Results

NEUROPEPTIDES IN HEALTHY HUMAN SKIN (I)

Immunocytochemistry

All neuropeptides except somatostatin were found in nerve fibers in the skin. CGRP was the most abundant of the neuropeptides. It had the same distribution pattern as SP and double staining procedures revealed coexistence of the two peptides in the same subepithelial nerve fibres. SP and CGRP were found in scattered fibers just beneath the epithelium, close to blood vessels or running freely in the connective tissue. In finger-tip skin SP and CGRP seemed to coexist in nerve fibers in the dermal papillae and in the corpuscules of Meissner. Nerves storing VIP and NPY were found deep in the dermis, usually around blood vessels. VIP - immunoreactive nerve fibers were found also around ducts and acini of sweat glands while NPY - immunoreactive fibers were associated with sweat glands and hair roots.

Immunochemistry

RIA was performed on skin samples from different body regions. The samples were pooled from 3 - 5 individuals (autopsy material). The concentrations of SP, somatostatin, VIP, CGRP and NPY were determined. Skin from fingers and toes contained the highest concentrations of neuropeptides, notably CGRP and NPY. 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.

The influence of physiological factors on neurogenic inflammation

Intracutaneous injection of SP was used as a standard challenge to induce neurogenic inflammation (II) (Fig. 3). The following physiological factors were studied: body region, age, sex, stress, environmental temperature and diurnal variation. The largest flare was recorded on the thorax and the largest wheal on the upper aspect of the foot. The most intense itch was noted from the nape of

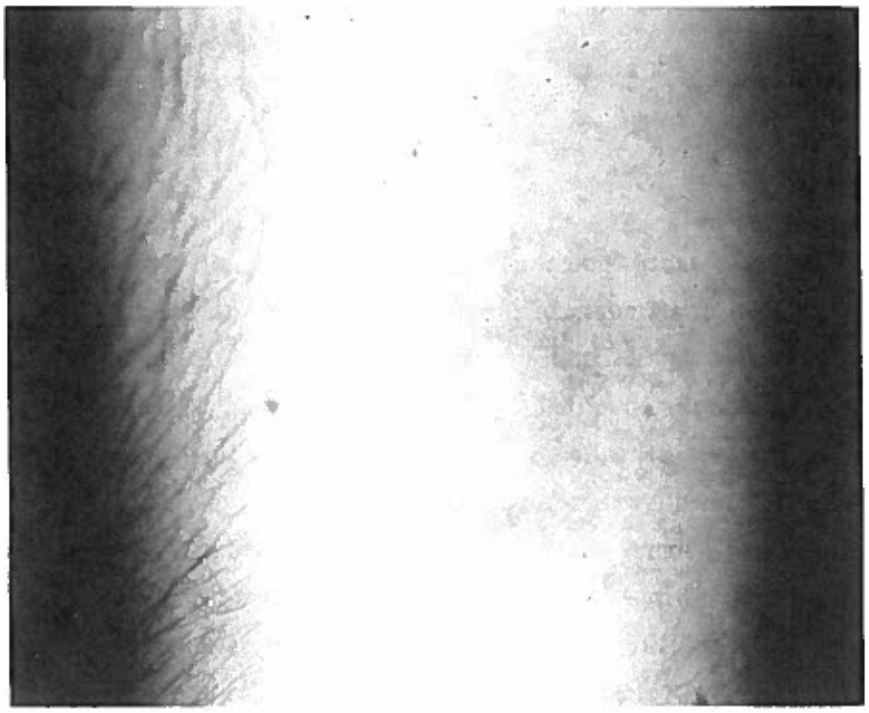

Fig. 3. *SP (50 pmol) injected ic (5 min) causes flare and wheal.*

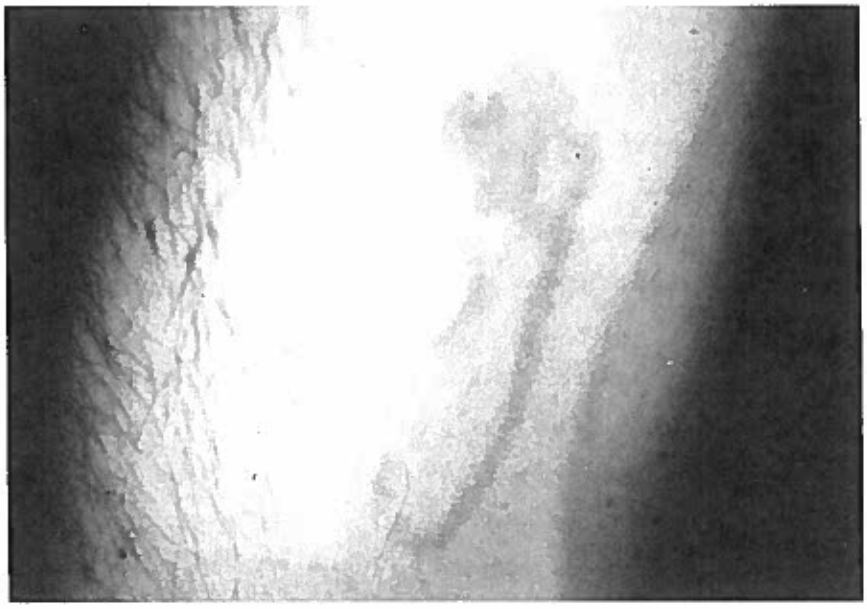

Fig. 4 *CGRP (13 pmol) injected ic (2 h) causes a longlasting erythema with pseudopodia.*

the neck and from the upper side of the foot. There was a marked decrease of the flare responses to SP with increasing age. Athletes displayed smaller SP-evoked flare responses after a competitive test of endurance than at rest. The flare responses to SP was larger during the night (11 p.m.) than during the day (11 a.m.), whereas the wheal did not differ. The sex of the subjects as well as the environmental temperature (17^{o}, 22^{o} or 28^{o} C) did not influence the neurogenic inflammation induced by SP.

