

Neuropeptides and the pathogenesis of allergy

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Following the discovery of histamine at the turn of this century, Dale and his associates were quick to note the parallel between the effects of histamine in animals and the manifestations of an anaphylactic reaction. The demonstration, that during an anaphylactic response in tissues histamine was released, was made at about the same time that Lewis, in his classical studies, pointed out the similarity between the responses to intradermal histamine injection and the triple response to injury. Once it was established that mast cells were the tissue stores of histamine and that these cells bound anaphylactic or reaginic antibody, studies of allergy focused on the mast cell. For several decades now, research has been directed at understanding how the antigen-IgE-mediated stimulation of mast cells is involved in the pathogenesis of allergic disease. There are now many pieces of evidence which have brought into question the importance of the antigen-IgE-mediated mast cell response in the pathogenesis of allergy. The demonstration that cells other than mast cells bear IgE receptors; the poor correlation between the inhibitory effect of cromoglycate-like drugs on mast cells and their anti-allergic properties; the heterogeneity of mast cells and basophil leucocytes are just some of the experimental observations which cast doubt on the importance of antigen-IgE-mediated mast cell responses in the pathogenesis of allergy. It is perhaps time to look again at the mast cell and the alternative ways of stimulating it, and the

purpose of this review is to look at two questions which appear to be related: is there a communication between mast cells and the nervous system and are neurotransmitters involved in the pathogenesis of allergy and inflammation?

To examine the hypothesis that the nervous system has a role in the pathogenesis of inflammatory and allergic reactions, certain questions have to be addressed. 1) What is the nature of the nerves involved? 2) How do inflammatory stimuli recruit such nerves? 3) What is the identity of the neurotransmitter which generates the inflammation? 4) What is the nature of the target cell for the neurotransmitter and how is the target cell involved in the generation of inflammation?

Do sensory nerves have an effector function in inflammation

The initial observation that vasodilator fibres are present in peripheral nerves was made by Golz (25) in 1874 and this was extended by Striker (67) who showed that such fibres left the spinal cord in the dorsal roots. Bayliss (2), critical of Stricker's experiments, went on to show that vasodilator fibres were indeed present in peripheral sensory nerves so that when such nerves were stimulated antidromically, blood vessels, largely in the skin, dilated. The possibility that the vasodilator fibres were spinal efferents was excluded by showing that 14 days

after section of the nerve between the spinal cord and the dorsal root ganglion the vasodilator response to antidromic stimulation of the peripheral part of the nerve was not lost. In contrast, section of the nerve distal to the dorsal root ganglion caused the sensory fibres to degenerate and the vasodilator response to antidromic stimulation was lost. Bayliss was also able to show that sympathetic nerves were not involved in the peripheral vasodilatation caused by stimulation of the sensory nerve in the antidromic mode. The observations of Bayliss together with those of Langley (42), who proposed the concept of an axon-reflex, led to the development of the hypothesis that sensory nerves could have a peripheral effector function by means of impulse spread through antidromic transmission in an axon-reflex arrangement. Lewis (45) developed this hypothesis in skin and showed that the flare or vasodilatation, which spreads for several centimeters from the point of injury in the skin and is part of the "triple response" to injury, is mediated by a neurogenic mechanism probably involving axon-reflexes in sensory nerves. Further work involving the measurement of conduction velocities in nerves demonstrated that the fibres carrying impulses which mediate peripheral vasodilatation are unmyelinated C fibres originating in the dorsal root ganglion (32).

When mustard oil is applied to the skin, an acute inflammation is produced and this inflammation is prevented if sensory nerves are transected distal to the dorsal root ganglion and allowed to degenerate (37). Section of the nerves proximal to the ganglion resulted in no degeneration of the peripheral process of the nerve and no loss of the inflammatory response to mustard oil. It was also shown that local anaesthetics could prevent the inflammatory response to mustard oil. In addition to this evidence showing that chemically induced inflammation depended on an intact sensory nerve supply to the area stimulated, it has been shown that stimulation, antidromically, of peripheral nerves results not only in vasodilatation but also in increased vascular permeability and plasma extravasation (37). Particles of colloidal silver introduced into the circulation

