The use of neuropeptide agonists and antagonists will open up new approaches to the treatment of itch, pain, vasoregulation, and immunological and irritative reactions.

Neuropeptides: Their Significance in the Skin

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Summary

The skin protects the body from noxious agents in the environment. Sensory nerve fibers of the skin transmit sensations of touch, pain and itch. Neuropeptides such as substance P (SP), neurokinin A (NKA), calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase-activating peptide (PACAP) are localized in epidermal and subepidermal C fibers that transmit these sensations. Opioid peptides involved in physiological pain control are also found in the peripheral and central nervous systems. Via antidromic nerve conduction, nerve fibers in the skin participate in neurogenic inflammation. Blood vessels and sweat glands are innervated by nerve fibers that contain SP, NKA, CGRP, PACAP and vasoactive intestinal peptide (VIP), which act as vasodilators. Neuropeptide Y is a potent vasoconstrictor in cardiac and cerebral vascular beds, but acts as a vasodilator when it occurs in the skin. Some vasoactive peptides such as endothelin and bradykinin, and other vasoactive molecules such as nitric oxide, seem to have a cellular rather than neuronal origin in the skin. Some neuropeptides co-exist in the same nerve fibers and are co-released upon nerve stimulation. They may then cooperate with each other or antagonize each other. Peptide-degrading enzymes ensure rapid elimination of mobilized neuropeptides. Many cells of the skin and immunocompetent cells residing in the skin have been shown to carry receptors for neuropeptides and sometimes to store or synthesize them. Interaction between the nerve fibers and cells of the immune system affects allergic skin responses. The immunological effect of a single neuropeptide, such as CGRP or VIP, may be inhibitory at one concentration but stimulatory at another. SP and NKA are thought to participate in tissue repair by stimulating the proliferation of keratinocytes, fibroblasts and endothelial cells. This review summarizes recent findings regarding the involvement of neuropeptides in the transmission of itch and pain sensations, and in vasoregulation, immunomodulation and tissue repair. Neuropeptide-releasing drugs, neuropeptides, and neuropeptide analogues and antagonists are used as vasoregulatory agents and to alleviate pain or itch. In the future, they may be used as therapeutic agents to control tissue repair and immune response.

The skin protects the body from noxious agents in the environment. Nerve fibers and nerve-derived agents participate in every step of this defense. In the first place, nerve fibers respond to the noxious stimuli and transmit danger signals to the central nervous system. The afferent function of sensory nerves is to inform the central nervous system of the presence of harmful (nociceptive) stimuli. In 1901, Bayliss showed stimulation of primary afferent nerve fibers to evoke vasodilatation in the skin. The term "axon reflex" was introduced to describe the conjunction of afferent (orthodromic) and efferent (antidromic) conduction of impulses.[1]

The efferent function of sensory nerves is to contribute to the local defense against nociceptive stimuli. Nerve fibers in the skin provide the chemical mediators that enable blood vessels and other structures to respond to external stimuli. Vasodilatation and

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plasma extravasation initiate an inflammatory reaction in order to combat the intruders and remove them from the site of trauma. Vasoconstriction, on the other hand, helps to limit the inflammation.

It was recognized many years ago that autonomic transmission in many organs could not be completely blocked by drugs that abolish responses to acetylcholine or norepinephrine and that other substances must be involved. The term "nonadrenergic noncholinergic transmission" was coined. Immunocytochemical methods have shown that many potential transmitters may co-exist in the same neuron. Some examples of nonadrenergic noncholinergic (NANC) transmitters in the skin are substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY) and nitric oxide (NO).[2]

There is abundant evidence that neuropeptides do not occur only in neurons but also in immune cells.[3] Once the skin has been damaged, neuropeptides contribute to tissue repair. This review deals with all the above-mentioned aspects.

Neuropeptides in nociception

Nerve fibers transmitting signals that trigger the sensation of itch seem to be located subepidermally, sometimes penetrating into the epidermis.[4] Immunocytochemical methods have enabled nerve fibers to be analyzed at this level. A large proportion of the free nerve fibers located in the epidermis or subepidermis seem to contain neuropeptides such as SP, neurokinin A (NKA) and CGRP. In 1953, Lembeck first suggested SP to be a transmitter of sensory nerves, on the basis of its occurrence in the dorsal rather than in the ventral spinal roots. Injection of SP intradermally causes itch.[5]

