, VIP, somatostatin, CGRP and NPY in human skin by immunocytochemistry and radioimmunoassay. We also analysed tissue fluid from spontaneous blisters in several inflammatory skin diseases for the presence of somatostatin, CGRP and NPY as an extension of previous observations on the presence of VIP and SP in such a fluid (17).

MATERIAL AND METHODS

Immunocytochemistry

One 3 mm punch biopsy was taken from normal skin of 24 out-patients at the dermatological department. Six biopsies were taken from axilla, five from the thigh, six from the leg, two from the arm, one from the back, two from the abdomen and two from the neck. In addition, fifteen 4 mm punch biopsies were taken from the fingertips of six individuals at autopsy within eight hours after death. Nothing exceptional was noticed on the skin of the autopsy subjects and the main course of death was myocardial infarction. All specimens were fixed by immersion overnight in 4% buffered formaldehyde, pH 7.2. They were then rinsed repeatedly in sucrose-enriched buffer and frozen on dry ice.

Cryostat sections (10 μm thickness) were processed for immunocytochemistry using antibodies against the following neuropeptides: SP, CGRP, VIP, NPY and somatostatin. Details on the antibodies are given in Table 1. The antigen-antibody reaction was visualized by fluorescein isothiocyanate (FITC)—or rhodamine isothiocyanate (TRITC)—conjugated anti-rabbit IgG and examined in a fluorescence microscope. For the monoclonal SP antibody FITC-labelled anti-mouse IgG was used as second antibody. The simultaneous demonstration of SP and CGRP-immunoreactive nerve fibers in the same tissue section was performed using the monoclonal SP antibody and FITC-labelled second antibody followed by the CGRP antiserum and TRITC labelled second antibody (Cf. 20).

Radioimmunoassay

Eighteen 6 mm punch biopsies were taken from toes and twenty 5 mm biopsies from several index fingers and several thumbs at autopsy within 8 hours after death. Several 3 cm skin specimens were

Table I. *Details of peptide antibodies used*

Peptide	Code	Working dilution	Source	Ref.
Vasoactive intestinal peptide	7852	1:320	MILAB, Malmö, Sweden	18
Substance P	SP-8	1:640	Dr P. C. Emson, MCR Cambridge, England	18
Substance P	NCI/34 (monoclonal)	1:80 from stock solution	Seralab, Oxford, England	11
Calcitonin gene-related peptide	8427	1:1280	MILAB, Malmö, Sweden	11, 19
Neuropeptide Y	NPYY/2	1:400	Dr P. C. Emson, MCR, Cambridge, England	18
Somatostatin	K 18	1:100	MILAB, Malmö, Sweden	18

taken from axilla, thigh and abdomen. All the tissue samples were stored at −20°C until analyzed. The skin samples were weighed, boiled in 0.5 M acetic acid 10 min homogenized (Polytron, 1–2 min). After centrifugation at 2000 g for 15 min the supernatants were collected and lyophilized. For radioimmunoassay the freeze-dried material was taken up in 4–8 ml 0.05 M phosphate buffer pH 7.4 containing 0.25% human serum albumin and 0.05% sodium azid.

In the NPY-RIA we used an antiserum raised against pure natural procine NPY conjugated to bovine serum albumin (a kind gift from Dr P. C. Emson, Cambridge, U.K.). This antiserum cross-reacts with porcine gut PYY to 100% but does not cross-react with PP, GIP, PHI, VIP and secretin. The detection limit is 12 pmol/l (21). The VIP-RIA has been described in detail (17) and the detection limit is 4 pmol/l. The VIP antiserum recognizes the N-terminal sequence of VIP and does not cross-react with PHI, GIP, secretin, CCK or glucagon. In the SP-RIA we employed antiserum SP2 (a kind gift from Dr E. Brodin, Stockholm, Sweden) which has been described in detail (22); the detection limit is 1 pmol/l. The SP antiserum does not detect any known tachykinin besides SP. Immunoreactive CGRP was quantitated using a rabbit antiserum (R-8429, Milab, Malmö, Sweden), raised against synthetic rat CGRP. The CGRP RIA has been described in detail (23). Human CGRP cross-reacts in this RIA to more than 100% on a molar basis. The CGRP RIA has a detection limit of 20 pmol/l.