Effects of suspected inflammatory mediators in healthy human skin

SP, NKA, histamine (III), and bradykinin (IV) injected intracutaneously induced flare and wheal (Fig 3). The flare was maximal after 3 - 5 min (histamine-induced flare was maximal after 10 min) and wheal after 15 min. Injection of histamine, NKA and SP induced itching while bradykinin induced pain rather than itch. The itch and pain were dose dependent. Intracutaneous injection of CGRP produced (III) a prominent long lasting, indurated erythema with pseudopodia surrounded by a pallor edge (Fig 4). The response to CGRP was dose dependent and lasted for several hours (6 - 12 h) (III). Intracutaneous injection of prostaglandin (PGE_2) also induced an indurated erythema with pseudopodia (IV).

Effects of capsaicin

Capsaicin (ic) (IV) induced a dose-dependent flare, wheal and pain. Capsaicin applied topically induced erythema, itch and burning.

MODULATION OF THE EFFECTS OF INFLAMMATORY MEDIATORS AND OF CAPSAICIN (III, IV)

Depletion of mast cell histamine

Pretreatment of the skin with repeated ic injections of compound 48/80 results in depletion of mast cell histamine.The flare response to SP (III) and bradykinin (IV) was greatly reduced by this pretreatment. The flare produced by

histamine (III) and NKA (III) was inhibited by about 50%. The flare response to PGE_2 (IV) was reduced by approximately 30%. The erythema evoked by CGRP was not suppressed by pretreatment with compound 48/80. The wheal response to SP was reduced (about 50%) by pretreatment with compound 48/80. The wheal responses to NKA, histamine and bradykinin were unaffected. The flare and wheal responses to capsaicin were unaffected.

Blockade of H_1 - receptors (mepyramine)

The flare responses to SP (III), NKA (III) and bradykinin (IV) were greatly reduced by pretreatment with mepyramine (ic). This pretreatment reduced the erythema evoked by PGE_2 (IV) by about 30% while the erythema evoked by CGRP was not affected.

The flare induced by capsaicin was not influenced by mepyramine. The wheal responses to bradykinin and capsaicin (IV) were not affected by mepyramine pretreatment.

Blockade of nerve conduction (lidocaine)

The flares produced by SP (III), NKA (III), histamine (III) and bradykinin (IV) were greatly reduced by the local anesthetic lidocaine (ic). The erythema produced by CGRP (III) was not or slightly reduced by lidocaine while that of PGE_2 (IV) was suppressed by approx 30 %. Lidocaine suppressed the wheal produced by histamine by about 30%. The wheal responses to SP (III), NKA (III) and bradykinin (IV) were unaffected by lidocaine pretreatment.

The flare response to capsaicin (IV) was greatly reduced by lidocaine pre-treatment while the wheal was unaffected.

Combination of mast cell depletion and blockade of nerve conduction

The combined pretreatment with compound 48/80 (ic) and lidocaine (ic) gave no additional inhibition of the flare and wheal responses to SP, NKA, histamine (III) and bradykinin (IV) and of the erythema induced by CGRP (III) and PGE_2 (IV).

Blockade of prostaglandin synthesis (indomethacin)

Pretreatment with indomethacin ic inhibited the flare response to SP to approx. 60% (IV) and to bradykinin to one third (IV). The wheal responses to SP and bradykinin were not influenced by pretreatment with indomethacin. Pretreatment with acetylsalicylic acid suppressed the flare response to SP to approx 30%, while the wheal response was unaffected.

The flare response to capsaicin was reduced to 50 % and wheal to about 30%.

Blockade of acetylcholine and serotonin receptors (atropine, ketanserin)

Pretreatment with atropine and ketanserin did not influence the flare and wheal responses to bradykinin and capsaicin.

Blockade of tachykinin receptors by the tachykinin antagonist, Spantide (VIII)

Intracutaneous injection of Spantide induced flare and wheal which subsided after 75 to 90 minutes. SP and histamine were injected 1.5 h after the injection of Spantide. The flare reaction to SP was suppressed to 23% by this pretreatment while the flare to histamine was reduced to 72%. Spantide reduced the wheal response to SP to 42% while the histamine evoked wheal was unaffected.

Pretreatment with capsaicin (IV)

Repeated injections of capsaicin (6 times) (IV) induced short lasting pain that did not seem to diminish with each injection. The flare reaction on the other hand was progressively suppressed until it was almost completely abolished. Subsequent injections of SP revealed a flare response that was only about 50% of the control response. After eight weeks the flare reaction was back to normal. Topical capsaicin (VI) evoked weaker pain with each application. There was a tingling or burning sensation when the treated area became warm.

Subsequent injection of bradykinin gave a reduced flare response (40% of that in the controls) while the wheal response was unaffected.