Also, intradermal injection of NKA causes itch while CGRP does not, although both peptides will give rise to inflammatory symptoms. Repeated administration of capsaicin, the pungent agent of hot pepper, induces anesthesia and it has been used for the past hundred years in the relief of toothache. Topical administration of capsaicin stimulates sensory C fibers to release CGRP, SP and VIP.[6] Repeated administration causes depletion of these peptides, which probably explains the anesthesia. Capsaicin has been used therapeutically to suppress localized pain and itch by acting on sensory fibers.[7]

SP was put forward as a candidate neurotransmitter in sensory nerves after the finding of large amounts of SP in the dorsal horn of the spinal cord.[8] Indeed, SP is present in primary sensory nerves that have their origin in the spinal and trigeminal ganglia.[9]

There is evidence that direct synaptic sensory transmission of itch sensation is carried by SP-containing sensory nerve fibers. Administration of SP intrathecally causes violent scratching which can be eliminated by an SP antagonist.[10] Hence, itch can be produced without peripheral input. Other neuropeptides such as VIP have also been shown to mediate itch.[11,12]

Opioid peptides with opiate receptor antagonist activity (β-endorphin, enkephalin, dynorphin) have been found in both the central and the peripheral nervous systems.[13] They are believed to have many diverse actions and to be involved in the endogenous control of pain. In the skin they have been traced to Merkel cells that have a mechanosensory function.[14] The opioid peptides interact with three distinct subclasses of receptors: μ, δ, and κ. Only μ receptors are relevant to the modulation of pain and itch in the central nervous system.[15] Opioids can potentiate the histamine-evoked itch response, and itch is one of the side effects seen upon administration of morphine and other opiates. Opiates seem to elicit itch also by a central mechanism. Injection of morphine into the fourth ventricle of the brain of laboratory animals produces violent scratching.[16] Spinal and epidural injection of opiates for pain relief exacerbates itch in 50% of patients.[17] The itch elicited by opioids upon spinal injection is effectively inhibited by the opioid antagonist naloxone. Naloxone has been used to alleviate pruritus in skin diseases, but the results are contradictory and difficult to interpret.[17,18]

To sum up thus far, it seems that sensory nerve fibers of the skin transmit sensations of touch, itch and pain. Nerve fibers containing SP, NKA, CGRP or pituitary adenylate cyclase-activating peptide (PACAP) are localized in epidermal and subepidermal C fibers. Opioid peptides are also found in the peripheral and central nervous systems and participate in endogenous control of pain. Through antidromic nerve conduction, nerve fibers in the skin participate in neurogenic inflammation.

Neuropeptides in vasoregulation and neurogenic inflammation

Blood vessels in the skin operate under nervous control and under the control of locally derived vasoactive substances. Each of the two mechanisms probably exerts different effects in different skin regions. The blood vessels of the skin of the extremities are controlled by the sympathetic nervous system, while those of the head and upper thorax (the "blush area") are predominantly controlled by the parasympathetic nervous system and by circulating vasoactive agents. Several endogenous systems intervene and interact in a complex manner. The contribution of the sensory nervous system to the symptoms of inflammation has been referred to by the term "neurogenic inflammation."[19] Increased concentrations of some neuropeptides have been found in inflamed skin.[20,21]

Tachykinins: Substance P and neurokinin A

SP, consisting of 11 amino acid residues, and NKA, consisting of 10 amino acid residues, are closely related and are in fact encoded by the same gene. Differential splicing of the RNA

transcript results in the production of two mRNAs and two precursor proteins, one of which includes both peptides, the other SP only. Tachykinin receptors are of three subtypes: NK_1, NK_2 and NK_3.[22]

Although each tachykinin has some affinity for all three receptor subtypes, SP mainly activates the NK_1 receptor. In the skin, SP-immunoreactive nerve fibers are distributed as single scattered fibers just beneath the epithelium or penetrating into it. They sometimes occur as bundles of fibers in the deeper layers, and sometimes as single fibers running either close to blood vessels or freely in the connective tissue. Generally, SP fibers are few or moderate in number, except, for instance, in the skin of the fingertips, where they are numerous. SP-containing nerve fibers are also known to form a network around sweat glands and blood vessels.