Immunoreactive somatostatin was quantitated using a radioimmunoassay not published previously. The somatostatin antiserum (K18, Milab, Malmö, Sweden) was used in a final dilution of 1 : 25 000. It does not cross-react with any other known neuropeptide besides cyclic somatostatin (100%), linear somatostatin (50%), [tyr^{1}]-somatostatin (100%), and [tyr^{11}]-somatostatin (38%). Two hundred microliters of antiserum was incubated first with 100 µl of standard or extract for 24 h at 4°C and then with 200 µl (~4000 cpm) of ^{125}I-tyr^{-1}-somatostatin for another 24 h at 4°C. Bound and free ^{125}I-tyr^{-1}-somatostatin were separated using dextran-coated charcoal (0.5% activated charcoal, 0.1% Dextran T-70 in phosphate buffer 0.05 M, pH 7.5 containing 0.25% human serum albumin). The radioactivity of the supernatants was counted in an LKB 1260 gamma counter. The detection limit is 5 pmol/l; intraassay variation is 6% and interassay variation is 12%. All samples were assayed in serial dilutions (duplicate samples).

RESULTS

Immunocytochemistry

All neuropeptides examined, except somatostatin, were found to occur in nerve fibers. CGRP was the most abundant of all neuropeptides. The skin from fingertips contained the richest supply of all neuropeptides, especially CGRP and SP. SP-immunoreactive nerve fibers were distributed as single scattered fibers beneath the epithelium, occasionally as bundles of fibers in the deeper layers and as single fibers running either close to blood vessels or freely in the connective tissue (Figs. 1 *c*, 2 and 3 *a*). Generally SP fibers were few or moderate in numbers except in skin from finger-tips where they were frequent, particularly in the papillae where they sometimes ramified within corpuscules of Meissner (Fig. 2). CGRP-immunoreactive fibers were found to have the same distribution pattern as the SP fibers, and simultaneous demonstration of CGRP and SP revealed coexistence of the two peptides in the same subepithelial nerve fibers (Fig. 3). Nerve fibers storing VIP were distributed deep in the dermis around blood vessels and around ducts and acini of sweat glands (Fig. 1 *a*). Only occasionally VIP fibers were seen close to the epithelium. Also NPY fibers occurred mainly in the deeper portions of the dermis, mostly around blood vessels (Fig. 1 *b*) and occasionally in association with sweat glands and hair roots. No nerve fibers could be demonstrated using the somatostatin antibodies.

Immunochemistry

Table II shows the concentrations of SP, somatostatin, VIP, CGRP and NPY in different regions based on pooled samples from 3–5 individuals. Skin from fingers and toes contained the highest concentrations of all neuropeptides. Among the neuropeptides CGRP and NPY were found in highest concentrations. Somatostatin was found in small amounts in all regions, including fingers and toes. VIP and CGRP were relatively abundant in the axilla region whereas NPY in this region was below the detection limit.