Pretreatment with bradykinin (IV)

Repeated injections of bradykinin (6 times) induced pain and flare that became progressively weaker with each injection. Subsequent injections of SP resulted in a reduced flare (40% of that in the controls), the wheal was unaffected. The response to SP was back to normal after eight weeks. Injection of capsaicin after pretreatment with bradykinin resulted in a reduced flare response (to 55%), the wheal was unchanged.

Cooperation of SP and CGRP (III)

A threshold dose of CGRP did not affect the response to SP (flare and wheal). A threshold dose of SP did not affect the CGRP-induced erythema.

CONTENT OF SP AND VIP IN SKIN DISEASES (V)

Spontaneous blisters in different skin diseases contained measurable levels of SP and VIP. The highest levels of SP were found in patients with eczema (mean 168 pmol/l) and bullous pemphigoid (106 pmol/l). The concentration of VIP was highest in burn blisters (265 pmol/l) and photodermatitis (79 pmol/l). Eczematous blisters were not analyzed for VIP. Suction blisters induced on inflamed skin (except patients with bullous pemphigoid) contained measurable levels of SP in patients with psoriasis (mean 86 pmol/l) and eczema (mean 33 pmol/l). Also VIP was found in blister fluid of patients with psoriasis (mean 10 pmol/l) and with eczema (mean 13 pmol/l). Patients with bullous pemphigoid (normal skin), acute urticaria and controls had non detectable levels of SP and VIP in the suction blister fluid. Of 56 patients with disseminated skin disease or pruritus (7 patients) only one with atopic dermatitis had increased plasma concentrations of VIP (200 pmol/l). All others had non-detectable levels of SP and VIP in plasma.

TREATMENT OF NOTALGIA PARESTHETICA WITH TOPICAL CAPSAICIN

During the first week following application of capsaicin there was increased itch or pain but after subsequent treatment such symptoms occurred less frequently and with lower intensity (Fig. 5). Symptom relief persisted for months after interrupting the treatment. Table III. Four patients relapsed and had to have the treatment repeated.

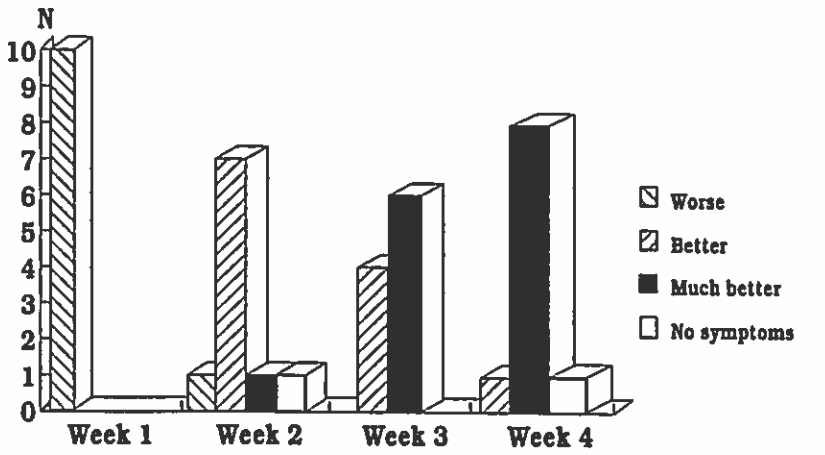

Figure 5.
Results of topical treatment of notalgia paresthetica with topical capsaicin cream (n=10)

NEUROPEPTIDES AS MODULATORS OF EXPERIMENTAL CONTACT ALLERGY (VII)

SP, VIP and somatostatin were injected (30 and 150 pmol) into the same skin sites as the antigen in patients with contact allergy to nickel at different points of time and in different doses. The peptides were injected 10 min after injection of nickel sulphate and the evaluation was made after 72 h. In another experiment 50 pmol of the peptides was injected three times (after 10 min, 24 h and 48 h) after the nickel deposition. The inflammatory reaction to injected nickel sulphate was the same whether SP, VIP of somatostatin were given concomitantly or not. This was regardless of dose or time schedule.

Table III. Follow up of the treatment of notalgia paresthetica with Zostrix®

No	1 month	5 - 6 months	9 months	1 year
1.	Symptomfree for about 2 weeks, then several attacks of weak itch, each lasting for few days	Weak itch 1-2 times a week	Occasional attacks of itch, symptom free for several weeks	Symptomfree for several months, attacks of weak itch, each lasting for few days
2.	Symptomfree	Symptomfree except for occasional itch	Symptomfree, a few attacks which were abolished promptly by Zostrix	Symptomfree, except for 4-6 mild attacks during the last couple of weeks. The itch disappears after application with Zostrix
3.	Symptomfree	Symptomfree	Symptomfree	Symptomfree
4.	Two attacks of itch. Zostrix alleviated the symptoms	Symptomfree	Symptomfree	Symptomfree
5.	Symptomfree	During short periods 1-2 attacks of itch/day. Applications of Zostrix causes burning sensation followed by symptom-relief	Renewed treatment with Zostrix, twice daily (two weeks), better for 4-6 weeks	Renewed treatment twice daily during 1 week, symptomfree for 14 days than again several attacks each day
6.	Weak itch two times a week	Started to use Zostrix once daily three months after the first treatment. Fewer attacks of itch	Mild itch sometimes in the evening after a bath	Mild itch about two times a week
7.	A few mild attacks of itch	Symptomfree	Symptomfree	Symptomfree
8.	Progressive relapse of the itch. Renewed treatment after two weeks for another three weeks. Symptom-relief	A mild attack of itch absent once a week (when warm)	One - two mild attacks of itch a week	Symptomfree for about one week, followed by mild attacks of itch for few days
9.	Relapsed after 2 weeks. Symptomfree following repeated treatment	2 - 3 mild attaks of itch a week. The second treatment period completed	1 - 2 mild attacks of itch each week	
10.	Symptomfree	Symptomfree (2-3 mild attacks during four months)	5 - 6 attacks of mild itch during the last two months	