Intradermal injection of SP produces flare, wheal and itch.[5] There is regional variation in the flare response, which is most prominent on the thorax and less so at more distal locations, whereas wheal is most manifest on the upper aspect of the foot.[23] The flare response diminishes with increasing age and in conditions of physical stress, and its magnitude varies with the time of day, being greater at night, whereas the wheal response appears to be independent of circadian rhythm.[23]

SP is known to release mast cell histamine *in vitro*. The depletion of histamine from dermal mast cells by compound 48/80 greatly reduces the flare response to SP, suggesting flare to be dependent upon histamine.[24] Moreover, SP-evoked flare is dependent upon an axon reflex, since pretreatment of the skin with lidocaine greatly diminishes the response.[24]

SP-evoked wheal, on the other hand, is only moderately affected by pretreatment with compound 48/80 and local anesthetics. Thus, the wheal response seems to be largely due to a direct local effect of the injected SP.[24] SP has strong vasoactive effects, causing vasodilatation and profound hypotension.

NKA was shown to activate NK_2 receptors.[18] It was found to co-exist with SP in primary sensory neurons. NKA exerts effects similar to those of SP, but is less potent.[25] NKA is less potent than SP in inducing flare and itch in human skin. The poor itch and flare response to NKA may reflect its weak histamine-releasing ability. Nonetheless, the flare evoked by NKA is reduced by pretreatment with compound 48/80 (by about 50%) and is virtually abolished by the local anesthetic lidocaine.[26] It is therefore conceivable that, like SP, NKA acts via sensory fibers and mast cells. The wheal-inducing capacity of NKA is similar to that of SP.[26]

Calcitonin gene-related peptide

CGRP, a 37-amino acid peptide, is encoded by the same gene that codes for calcitonin. Differential splicing allows cells to produce either pro-calcitonin (expressed in thyroid C cells) or pro-CGRP (expressed in many neurons and in thyroid C cells) from the same gene.[27] There is pharmacological evidence supporting the existence of several CGRP receptors.[28]

CGRP co-exists with SP and NKA in sensory nerve cell bodies.[9] CGRP is the most abundant of all neuropeptides in human skin and is often found to be co-localized with SP.[21]

In human skin, CGRP induces slowly developing local reddening (duration of several hours).[26] The erythema does not seem to be mediated by mast cell histamine or by C fiber tachykinins, as it is not suppressed by pretreatment with either mepyramine (an H_1 receptor antagonist) or compound 48/80 (a mast cell histamine liberator) or by lidocaine treatment. The long-lasting and widespread vascular effects of CGRP may reflect a gradual diffusion of the peptide, which conceivably exerts direct effects on blood vessels.

Although SP and CGRP may be released concomitantly from the same C fibers, they differ from each other in their duration of action, CGRP being more long acting. In the immediate phase of inflammation, it is likely that SP and histamine play crucial parts in the development of flare and wheal. The CGRP-evoked erythema continues to develop as the effects of SP and histamine subside. Hence, CGRP may be expected to play a greater part in subsequent stages of inflammation.

Clinical inflammatory reactions are usually more prolonged than the rapidly developing flare and wheal reactions that can be induced experimentally, and hence resemble the effects of CGRP more than those of SP. When injected concomitantly with CGRP, SP is found to shorten the duration of the reddening induced by CGRP.[29] NKA does not shorten the duration of the CGRP response.[30] Local depletion of mast cell histamine by treatment with compound 48/80 causes SP to lose its ability to shorten the duration of CGRP-evoked erythema. These observations suggest an SP-evoked release of proteolytic enzymes from mast cells to cause accelerated degradation of CGRP.[29]

Vasoactive intestinal peptide

VIP consists of 28 amino acids and belongs to the same family of peptides as secretin and glucagon. Recent reports have identified two distinct VIP receptors, VIP_1 and VIP_2, which belong to a family of G-protein–linked receptors.[31]

VIP was so named because of its hypotensive actions and its potent vasodilatory activity in the splanchnic, coronary and pulmonary vascular beds, where it is present in nerve structures.[32] VIP and acetylcholine co-exist in postganglionic parasympathetic nerve fibers. In the skin, VIP-containing nerve fibers are perivascular and distributed preferentially in the deeper parts of the dermis.[21] VIP relaxes the smooth muscle of blood vessels and has been suggested to be involved in the regulation of vasodilatation and

sweat gland function. It induces histamine-dependent flare and wheal when injected intradermally.[33] VIP is found at high concentrations in fluid from spontaneous blisters in inflamed skin.[20]

Pituitary adenylate cyclase-activating peptide

PACAP exists in two forms, one with 38 amino acid residues and the other with 27. Both peptides arise from a common precursor and belong to the same peptide family as VIP.[34] Several receptors interact with both PACAP and VIP; however, the PACAP type I receptor seems to be highly specific for PACAP.[31]

PACAP is a powerful activator of adenylate cyclase and seems to regulate hormone production and secretion in the pituitary gland, thyroid gland, gastrointestinal tract and pancreas, as well as endothelium-independent vasodilatation. In human skin PACAP induces a slowly developing erythema resembling that induced by CGRP (unpublished observations). In the skin of the rat, PACAP-positive nerve fibers have a distribution similar to those of VIP and CGRP.