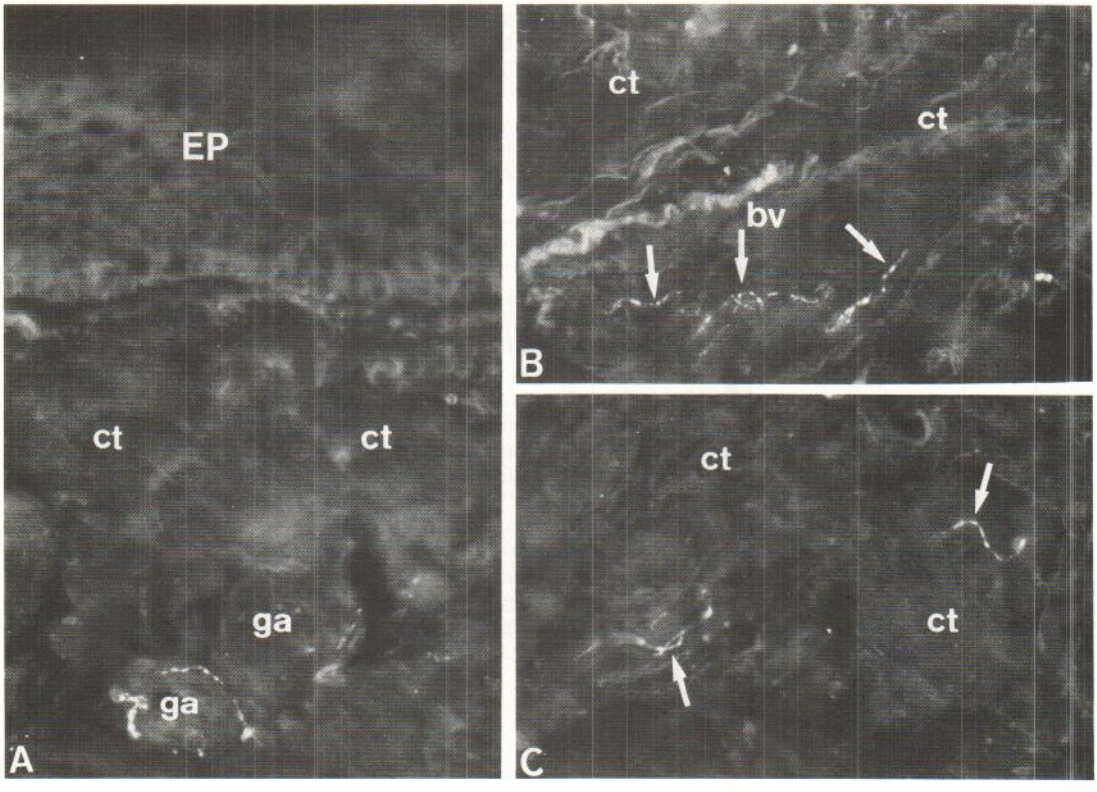

Fig. 1. Sections from abdominal skin. (*A*) VIP-immunofluorescent fibers surrounding sweat gland acinus. EP = epithelium (×160), ct = connective tissue, ga = glandular acinus. (*B*) NPY-immunofluorescent nerve fibers (arrows) in dermal connective tissue close to the wall of an obliquely cut blood vessel (bv). ct = connective tissue (×160). (*C*) SP-immunofluorescent nerve fibers (arrows) running freely in dermal connective tissue. ct = connective tissue (×120).

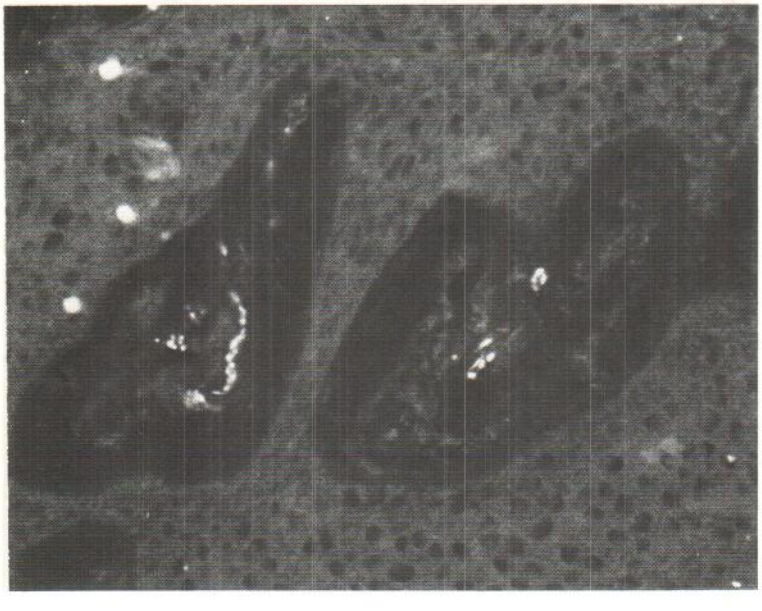

Fig. 2. Tangential section from dermal papillae of finger tip. SP-immunoreactive nerve fibers are associated with Meissner's corpuscle in two adjacent papillae (×200).