THE EFFECT OF SPANTIDE ON EXPERIMENTAL INFLAMMATIONS (VII, VIII)

Delayed hypersensitivity reactions (type IV). Nickel allergy and tuberculin reaction. Spantide in a dose of 800 pmol injected 10 min after nickel sulphate in patients with nickel allergy reduced the contact allergy reaction to 20%. A single dose of 1600 pmol or 800 pmol injected three times failed to reduce the contact allergy (VII). Spantide (800 pmol) greatly suppressed the response to tuberculin (Fig. 6).

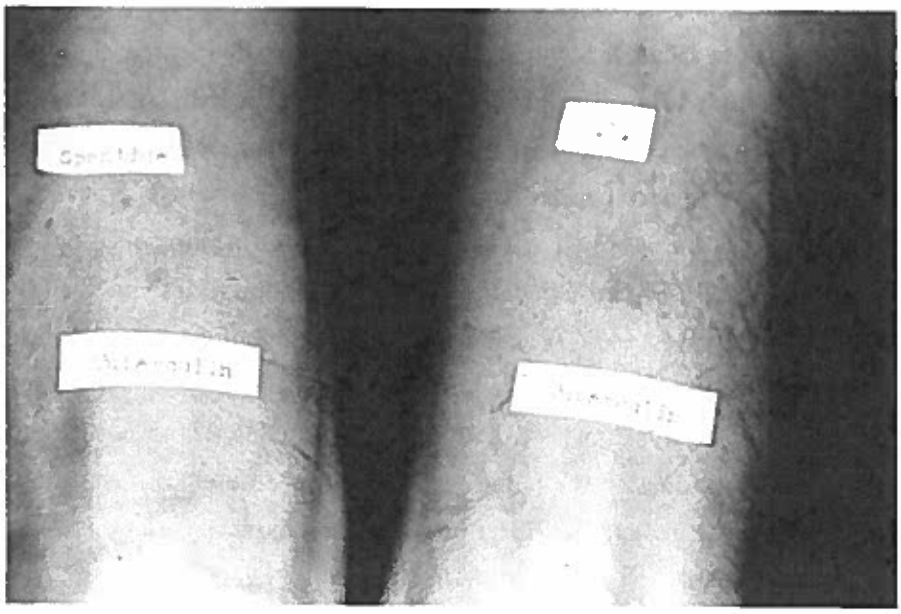

Fig. 6. Spantide (dx) suppresses the response to tuberculine.

Immunologic contact urticaria (type I reaction).
Spantide (800 pmol) suppressed the urticarial response to different foods and latex.

Non - immunologic inflammatory reactions. The delayed irritant reactions (benzalkonium chloride), moderatly delayed irritant reaction (UVB irradiation) and the non-immunologic contact urticaria (NICU - benzoic acid) were unaffected by Spantide (800 pmol).

Discussion

WHAT DETERMINES THE FLARE RESPONSE TO NEUROPEPTIDES?

The flare response to ic neuropeptides is usually well demarcated. It is dose-dependent (II, III, IV) and dependent on the body region (III). This may reflect the local organization of *innervation territories*. Arthur and Shelley (1958) counted the number of nerve fibers that entered the epidermis (probably mainly SP and CGRP fibres) and found different numbers in different body regions (largest on the cheek, lower on the neck and pre-sternal and lowest on the lateral part of a finger). Some small areas of the skin seemed to receive no nerve supply at all while other areas were richly innervated. At times (usually when small amounts of peptides are used to provoke a response) the periphery of the flare has a "salt and pepper" pattern (Fig 2). The white areas probably represent areas that are supplied with fibers from an adjacent nerve not excited by the local stimulus. The concentration of neuropeptides in the skin of different regions varies too (I). Blockade of nerve conduction by lidocaine greatly suppressed the flares produced by SP (III), NKA (III), histamine (III), capsaicin (IV) and bradykinin (IV). The response to CGRP was not affected by blockade of nerve conduction while the erythema induced by PGE_2 was inhibited by 30%. The erythema evoked by CGRP and PGE_2 is characterized by pseudopodia suggesting involvement of the *lymphatic system*. There are also regional variations in the *blood flow* (Tenland et al 1983).

The *endogenous histamine* is very important for the flare response to SP and bradykinin. In fact pretreatment with repeated injections of 48/80 or mepyramine is as effective as neuronal blockade in abolishing the flare to SP, less important for the flare response to NKA and to histamine and of little importance for the erythema response to PGE_2 and CGRP.

The role of histamine in neurogenic inflammation is still a subject of discussion. All agree that histamine is released at the point of the stimulus (primary mediator). The crucial point is if histamine participates in the progressive spreading of the flare by activation of sensory nerve endings to release neurogenic mediators (e.g. tachykinins) followed by further histamine release. According to this scheme histamine is also a secondary or final mediator. This cascade of responses has been called *"axon response"* (Arvier et al 1977, Hägermark et al 1978, Lembeck and Holzer 1979, Jordan and Foreman 1983) to be distinguished from the classical *"axon reflex"*. The latter concept implies that the spread of flare is mainly dependent on the antidromic passage of impulses and that the flare is limited by the extent of the collateral network as was initially demonstrated by Bayliss (for a review see Holzer 1988).