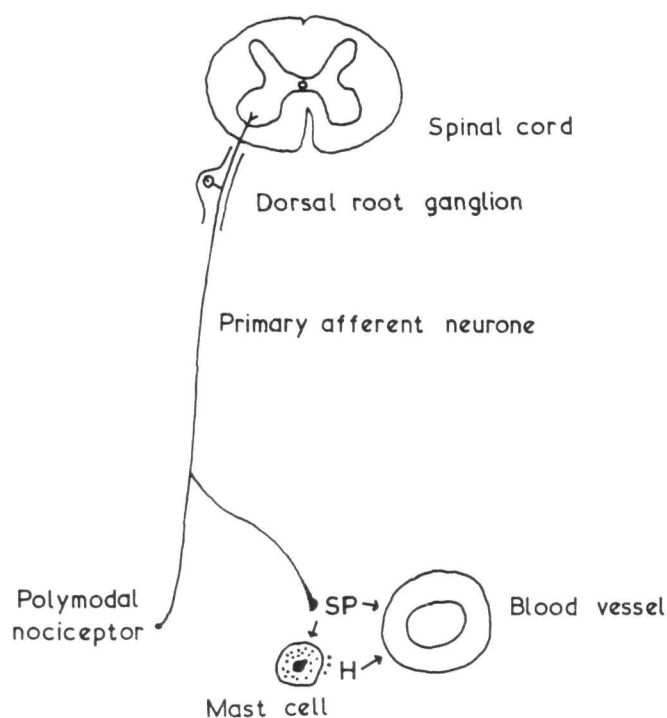
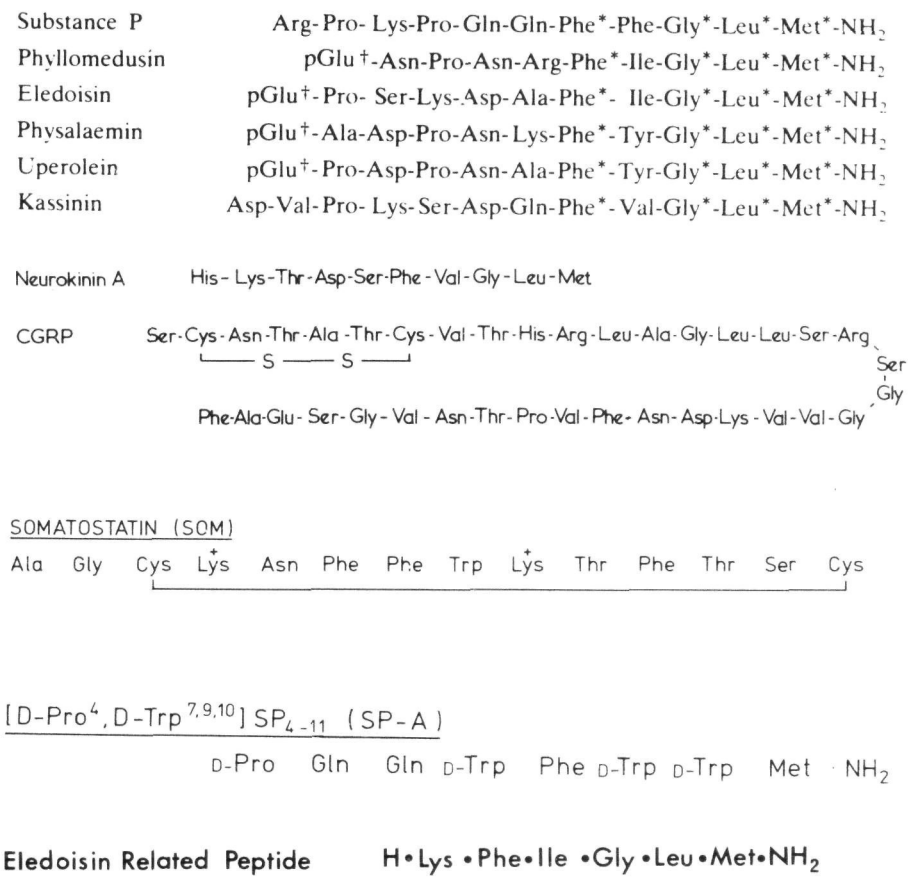


Fig. 1. Axon-reflex model of neurogenic inflammation. Stimulation of polymodal nociceptors by heat, firm pressure, or some chemicals, sets up impulse transmission in the primary afferent neurone towards the spinal cord but such impulses also pass antidromically into other branches of the neurone to cause release of substance P (SP) or other neuropeptides. The neuropeptides then mediate the effects of inflammation either directly or indirectly by releasing mast cells.

become fixed to vascular endothelium upon peripheral nerve stimulation and such particles are then phagocytosed by tissue macrophages.