It appears that PACAP is a constituent of C fiber neurons (together with SP and CGRP) and of parasympathetic neurons and fibers (together with VIP and sometimes with NPY). It stimulates the secretion of NPY and catecholamines.[35] It can thus be considered a sensory neuropeptide and a vasomodulator.

Neuropeptide Y

NPY consists of 36 amino acid residues and has been found in neuronal elements in both brain and periphery: respiratory tract, urogenital tract, heart, gut and pancreas. Numerous NPY receptors have been identified. The binding properties of two of the NPY receptors (Y_1 and Y_2) have been described in some detail.[36]

NPY is known to co-exist with norepinephrine in noradrenergic sympathetic fibers.[37] In these vascular beds, the vasoconstrictor response to exogenous norepinephrine is greatly enhanced by NPY.[38] In the skin, NPY fibers occur mainly in the deeper portions of the dermis, mostly around blood vessels and occasionally in association with sweat glands and hair roots.[21] We have found concomitant intracutaneous injection of NPY and norepinephrine in equimolar concentrations to be associated with less bleaching than norepinephrine given alone (unpublished observations), suggesting that in the skin the two substances do not act synergistically. The flare induced by intracutaneous injection of NPY is probably mediated by histamine, as NPY releases histamine from cutaneous mast cells.[39] NPY is found in fluid from spontaneous blisters in inflamed skin.[21] NPY occurs together with norepinephrine in sympathetic fibers and together with VIP in a subpopulation of parasympathetic fibers. If NPY participates in the neurogenic inflammation in the skin, it probably does so as a transmitter released from sympathetic nerve fibers together with norepinephrine.

Somatostatin

Somatostatin consists of 14 or 28 amino acid residues. Specific receptors for somatostatin are expressed on normal and activated monocytes and on lymphocytes, but not on granulocytes or red blood cells.[40] In normal human skin, the concentration of somatostatin is low.[21] The main origin of somatostatin in human skin is probably cellular and not neuronal; it has been found in Langerhans cells.[41]

Intracutaneous injection of somatostatin induces flare and wheal that are fainter than those induced by SP. The two peptides given in equimolar doses act synergistically, producing a greater flare response together than either of them does alone. Somatostatin is found in blister fluid from inflamed skin, a finding which suggests that it functions as an inflammatory mediator.[21]

Nitric oxide

NO, a short-lived free radical,[42] appears to be involved in diverse types of neuronal transmission. One of its functions is as a second messenger acting within the cell or neuron in which it is produced. Another pathway involves cell-to-cell signaling, possibly by interacting with *N*-methyl-D-aspartate (NMDA)-type glutamate receptors.[43] The most important stimulus controlling endothelial NO synthesis under physiological conditions is probably mechanical, namely, the pulses of the blood flow. Endothelial cells possess receptors for vasodilators including acetylcholine, SP, CGRP, adenosine diphosphate and bradykinin. Occupation of these receptors stimulates the formation of NO.[44] Following its induction, the activity of NO synthase (NOS) first increases and then decreases again over a period of hours or days, one factor of importance in the activity decline being irreversible feedback inhibition of NOS by NO itself.

NO has also been implicated as a neuromediator in the peripheral nervous system, acting as a NANC transmitter. Neuronal NO is produced on demand in the neuronal cytoplasm and diffuses freely across cell membranes.[42] There have been reports of the co-existence of NOS and neuropeptides in both C fibers and parasympathetic fibers.[42] NO has been shown to release vasodilator quantities of CGRP from capsaicin-sensitive nerves in rabbit skin.[45]

Endothelins

The endothelin (ET) system comprises three peptides, ET-1, -2 and -3, each consisting of 21 amino acid residues. ET-1, which is synthesized by blood vessels, occurs in both endothelial and smooth muscle cells.[46] Two mammalian ET receptor isoforms have been cloned: ET_A receptors, which mediate contraction of vascular smooth muscle cells; and ET_B receptors of vascular endothelial cells, which exert vasodilatory effects via release of NO and prostacyclin.[47] The endothelins constitute a family of vasoconstrictor peptides that may cause vascular hypertrophy and hypertension.[47]