Our experiments with *capsaicin* (IV) revealed that the flare and wheal responses induced by ic injection can be inhibited by local anesthetics and indomethacin, but not by compound 48/80 or antihistamines. Capsaicin releases neuropeptides from C-fibres and inhibits axoplasmic transport of sensory transmitters (Gamse et al 1982). It has been used as a model for neurogenic inflammation also by Barns et al (1986) who concluded that enogeneous histamine is not the final vasodilatator in the axon reflex as it is not suppresed by a pretreatment with peroral antihistamines. This supports the "axon reflex" theory (with initiation of the reflex by histamine) before the "axon response" where histamine is supposed to play a role as a secondary mediator of the neurogenic inflammation.

The mast cells which degranulate in response to noxious stimuli release not only histamine but also proteolytic enzymes which may contribute to the size and duration of the flare and wheal response (Brain & Williams 1988).

The wheal responses were unaffected by nerve blockade suggesting that the wheal response reflects a direct local effect of the challenging agent. The wheal response is unaffected by pretreatment with the histamine liberator compound 48/80 or the histamine antagonist mepyramine. The intensity of the *flare, wheal*

and *itch* did not vary in parallell (II). Flare was most prominent on the upper thorax, wheal on the upper aspect of the foot, and the most intense itch was reported from the nape of the neck and upper part of the foot. The flare response to SP was greater during the *night* (11 p.m.) than during the *day* (11 a.m.). Also itch was reported to be stronger at night while the wheal response was the same regardless of the time of day. This is in line with the clinical experience that itch is more intense during the night than during the day and that the nape of the neck and upper part of the foot are common places for neurodermatitis.

The *athletes* had smaller SP-evoked flare responses compared with the age-matched controls and even smaller responses immediately after a competitive endurance test than at rest. We did not observe any sex differences in the flare and wheal response to SP. There is no obvious explanation of this finding. Conceivably, sympathetic discharge or stress-released endorphins may impair impulse flow in the C-fibres. However, numerous other alternative explanations are possible.

The *age* of the individual is decisive for the flare response and possibly for the wheal response to SP (II). As early as 1955 Tuft and his associates found that the wheal response to intradermal histamine decreased after the age of 60. The flare and wheal responses to histamine were found to diminish with age (Magerl et al 1990) as did the response to topical capsaicin (Helme 1985) and ic bradykinin (Juhlin and Michaëlson 1969). Cutaneous nerves are not thought to be much affected by age (Montagna 1979). The superficial blood vessels are reduced in number by 35% with age and the number of mast cells is reduced by 50% (study based on two groups mean age 24 and 63 years respectively) (Gilchrest et al 1982). The microvessel reactivity to an applied stimulus (methyl nicotinat) was shown, however, to be unchanged by age (Roskos et al 1990). Still it cannot be excluded that it is the impulse traffic in the C-fibers that deteriorates with age, manifested by smaller flare and wheal responses.

COEXISTENCE AND COOPERATION OF INFLAMMATORY MEDIATORS

We have shown (I) that *SP* and *CGRP* coexist in sensory nerve fibers in human skin as has been previously shown by Gibbins (1986) in guinea pig skin. Also *NKA* has been found to coexist with SP in the same sensory fibres (Sundler et al 1985).

All peptides in the C-fibres are probably released concomitantly when the nerves are stimulated (Terenghi et al 1985). SP and NKA induce flare and wheal responses which are greatly inhibited by local anesthetics and in the case of SP by antihistamines (III). These findings are consistent with reports of other authors on the effects of SP (Hägermark et al 1978, Fjellner and Hägermark 1981, Foreman and Jordan 1983) and on NKA (Fuller et al 1987). Spantide, which is a tachykinin antagonist, suppresses the flare induced by histamine (VIII) indicating that the response to histamine is mediated by tachykinins. The effect of CGRP differs in that it can not be inhibited by either local anesthetics or mast cell histamine depletion (III). Like the response to PGE_2 that to CGRP develops slowly with the formation of pseudopodia, apparently involving the lymphatic vessels. The response to CGRP is not inhibited by indomethacin (Brain and Williams 1985) or acetylsalicylic acid (Fuller et al 1987). The response to CGRP is more long lasting than the responses to the other inflammatory mediators. Conceivably, CGRP is responsible for the delayed phase of inflammatory reaction which is probably initiated by other peptides such as SP and NKA. CGRP is chemotactic and stimulates invasion of polymorphonuclear leukocytes to the inflammatory site (Piotrowski and Foreman 1986). Brain and Williams (1988, 1989) have reported that the CGRP erythema disappears sooner by the concomitant injection of SP. That was thought to be a consequence of SP-induced release of proteases (tryptase and chymotryptase) from mast cells. CGRP has also been reported to potentiate the oedema (wheal reaction) induced by other mediators such as SP and histamine (Gamse and Saria 1985, Brain and Williams 1985, 1989). Skin mast cells release the protease chymase along with histamine during degranulation. Chymase has been reported to greatly augment histamine induced wheal formation (Rubinstein et al 1990).

Our data do not show any potentiation of SP by threshold doses of CGRP or potentiation of CGRP response by threshold doses of SP (see also Fuller et al 1987).