Whilst nerve stimulation can produce several components of the inflammatory response and some types of inflammatory stimuli require an intact sensory nervous system for the manifestations of the inflammatory response, there is evidence that not all inflammatory stimuli invoke a neurogenic mechanism (16, 37).

Returning to the C fibres and their involvement in inflammation, it has been shown that in the periphery such fibres may be associated with polymodal nociceptors and Celander & Folkow (9) have suggested an arrangement in which the C fibre branches in the periphery: one branch is associated with a polymodal nociceptor, the input, and the other branch forms a neuroeffector junction with some target cell (Fig. 1). Direct recording from human C



the radioimmunoassay of substance P levels in biological fluids and tissues.

Substance P has been shown to be present in the small, unmyelinated fibres of sensory nerves both in the central and the peripheral endings (34). In fact, from the site of synthesis of the peptide in the cell bodies lying in the dorsal root ganglion, the rate of transport is greater by an order of magnitude towards the peripheral endings than towards the central endings in the spinal cord (39). Apart from peripheral sensory nerves, substance P has been localized in fibres of the vagus where it is present in up to 10 % of vagal fibres (23) and also in the trigeminal nerve (13) and the nerve supplying the pars flaccida (74) of the tympanic membrane. Substance P is, however, not the only peptide to be found in sensory nerves. Somatostatin (33), neurokinin A (substance K) (52, 53) and calcitonin gene-related peptide (CGRP) (59) have also been localized in sensory nerves (Fig. 2). Neurokinin A is a decapeptide derived from a larger precursor called neuropeptide K which is itself part of a long pretachykinin from which substance P is also derived. It seems likely that substance P and neurokinin A co-exist in sensory nerves within the same C fibres. CGRP, containing 37 amino acids is produced from a mRNA transcribed from the calcitonin gene but expressed, not in thyroid C cells, but in neural tissue (77). There is evidence that CGRP co-exists with substance P in primary afferent neurones (18). On the other hand, somatostatin appears to be located in sensory neurones different from those that contain substance P (33).

In addition to localization of neuropeptides within sensory neurones, it has been shown that antidromic stimulation of these neurones results in the release of substance P (7, 73).

Thus, neuropeptides such as substance P, neurokinin A and CGRP have been localized in the nerves which are involved in axon-reflex, neurogenic inflammation and stimulation of such nerves results in release of neuropeptides.

Inflammatory actions of neuropeptides. To examine the question of whether neuropeptides are mediators of inflammation, three principal experimental approaches have been employed.

1) The effect of applying neuropeptides to tissues, 2) the effects on inflammatory responses of depleting nerves of their neuropeptide content with the drug capsaicin, and 3) the effect on inflammatory reactions of neuropeptide antagonists.

Capsaicin. Capsaicin (trans-8-methyl-N-vanillyl-6-noneamide) has a mechanism of action which is not completely understood but it causes the initial release of and the depletion of the content of substance P and other neuropeptides from some primary afferent neurones (63, 68). Capsaicin also inhibits the axoplasmic transport of neuropeptides in these nerves (24). Application of capsaicin to skin produces an acute inflammatory response, apparently by releasing neuropeptides since prior denervation of the area of skin abolishes the inflammatory response to capsaicin (37). Repeated application of capsaicin reduces the neuropeptide content of the neurones and the inflammatory response attenuates at the same time so that ultimately there is complete desensitization to the inflammatory effects of capsaicin and, importantly, to other inflammatory agents. Furthermore, in skin desensitized by repeated application of capsaicin and depletion from nerves of neuropeptides, there is no inflammatory response to thermal injury or to antidromic nerve stimulation (8, 37). It is important to point out that capsaicin has a selective action on neuropeptide-containing C fibres linked to the polymodal nociceptors described above since after application of capsaicin there is not complete anaesthesia and continued response to pressure and temperature changes occurs (27, 36).