Immunostaining for ET-1 has been observed in all cutaneous blood vessels

of normal human skin, including those of the papillae, hair follicles and sweat glands in the dermis.[48] Intradermal injection of ET-1 into human skin has been shown to reduce cutaneous blood flow.[49] Because of its prolonged vasoconstrictor effect, it has been suggested that ET-1 may modulate the vasospasmic episodes involved in Raynaud's phenomenon.[50]

Bradykinin

Bradykinin consists of nine amino acid residues and belongs to the kinin family.[51] There is more than one type of bradykinin receptor—B_1 and B_2 subtypes—although the effects mediated by these receptors are very similar. The precursors of bradykinin, kallikreins and prekallikreins, are released from exocrine glands (pancreas, kidney, intestine, salivary glands and sweat glands) upon stimulation by autonomic nerves.[51] Bradykinin exists in the circulation at very low concentrations. It is a powerful stimulant of C fibers and induces triple response upon intradermal injection.[52] The flare response reflects axon reflexes and the release of mast cell histamine.[53]

To sum up thus far, NANC nerve transmission occurs in autonomic nerve fibers around blood vessels and sweat glands. These are innervated by peptidergic nerve fibers containing neuropeptides—SP, NKA, CGRP, PACAP and VIP—that act as vasodilators. NPY is a potent vasoconstrictor in cardiac and cerebral vascular beds, but acts as a vasodilator when it occurs in the skin. Some vasoactive peptides such as endothelin, somatostatin and bradykinin, and other vasoactive molecules such as NO, are of cellular origin. The vascular effects of neuropeptides are dose dependent. Some neuropeptides co-exist with each other in the same nerve fibers and are co-released upon nerve stimulation. They may then cooperate with each other, antagonize each other or inactivate each other.

Neuropeptides in cutaneous hypersensitivity reactions

There is growing evidence of cross-talk between the nervous and the immune systems. In the skin this communication is made possible by the presence of free nerve endings close to immunocompetent cells. Neuropeptides can activate a number of target cells, including keratinocytes, Langerhans cells, mast cells, endothelial cells, granulocytes, eosinophils, macrophages and lymphocytes. These cells have been shown to have receptors for neuropeptides and in some cases even to synthesize and release neuropeptides and NO.[54–59]

Capsaicin depletes the skin of C fiber neuropeptides upon repeated topical application.[53] Capsaicin pretreatment has been shown to abolish the flare response to prick tests in human subjects.[60] It also suppresses the symptoms of cold and heat urticaria.[61]

We have explored the involvement of SP in immunologic contact urticaria, using spantide, an antagonist to SP.[62] A single dose (800 pmol) of spantide was used prior to induction of immunologic contact urticaria (scratch test in patients sensitized to fish, egg and flour). Of 11 atopic patients, seven manifested reduced immunologic contact urticaria reactions after pretreatment with spantide.

Immunologic contact urticaria is due to specific IgE-mediated degranulation of mast cells.[63] Mobilized histamine will excite adjacent C fibers until all ramifications of the nerve are engaged in axon reflexes. When additional nerves become engaged, the contact urticaria becomes generalized. Another hypothetical route for cross-talk between different organs, elicited by percutaneous absorption of an allergen, would be a nervous pathway initiated by C fiber-dependent reflexes originating in the skin. Such a nerve-mediated reflex may result in efferent nerve stimulation in a number of organs. SP may induce bronchoconstriction, vasodilatation of blood vessels (flushing and tachycardia) and gastrointestinal symptoms like diarrhea, mimicking generalized urticaria. Urticaria is supposed to be due to the humoral action of released histamine. Apparently nervous mechanisms are involved, which may explain the role of psychological factors triggering urticaria.

CGRP has been shown to attenuate histamine-induced wheal, indicating that it has a down-regulating role in immediate-type reactions.[64] SP and CGRP, when co-released, seem to have opposite effects in immediate type reactions. A role for NO in immediate immunologic reactions has been suggested by the finding that NO can be synthesized in response to antigen provocation and after histamine administration in allergic conjunctivitis.[65]

Delayed-type immunologic reactions are mediated by Langerhans cells.[66] We induced contact dermatitis in guinea pigs by means of dinitrochlorobenzene (DNCB). One group pretreated with capsaicin received the pretreatment prior to sensitization, others prior to challenge with DNCB. In both cases capsaicin pretreatment enhanced delayed-type allergic reactions,[67] results consistent with those obtained by Girolomoni and colleagues in mice.[68] We conducted similar experiments with contact allergen (in patients with known contact allergy) and tuberculin reaction, with analogous results, namely, capsaicin pretreatment enhanced delayed-type immunologic reactions.[69] Capsaicin pretreatment causes collective depletion of most neuropeptides from the skin, the net result being the opposite of the effects of the most effective neuropeptides. Girolomoni and collaborators, who investigated the effect of VIP on contact sensitivity in mice, found VIP to inhibit this reaction.[70] Their results were confirmed by Bondesson and co-workers in human skin.[71]