Is there any interaction between *prostaglandins* and SP? Indomethacin suppressed the flare and wheal response to SP. Also pretreatment with local anesthetics and depletion of mast cell histamine by compound 48/80 suppressed the PGE_2 induced erythema by 30%. These findings suggest that prostaglandins interfere with the neurogenic mechanisms behind inflammation in the skin. These data are consistent with those reported by Fuller et al (1987) who found acetylsalicylic acid to suppress the SP-evoked flare and those reported by Ståhle and Hägermark (1985) who found indomethacin to suppress the histamine evoked flare without affecting the itch. Prostaglandin E_1 has been reported to lower the threshold of human skin to histamine-evoked itching (Greaves and Mc Donald-Gibson 1973). However another report by Albro et al (1972) found no evidence that prostaglandins act on mast cells to release histamine in vitro. Aspirin and indomethacin have also been tested in different pruritic and inflammatory disorders with varying effectiveness (for a review se Greaves 1987). We found that the *bradykinin-induced* flare is dependent on the sensory nerve supply (which confirms the findings by Juhlin and Michaëlson 1969), on mast cell histamine, and on the prostaglandin system. The latter conclusion is at odds with the data presented by Crossman and Fuller (1988) who found no inhibition of the bradykinin response following peroral treatment with terfenadine and acetyl-salicylic acid. The difference in results may perhaps be explained by the difference between local injections (high local concentration) and peroral treatment (subthreshold local concentration).

Several endogenous systems intervene and interact in inflammation in a complex manner. The actions and interactions of the different agents investigated in this study are schematically illustrated in Fig. 7.

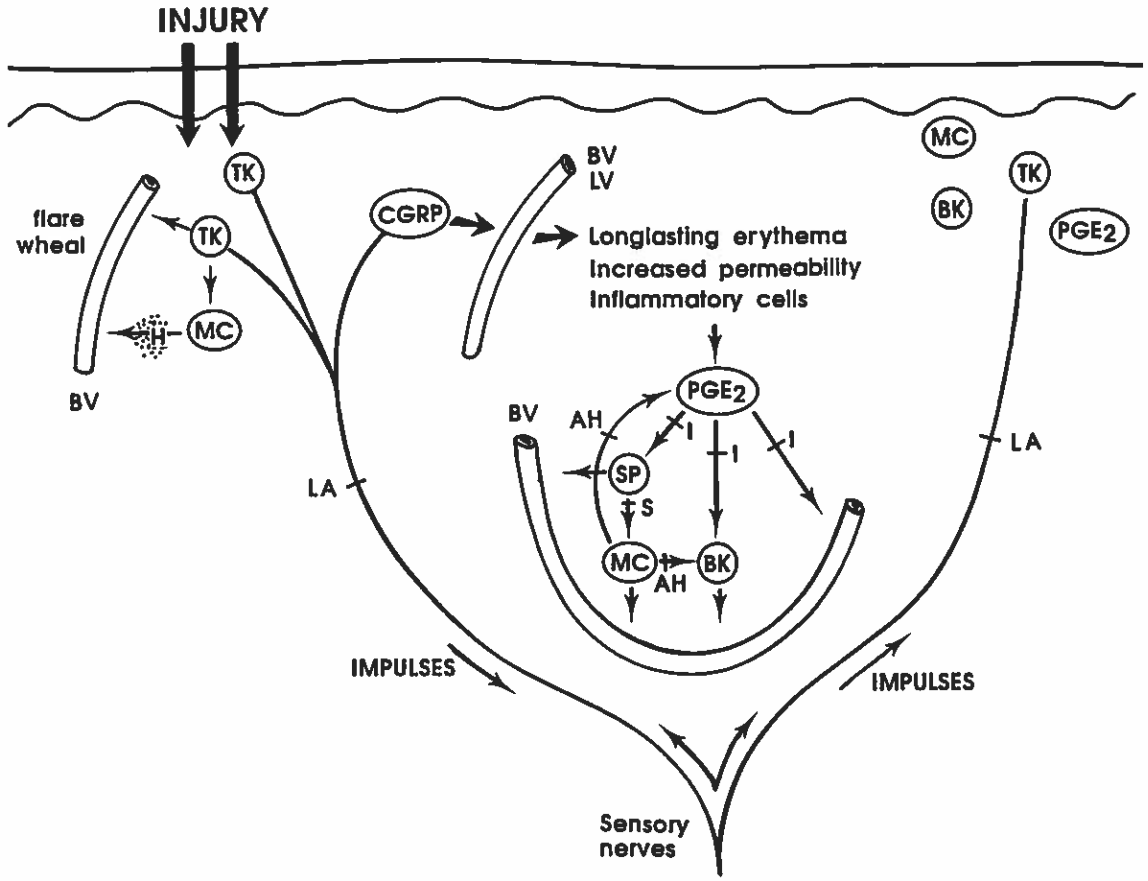

Fig. 7. *Hypothetical scheme of events caused by intradermal SP injection. Tachykinins (TK) stimulate mast cells (MC) to release histamine (H). Also bradykinin (BK) and prostaglandins (PGE_2) are formed in the process. Together they affect blood vessels (BV) causing vasodilatation (flare) and extravasation (wheal). CGRP and PGE_2 stimulate both blood vessels and lymphatic vessels (LV), causing extravasation and recruitment of inflammatory cells. As a result more PGE_2 is released which stimulates SP release and BK formation. The steps which are inhibited by antihistamines (AH), local anesthetics (LA) or indomethacin (I) are indicated.*