In human skin, the application of capsaicin induces an acute inflammatory response which attenuates with repeated application until complete desensitization has occurred. Injection of histamine into human skin desensitized to capsaicin abolishes the flare but not the wheal (Fig. 3), indicating that the flare requires intact neuropeptide-containing nerves for its generation (3, 20). Also, desensitization of skin to capsaicin in patients with cold urticaria leads to an attenuation of the urticarial response, again indicating the involvement of neuropeptide

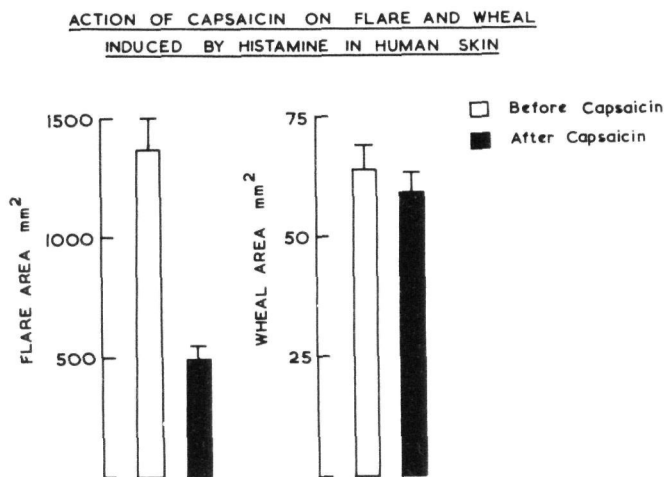


Fig. 3. Action of capsaicin on flare and wheal responses to histamine in human skin. Skin was lightly abraded and painted with a solution of capsaicin (1% in ethanol) 2 to 3 times daily for 4 days. After such pretreatment histamine (675 p-mole) was injected intradermally and wheal and flare responses measured. Responses to histamine are shown in skin before treatment with capsaicin (□) and after such treatment (■) ($n = 7$).

release from nerves in the pathogenesis of cold urticaria (70). In rat skin, capsaicin pretreatment has been shown to prevent heat-induced oedema (61).

In lung, capsaicin increases the vascular permeability of airways mucosa and this response to capsaicin, like those in skin, desensitizes with repeated application. Depletion of neuropeptides from the vagus by means of capsaicin partially prevents anaphylactic bronchoconstriction and also prevents increases in mucosal vascular permeability caused by cigarette smoke (48, 62). Capsaicin itself induces an atropine-resistant bronchoconstriction which exhibits tachyphylaxis (47).

Thus, in both lung and skin, experiments with capsaicin indicate that neuropeptide release causes inflammation and bronchoconstriction, while depletion of neuropeptides from these tissues attenuates or prevents inflammatory changes and bronchoconstriction induced by nerve stimulation and by chemical or thermal stimuli.

Effects of substance P. Injection of substance P intradermally into human skin produces a flare and wheal reaction comparable to that seen

following a similar injection of histamine (28). However, substance P is about 100 times more potent on a molar basis than histamine in producing wheal and flare reactions (20). Comparison of other tachykinins with substance P in respect of their ability to produce flare and wheal indicates that the mechanism of production of wheal or plasma extravasation differs from the mechanism by which flare or vasodilation is produced (20). Physalaemin, for example, is about twice as active as substance P in producing wheal in human skin whereas it fails to produce a flare. Eledoisin-related peptide (ERP) produces flare and wheal but has only a tenth of the activity of substance P (Fig. 4). Structure-activity studies of a number of related peptides have shown that the ability to produce vasodilatation or flare depends upon the presence of N-terminal basic amino acids such as the arginine and lysine residues of substance P or the lysine residue of ERP (Fig. 2). In contrast, the wheal-producing action of the peptides is a property of the C-terminal sequence of the peptide. Peptides like physalaemin or the octapeptide fragment of substance P (SP_{4-11}) which have C-terminal homology with substance P but lack N-terminal basic amino acid residues produce no flare but do produce wheal (20).

The flare induced by intradermal injection of substance P into human skin is prevented by pretreatment of the subject with an antagonist of histamine at H_1 receptors (12, 20, 28). The wheal is, however, only partially inhibited by such antihistamine pretreatment. A combination of H_2 and H_1 antagonists also fails to inhibit totally the wheal response to intradermal injection of substance P. There are two possible explanations for these observations. Firstly, when injected intradermally, substance P may release histamine from mast cells local to the site of injection. The histamine so released may then stimulate polymodal nociceptors to activate an axon-reflex which produces a flare distant from the site of injection. In this case, the flare would actually be produced by neuropeptides released from the sensory nerve through the axon-reflex. A second possibility, which may in fact operate together with that just out-