We performed experiments with spantide, a substance P antagonist, prior to eliciting allergic contact dermatitis, and observed inhibition of contact dermatitis and tuberculin reactions.[67] This supports the view that SP augments delayed immunologic reactions. CGRP, on the other hand, was

found to suppress delayed hypersensitivity reactions and to inhibit the antigen presenting capacity of the Langerhans cell.[72] The CGRP antagonist CGRP-(8–37) exerted dual effects on allergic contact dermatitis, potentiating it at high doses and inhibiting it at low doses (unpublished observations). An analogous dual effect of a peptide has been reported for VIP.[73]

There is evidence that NO is involved in the formation of sunburn erythema, as ultraviolet B activates neuronal NOS in keratinocytes and endothelial cells.[74] Even nonimmunologic reactions can be affected by nervous mechanisms. We found that 0.1 µg L-NAME, an inhibitor of NOS, inhibited nonallergic reaction to benzalkonium chloride in human skin (unpublished observations).

To sum up thus far, the interaction between neuropeptides and cells of the immune system affects allergic and irritative skin responses. Capsaicin depletes C fibers of their neurotransmitters, the end result being a potentiation of the delayed allergic immunologic reaction and reduction of the immediate immunologic reaction. A selective release of neuropeptides from different subsets of sensory nerve endings may initiate and modulate the symptoms of contact dermatitis. The immunological effect of a single neuropeptide such as CGRP or VIP may be inhibitory at one concentration but stimulatory at another.

Neuropeptides in tissue repair

Nerve fibers may contribute to tissue reconstruction by releasing trophic factors or, alternatively, neuronal messengers may stimulate the local production of such factors.[75] There is increasing evidence from *in vitro* experiments that several neuropeptides stimulate cellular proliferation and vascularization. SP and NKA, which co-exist in C fibers, stimulate the growth of cultured fibroblasts[76] and endothelial cells.[77] SP has been shown to up-regulate the synthesis of interleukin-1γ, interleukin-6 and transforming growth factor-α in keratinocytes and to augment interferon γ-mediated induction of keratinocyte ICAM-1 expression.[78,79] CGRP stimulates the formation of cAMP in keratinocytes and their proliferation.[80] CGRP has also been shown to stimulate proliferation of human endothelial cells.[81] VIP has been shown to act as a growth factor for keratinocytes[82] and as a modulator of their migration.[83] Hence, neuropeptides may stimulate proliferation of cutaneous epithelial, vascular and connective tissue.

The concentration of SP and the density of SP-immunoreactive nerve fibers in the guinea pig skin increase during wound healing *in vivo*.[84] SP and NO have been shown to stimulate keratinocyte proliferation in wound healing after ultraviolet light-induced damage of the rat skin.[85] Systemic capsaicin pretreatment of newborn rats has been reported to result in lifelong impairment of certain sensory functions.[86] Such pretreatment induces "spontaneous" cutaneous lesions in the head and neck area.[87]

In a recent study, we examined the part played by local nerve fibers (C fibers in particular) in wound healing in adult rats.[88] Rats were treated with capsaicin and/or unilateral surgical destruction of the sciatic nerve to reduce the number of nerve fibers in the skin prior to the infliction of wounds in the thigh area. Rats treated with capsaicin developed "spontaneous" ulcers in the neck area. In one group of rats, both capsaicin treatment and surgical denervation were combined in an attempt to eliminate or incapacitate all sensory nerve fibers. Capsaicin treatment and/or sciatic nerve sectioning reduced the density of CGRP immunoreactive nerve fibers by 70%. The nerve fibers regenerated partly by collateral sprouting during the wound healing process. However, neither the reduction in nerve fiber density following sciatic nerve sectioning nor the impairment of sensory nerve functional capacity following capsaicin treatment affected the healing rate of inflicted wounds, suggesting that the remaining innervation suffices to ensure conditions for normal tissue repair.[88] Also, the spontaneous ulcers in the neck region healed. This is in agreement with the finding that newborn rats treated with capsaicin and developing ulcers in the skin of the neck still can produce neurogenic inflammation.[87]