NEUROPEPTIDES AND SKIN DISEASE

We have found high concentrations of SP and VIP in spontaneous bullae from pathogenetically different skin diseases like bullous pemphigoid, eczema, burn, phototoxic reaction, photoallergic reaction and infection. Fluid from suction blisters induced on inflamed skin, due to different disorders, contains measurable levels of SP and VIP but not fluid from suction blisters induced on normal skin of healthy individuals and of patients with bullous pemphigoid. These results (see also Table I) seem to provide some evidence that neuropeptides are released locally in *inflammatory diseases*. The connection between the nervous system and the skin is reflected also in the fact that many dermatoses worsen during conditions of stress. Stress and mental illness are all thought to have an effect on skin diseases and immune function. A possible role of neuronal elements in psoriasis has been proposed by Farber (et al 1986) on the basis of two striking clinical features: the symmetric distribution of psoriatic plaques and the role of stress as a triggering factor in the exacerbation of psoriasis. There is an increased density of SP containing nerves (Naukkarinen et al 1989), and VIP-containing nerves (Pincelli et al 1990) in psoriatic lesions. Prompt remission of a psoriatic plaque has been reported following cutaneous nerve sectioning (Weddel et al 1965, Dewing 1971). Somatostatin infusion improved psoriasis (Weber et al 1981, MacKie et al 1983). This might be explained by the inhibiting effect of somatostatin on the release of neuromediators (Brodin et al 1984). There is good evidence that emotional stress aggravates psoriasis (Braun-Falco et al 1972, Farber and Nall 1974, Newbold 1977). This reasoning is also valid with regard to atopic dermatitis which also worsens in situations of stress (Roth and Kierland 1984, Rajka 1975, Medansky and Handler 1981, Koblenzer 1983). The increased density of sensory nerve fibres and the increased levels of neuropeptides in the skin provide evidence for a role of nerves also in atopic dermatitis (Table I). Also the increased number of mast cells in psoriasis (Cox 1976) and atopic dermatitis (Sagher and Even-Paz 1967, Mihm et al 1976) provide evidence for a role for neurogenic inflammation since mast cell histamine is essential for the axon reflex.

Interestingly, the flare response to inflammatory mediators (bradykinin - Juhlin and Mikaëlsson 1969, histamine - Möller and Rorsman 1958, Heyer et al 1989, and SP - Gianetti and Girolomoni 1989) is lower in atopic patients than in controls. Other workers, however, failed to observe a significant difference in the flare response to SP or histamine while the wheals were larger in atopic patients (Coulson and Holden 1990).

SP and NKA have been reported to stimulate cell growth in fibroblast cultures (Nilsson et al 1985) which may initiate wound healing in connection with tissue injury. The itch which patients with leg ulcers report and recognize as a good sign of wound healing might reflect sprouting of SP- and NKA-containing nerve fibers. SP containing nerves regenerate in the process of burn wound healing (Kishimoto 1984), while systemic capsaicin treatment causes impaired wound healing (Mantyh et al 1989).

Certain viruses (herpes simplex and herpes zoster) seem to invade sensory nerves and to be stored in the dorsal roots (quoted by Hökfelt et al 1975). Recurrence of the infection results in transport of the virus to the skin. We have found fairly high levels of SP (97 pmol) (Wallengren, Ekman, unpublished) and CGRP 156 pmol, V) in blister fluid from patients with zoster infection . Treating herpes simplex infections with topical application of local anesthetics (Emla®) inhibited the development of a recurring infection (Cassuto 1989). This can be explained by an inhibition of the release of both the virus and the neuropeptides (which normally induce flare, wheal, itch and pain) from sensory nerves.

Acute herpes zoster infection is followed by marked destruction of cutaneous nerves (Hasegawa 1971). Axon reflex vasodilatation was found to be impaired indicating a loss of C-fibres (Jancsó et al 1983). The pain associated with herpes zoster infection probably reflects the destruction or the regeneration of cutaneous nerves and has been found to be substancially reduced by continuous

topical application of capsaicin (Bernstein et al 1987, 1989). The paroxysmal attacks of itch in patients with notalgia paresthetica might reflect abnormal firing of peripheral nociceptors. The attacks often are of short duration which may explain why there is no inflammatory reaction in the skin which would be expected if excitation of nociceptors leads to neuropeptide release. The axon reflex is not impaired in notalgia paresthetica i.e. ic injection of SP in the afflicted patch revealed the same flare and wheal but more itch than in control skin (same region) (Wallengren, Håkanson unpublished). The local functional changes in C-fibres of such patients do not seem to correspond to morphological nerve changes. This may explain why a short treatment period with capsaicin may be enough to restore a normal function in patients with notalgia paresthetica (VI).

CAPSAICIN AND SKIN DISEASES

Capsaicin has been used in animal experiments in order to elucidate the role of C-fibre-mediated mechanisms in a variety of organs and the role of these mechanisms in the pathogenicity of different disorders. Parenteral administration of capsaicin causes severe bronchoconstriction (which may be prevented by administration of terbutalin) and severe itch leading to scratching. Trophic skin lesions described in rats treated with capsaicin as newborn probably reflect degeneration of C-fibres (Maggi et al 1987). Capsaicin produces degeneration of C-fibres in neonatal rats up to 10-14 days of age. When administred to adult rats there is no destruction of primary sensory neurons (Fitzgerald 1983). The effects of capsaicin on cutaneous nerves are species dependent (Lynn and Pini 1984) with respect to both immediate and long term effects.