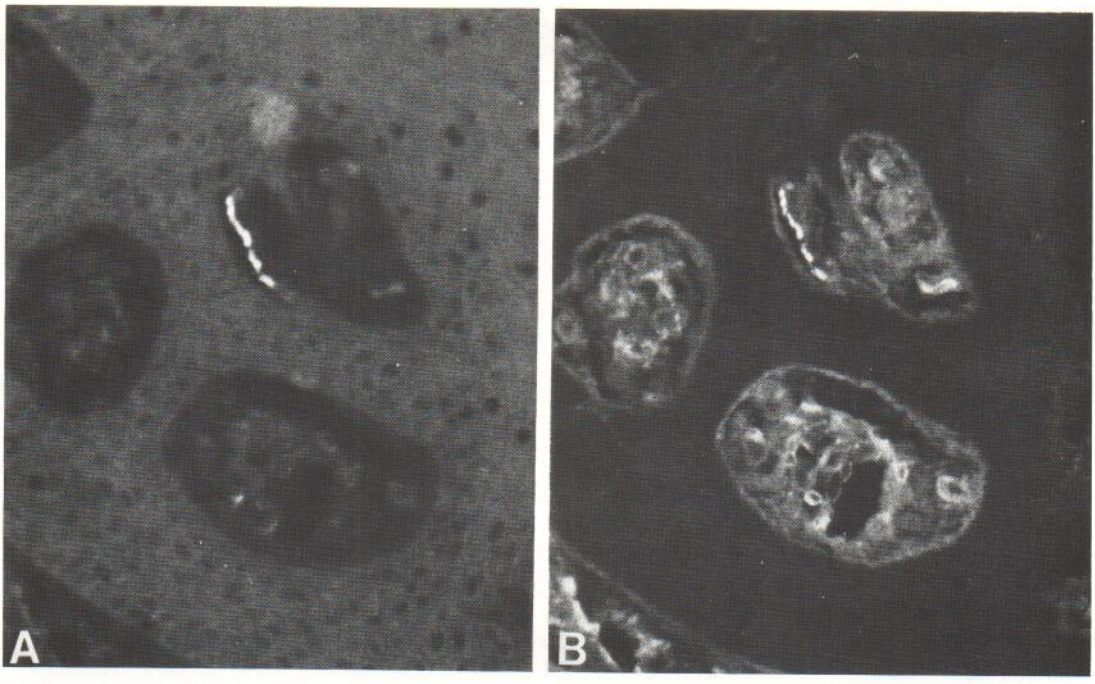

Fig. 3. Tangential section from dermal papillae of finger tip. The section was immunostained simultaneously for SP (*A*) using FITC as fluorescent marker and for CGRP (*B*) with TRITC as fluorescent marker (for technical details see text). Single fiber in dermal papilla displays SP and CGRP immunofluorescence indicating co-existence of the two peptides in the same nerve fiber. In *B* the papillae display unspecific background fluorescence (×200).

Blister fluid

Tables III, IV and V show concentrations of CGRP, somatostatin and NPY in the fluid of spontaneous blisters on inflamed skin. In all cases (with a single exception) there were significant levels of all peptides, well above the detection limit.

DISCUSSION

In almost all regions of human skin examined significant amounts of immunoreactive SP, VIP, CGRP, somatostatin and NPY were found, CGRP being the most abundant of the

Table II. *Concentration of various neuropeptides in biopsies from human skin as determined by radioimmunoassay*

Autopsy material. Samples pooled from 3–5 individuals. pmol equivalents/g. ND = not detectable, NA = not analysed

Region	SP	CGRP	NPY	VIP	Somato-statin
Finger	2.45	5.1	4	2.3	0.23
Axilla	0.07	0.9	ND	0.7	0.17
Thigh	0.07	0.5	0.5	0.2	0.05
Toe	0.59	1.9	0.7	0.8	NA

Table III. *Concentrations of CGRP in blister fluid from spontaneous blisters in inflamed skin*