This indicates that sensory nerves operate physiologically at a low level, and only a part of the existing nerve fibers is enough to maintain tissue integrity.[87] Reinnervation of the wounded skin by collateral sprouting has been shown to be dependent on nerve growth factor (NGF).[89] NGF has been shown to reverse the decrease of neuropeptide content caused by capsaicin, and even to restore the peripheral function of primary afferent neurons.[90] In a preliminary study, Spevak and colleagues found wounds in rat skin to heal faster when treated with SP.[91]

In summary, chemical or surgical denervation knocks out a part of, but not all, peripheral innervation. The remainder suffices to ensure normal wound healing. Exogenous neuropeptides and proteins such as NGF promote healing in damaged skin.

Peptide-modulating strategies, therapeutic perspectives

As mentioned above, neuropeptides may exert different effects depending on their concentration. The concentration in turn will depend on synthesis, release and degradation. Inflammation causing chronic activation of sensory nerves evokes enhanced neuropeptide synthesis in experimental arthritis and after aerosol immunization of rats.[92–94]

Even in the skin neuropeptides will be synthesized on demand in inflammatory reactions, owing to a positive feedback mechanism. Different neuropeptides may cooperate or antagonize each other. Somatostatin has been shown to inhibit SP release.[95]

TABLE I: NEUROPEPTIDES IN THE SKIN

PEPTIDE, STRUCTURE	RECEPTORS	ANTAGONISTS	DEGRADING ENZYMES	CUTANEOUS DISTRIBUTION	VASCULAR FUNCTION	HISTAMINE RELEASE	EFFECT IN CONTACT DERMATITIS
SP, 11 aa	NK_1 (NK_2, NK_3)	Spantide I, II, III, SP-(4–11), etc.	Tryptase, chymase, NEP, ACE	Free nerve endings in dermis and epidermis. Around blood vessels	Vasdoilation, flare and wheal	Yes	Potentiates immediate and delayed hypersensitivity
NKA, 10 aa	NK_2 (NK_1, NK_3)	Multiple, ([β-Ala^8)-NKA-(4–10)	Tryptase, chymase, NEP	Free nerve endings in dermis and epidermis. Around blood vessels	Vasodilation, flare and wheal	Yes	?
Bradykinin, 9 aa	B_1, B_2	Multiple, potent: (1-adamantane-carbonyl-D-Arg^0, Hyp^3,β-(2-thienyl)-Ala^{5,8}-D-Phe^7)-bradykinin	ACE, NEP	Circulating blood	Vasodilation, flare and wheal	Yes	Potentiates immediate hypersensitivity
CGRP, 37 aa	CGRP 1R, CGRP 2R	CGRP-(8–37)	Tryptase, chymase, NEP	Free nerve endings in dermis and epidermis. Around blood vessels and hair follicles	Vasodilation, persisting erythema	No	Inhibits delayed hypersensitivity
VIP, 28 aa	VIP_1, VIP_2, PACAP type I	VIP-(6–28)	Tryptase, chymase	Around blood vessels, hair follicles and sweat glands	Vasodilation, flare and wheal	Yes	?
PACAP, 27 aa, 38 aa	PACAP type I, VIP_1, VIP_2	PACAP-(6–27), PACAP-(6–38)	?	Free nerve endings in dermis and epidermis. Around blood vessels and hair follicles	Vasodilation, persisting erythema	?	?
NPY, 36 aa	Y_1, Y_2, Y_4, Y_5, Y_6	NPY-(27–36)	?	Free nerve endings around blood vessels, Langerhans cells?	Vasodilation, flare and wheal	?	?
Somatostatin, 14 aa, 18 aa	sst_1, sst_2, sst_3, sst_4 sst_5	*cyclo*-Somatostatin	NEP	Merkel cells (?)	Vasodilation, flare and wheal	Yes	?
Endothelins, 38 aa	ET_A, ET_B	Endothelin-1, ET-(11–21), ET-(16–21) ET-(19–37)	ECE	Nerve fibers around blood vessels. Epithelial and endothelial cells	Vasodilation, pallor	No	?
Nitric oxide	Glutamate receptors: ionotropic and metabotropic	NOS inhibitors: L-NMMA, L-NMA, L-NMAA, L-NA, L-NNA, L-NAME	Short-lived molecule	Nerve fibers around blood vessels. Epithelial and endothelial cells	Vasodilation	?	Contributes to sunburn erythema and to irritant contact dermatitis

Abbreviations: aa, amino acids; ACE, angiotensin-converting enzyme; ECE: endothelin-converting enzyme; NEP, neutral endopeptidase.