The response to ic injected capsaicin (flare and wheal) in human skin is dose dependent (IV). Repeated injections induced tachyphylaxis. The axon reflex recovered after eight weeks indicating that there are no long-term degeneration effects on the neurons. Repeated topical application of capsaicin in patients with notalgia paresthetica caused a permanent improvement in nine out of ten

patients (VI). The efficacy of this treatment speaks for a pathogenetic role of capsaicin-sensitive, unmyelinated sensory fibres. Topical capsaicin treatment has previously been shown to be efficient in postherpetic neuralgia (Bernstein et al 1987 and 1989, Bucci et al 1988), apocrine chromohidros (Marks 1989), severe psoriasis (Bernstein et al 1986) and stump pain (Rayner et al 1989).

Capsaicin treatment reduces the itch associated with different dermatological affections e.g. lichen planus, nummular psoriasis, aquagenic urticaria, atopic dermatitis, and contact dermatitis to nickel sulphate (Cappugi et al 1990). Topical capsaicin seems, however, to enhance immunologic and non-immunologic inflammatory reactions in human skin (Wallengren and Möller 1987) and contact dermatitis in mice (Girolomoni 1990). Systemic treatment of guinea pigs with capsaicin enhances both induction and responses to provocation of contact dermatitis to DNCB (Wallengren et al 1990). However, pigs sensitized with ascaris and pretreated with capsaicin responded to ascaris challenge with reduced nasal and tracheobronchial bloodflow (equivalent to the flare reaction) (Alving et al 1988). Topical pretreatment with capsaicin reduces also the cutaneous allergy reaction to rat antigen in sensitized persons or to anti-IgE in non-allergic persons (Lundblad et al 1987).

IMMUNOLOGICAL REACTIONS AND NEUROPEPTIDES

The tachykinin antagonist Spantide has been shown to inhibit the immunologic reactions of both immediate (contact urticaria, VIII) and delayed cutaneous hypersensitivity (contact dermatitis, VII) and tuberculin reactions (VIII) while had no effect on non-immunologic inflammatory reactions. This suggests that SP might be involved in the pathogenesis of immunologic skin reactions. There was no enhancement of contact allergy to nickel by local pretreatment with SP, neither was there inhibition following the local pretreatment with VIP or somatostatin as might perhaps be expected from in vitro studies (Payan and Goetzl 1986). The proliferation of both human (Payan et al 1983) and murine

(Stanisz et al 1986) T-lymphocytes is stimulated by nanomolar concentrations of SP. The poliferation of T-lymphocytes in response to nickel sulphate or mercuric chloride can both be enhanced and suppresed by the same neuropeptide dependent on the concentration used (Nordlind and Mutt 1986 a, b). It appears that Spantide acts independently of mast cells since it inhibited only slightly (to 72%) the flare response to histamine and not at all the wheal (VIII).

General summary and conclusions

1. SP, CGRP, somatostatin, VIP and NPY have been demonstrated in nerve fibres in the human skin by immunocytochemistry and immunochemistry. There were regional and topographical differences in the occurrence and distribution of the neuropeptides. Skin from fingers and toes contained the highest concentrations of the neuropeptides, notably CGRP and NPY. VIP and CGRP were relatively abundant in the axilla. We found SP and CGRP to coexist in a subpopulation of nerve fibres; however, fibres containing CGRP alone were more numerous. SP/CGRP occurred in numerous free subepitelial nerve endings, VIP and NPY were fond in nerve fibres deep in the dermis, usually around blood vessels. VIP-containing fibres were also found around ducts and acini of sweat glands while NPY fibres were associated with ducts and acini of sweat glands and hair roots.

2. Certain physiological factors were found to influence neurogenic inflammation (standardized challenge with SP). Different body regions differed in the flare and wheal response to SP. The largest flare was recorded on the thorax, the largest wheal on the upper aspect of the foot. The flare decreased dramatically with increasing age and was greater during the night then during the day. Athletes displayed smaller SP evoked flare responses immediately after a competitive endurance test than at rest.

3. Mast-cell histamine plays an important role in the flare response induced by tachykinins but not in the response induced by CGRP.

4. Prostaglandin E_2 and bradykinin interfere with the neurogenic inflammation. Their effects are partly inhibited by local anesthetics and antihistamines.

5. Capsaicin (injected intradermally) seems to cause flare and wheal without the participation of mast-cell histamine. The flare was inhibited by local anesthetics, indicating the involvement of sensory fibres and axon reflexes. Repeated application of capsaicin caused desensitization to both capsaicin and bradykinin. The desensitization was reversed after about 8 weeks.

6. Fairly high concentrations of SP, VIP, CGRP and NPY were found in blister fluid from inflamed skin (many different types of disorders) indicating that neuropeptides play an important role in inflammation regardless of its primary cause.

7. Ten patients with notalgia paresthetica were successfully treated with topical capsaicin (1 x 5 in one week, 1 x 3 in 4 weeks). Five of ten patients treated with capsaicin became symptom-free, four were greatly improved but reported minor relapse. Renewed capsaicin treatment alleviated the itch also in these patients. Capsaicin sensitive sensory neurons seem to play a pathogenetic role in the development of notalgia paresthetica.

8. A tachykinin antagonist, Spantide, was shown to inhibit immediate and delayed hypersensitivity reactions in human, while it failed to influence non-immunologic inflammatory reactions. These speaks for a stimulatory role of SP in the immunologic process.

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