Age (yrs)	Sex F/M	Diagnosis/localization	Peptide concentration (pmol/l)
80	F	Herpes zoster (back + buttocks)	156
21	F	Microbiol eczema (foot)	154
18	F	Bite of insect (leg)	104
17	F	Phototoxic blister (leg)	112
55	F	Phototoxic blister (leg)	119
36	F	Porfyria (foot)	120
80	M	Hypostasis (leg)	75
78	F	Hypostasis (leg)	82
51	M	Infected eczema (hand)	59
33	F	Toxicodermia (general)	60
79	M	Bullous pemphigoid (general)	85
82	F	Bullous pemphigoid (general)	81
71	F	Pemphigus (general)	120

Table IV. *Concentrations of somatostatin in blister fluid from spontaneous blisters on inflamed skin*

Age (yrs)	Sex F/M	Diagnosis/localization	Peptide concentration (pmol/l)
82	F	Bullous pemphigoid (general)	42
41	F	Phototoxic blister (legs, arms)	21
33	F	Toxicodermia (general)	51
71	F	Pemphigus (general)	78
49	F	Inflammatory blisters (hand)	66
80	M	Hypostasis (leg)	48
78	F	Bullous pemphigoid (general)	6
80	M	Bullous pemphigoid (general)	54
80	M	Bullous pemphigoid (general)	46

Table V. *Concentrations of NPY in blister fluid from spontaneous blisters on inflamed skin*

Age (yrs)	Sex F/M	Diagnosis/localization	Peptide concentration (pmol/l)
55	F	Photodermatitis (leg)	73
84	F	Bullous pemphigoid (leg)	338
97	M	Herpes zoster (back)	363
41	F	Phototoxic blister (leg)	39
60	F	Bullous pemphigoid (general)	102
80	M	Hypostasis (leg)	<12
82	F	Bullous pemphigoid (general)	72
33	F	Toxicodermia (general)	143

neuropeptides examined. Fingers and toes were richest supplied by all these peptides. The finding of SP in free nerve endings and in Meissners corpuscles in human digital skin is in agreement with previous observations (5). Available data suggest that SP fibers in the skin are sensory and play a role in nociception. Our finding of SP and CGRP co-existence in the same nerve fibers superficially in dermis suggests a role in nociception also for CGRP. It is notable that the highest concentrations of SP and CGRP were found in fingers and toes which are known to receive a heavy sensory innervation. Coexistence of SP and CGRP has previously been reported in primary sensory neurons of several mammals (11).

Also NPY occurred in high concentrations in fingers and toes. Nerve fibers storing NPY were localized mainly in deeper parts of dermis around blood vessels and acini of sweat glands. The rich supply of NPY fibers around certain blood vessels suggests that NPY may be involved in the control of local blood flow. The fact that NPY and norepinephrine coexist in the same nerve fibers in other organs (13) may suggest that the NPY fibers in the skin are identical with sympathetic adrenergic fibers. There are several recent reports indicating a vasoconstrictor effect of NPY in vivo and enhancement of adrenergically mediated vasoconstriction in vitro (13). Nerve fibers storing VIP were found in deeper parts of dermis with a distribution similar to that of NPY fibers, i.e. around acini of sweat glands and around blood vessels as previously described (4, 8). A role for VIP in sweat secretion is supported by the relatively rich supply of VIP in axilla.

Somatostatin was found in low concentrations compared to the other peptides studied and immunocytochemically somatostatin could not be demonstrated.

When injected intracutaneously VIP, SP and somatostatin evoke flare and wheal accompanied in the case of SP and somatostatin by itching (14 and unpublished observations). CGRP injected intracutaneously induces a slowly spreading flare and induration which subsides after several (1–6) hours depending on the concentration (15, 16 and own unpublished observations). Also NPY when injected intracutaneously induces an erythema but no wheal (unpublished observations). This effect is somewhat surprising in view of the vasoconstrictive effect demonstrated upon intravascular injection (13).

It has been speculated that under pathological conditions in some prurigenous dermatoses enhanced release of VIP and SP may give rise to local inflammation and itching (8). Interestingly, significant amounts of SP and VIP (17), as well as CGRP, somatostatin and NPY are found in spontaneous inflammatory blisters.

By contrast, in control blisters raised by suction SP, VIP and CGRP concentrations were usually below detection limit (17, unpublished observations).

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