Degradation of peptides may be caused by mast cell degranulation. Thus, the enzymes tryptase and chymase, released from mast cells, seem to contribute to the degradation of SP, CGRP and VIP, thereby limiting their duration of action.[29,96] Peptidases are also present in the airways, gastrointestinal tract, brain and lymph nodes. Neutral endopeptidase (NEP) is expressed on the cell surface of multiple cell types including neurons, epithelial cells, endothelial cells and smooth muscle cells.[97] The cell surface NEP is anchored to the plasma membrane in the vicinity of neuropeptide receptors.[98] The degradation of neuropeptides will restrict the number of intact peptide molecules that are available to interact with receptors and initiate signaling. NEP inhibitors such as phosphoramidon or thiorphan can be used *in vivo* or *in vitro* to suppress peptide degradation.[97,98] This degradation is relatively nonspecific and universal compared to the very selective inactivation processes regulating the degradation of classic neurotransmitters. Thus phosphoramidon prevents degradation of both tachykinins and CGRP.[99]

Another enzyme that degrades substance P and bradykinin is angiotensin-converting enzyme (ACE). Therefore, enzyme inhibitors like captopril attenuate SP metabolism and may induce skin inflammation and urticaria.[100]

Cellular responses to neuropeptides may also depend on chemical modification of the activated receptor and on levels of receptor expression.[101]

Chemically, capsaicin is *trans*-8-methyl-*N*-vanillyl-6-nonenamide. It was introduced to pharmacology by Jancso and collaborators in the 1960s in order to elucidate the function of sensory nerve endings in neurogenic inflammation. Capsaicin produces irreversible damage to C fibers in rats only if administered neonatally. Systemic administration of capsaicin to adult animals may cause transient depletion of SP from small sensory neurons and their terminals (C fibers), resulting in bronchospasm and skin irritation, but the nerves do not degenerate.[6] Applied locally to the sciatic nerve, capsaicin blocks axonal transport of peptides. The effect is specific to sensory C fibers and is not due to local damage of those fibers, since they still are capable of conducting action potentials. A single application of capsaicin causes flare and wheal. Repeated topical administration of capsaicin in human skin induces tachyphylaxis and abolishes the axon reflex to a number of noxious agents. The axon reflex recovers after eight weeks, indicating that there are no chronic effects.[53]

Capsaicin treatment reduces pain and itch and has been used therapeutically in dermatology.[7] Ovanil is a capsaicin analogue that is a tenfold more potent vasodilator than capsaicin itself.[102] High concentrations of SP, VIP, CGRP, NPY and somatostatin are found in spontaneous bullae from pathogenetically different inflammatory diseases.[20,21] This emphasizes the part played by sensory and autonomic nerves in the inflammatory process.

Capsaicin pretreatment results in collective depletion of most neuropeptides from the skin, the net result being the opposite of the effects of the most effective neuropeptides. NGF has been shown to reverse the decrease of neuropeptide content caused by capsaicin and even to restore the peripheral function of primary afferent neurons.[90] NGF has been reported to up-regulate the expression of SP and CGRP and to modulate their synthesis in sensory neurons.[103] The net result is cooperation between released neuropeptides.

The discovery of the existence of multiple receptors that mediate the actions of neuropeptides has enriched the field. Following the development of potent receptor-selective antagonists, it has become possible to precisely estimate the importance of neuropeptides as mediators of different physiological and pathophysiological events. Powerful and selective neuropeptide receptor antagonists will serve as useful tools to shed more light on involvement of different neuropeptides in various pathological conditions. Endothelin receptor antagonists have been used to treat hypertension.[46] NOS inhibitors (such as L-NMMA) have been used in the treatment of hypotensive shock.[43]

The special features of the circulation of the skin make it important in the regulation of body temperature. In some pathological conditions peptidergic drugs may be useful. CGRP has been given intravenously to patients with Raynaud's phenomenon in order to cause vasodilatation.[104]

In summary, the nervous system interferes with many physiological functions of the skin (Table I). The use of neuropeptide agonists and antagonists will open up new approaches to the treatment of itch, pain, vasoregulation, immunological and irritative reactions. These processes can be modified by administration of augmenting agents such as NGF and neuropeptide-depleting agents such as capsaicin and its analogues, as well as neuropeptide- degrading agents such as NEP. Neuropeptides, and their agonists and antagonists, will probably be used therapeutically in the future.

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