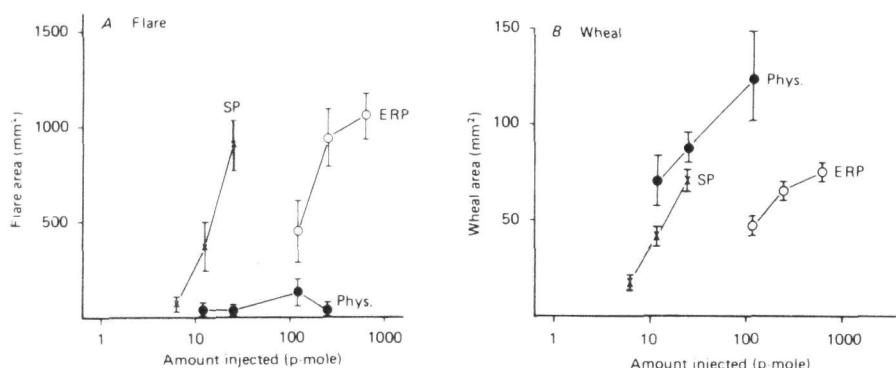


Fig. 4. Flare and wheal reactions induced by intradermal injection of peptides in human skin. Peptides were injected in the amounts shown intradermally in a volume of 25 μ l. Flare was measured 3 min after injection and wheal 12 min after injection. ($n = 8$).

lined, is that the neuropeptide released from the sensory nerve by the axon-reflex does not itself act directly to produce the vasodilatation of flare but acts indirectly by the release of histamine from mast cells. In this case, it is histamine which is the direct mediator of flare and there are several pieces of evidence which suggest that this is likely.

Role of mast cells. Mast cells are predominantly located around the blood vessels in human skin and several studies have now revealed that neuroeffector junctions exist between these mast cells and nerves in the skin and elsewhere (31, 54, 65, 66, 75). Recently it has been shown that fibres containing substance P form neuroeffector junctions with mast cells around blood vessels but rarely form such relationships with mast cells elsewhere in the tissues (65). In addition, antidromic stimulation of sensory nerves induces mast cell degranulation (41) and nerve stimulation has also been shown to induce histamine release in lung and gut (1, 4). Depletion of mast cell histamine by pretreatment with the mast cell degranulating agent, compound 48/80 reduces the inflammatory response normally seen on antidromic stimulation of nerves (44), and H_1 antagonists of histamine also prevent the vasodilatation and plasma extravasation which occurs in response to antidromic stimulation of nerves (26, 44).

All of this suggests that substance P or another neuropeptide can release histamine from tissue mast cells, and indeed injection of

substance P into human skin or into the perfused rat hind limb has been shown to release histamine into the circulation (30, 64). Application of substance P to rat peritoneal mast cells causes a dose-dependent release of histamine (17, 38). CGRP, which co-exists with substance P also releases histamine from rat mast cells but is less potent than substance P (56) whereas neurokinin A does not release histamine. Structure-activity studies of the histamine releasing effect of substance P on rat peritoneal mast cells have shown that N-terminal basic amino acid residues of the peptide are essential (17). The octapeptide SP_{4-11} is devoid of histamine-releasing activity as is physalaemin, which is similar to substance P in the C-terminal region but contains no basic amino acid residues (Fig. 2). The first four amino acid residues of substance P, SP_{1-4} (Arg-Pro-Lys-Pro), releases histamine from mast cells but is only about 0.06 times as active as substance P itself (17). Thus, the C-terminal sequence of substance P is also important for the maximum expression of histamine releasing activity. Progressive shortening of the C-terminal sequence of substance P in the series of peptides SP_{1-9} , SP_{1-8} , SP_{1-7} and SP_{1-6} leads to a corresponding reduction in the potency of the molecules for the release of histamine from rat mast cells. Moreover, certain amino acid substitutions in the C-terminal sequence have been shown to increase the activity of the peptide on rat mast cells (17, 21). For example, $[D-Trp^7, ^9] SP_{1-11}$ is about 10 times more active than substance P. Substance

P receptors exist in a variety of smooth muscle preparations and in salivary gland, and receptors mediating the effects of substance P have been classified into P and E types on the basis of the relative activities of agonist peptides in these tissues (35). The rat mast cell receptor, however, appears to belong to neither of these types. Recent studies with a hybrid molecule formed from the N-terminal sequence of substance P and dodecylamine indicate that the properties of substance P which relate to histamine release are (i) the specific structure of the N-terminal region in which basic amino acids are essential and (ii) a non-specific hydrophobic property of the C-terminal sequence.

Apart from an understanding of the substance P interaction with the mast cell membrane, these structure-activity studies revealed an interesting parallel between the ability of peptides to release histamine from rat mast cells and their ability to produce flare in human skin. The histamine release data is, therefore, compatible with the other evidence cited above which has been used to argue that substance P released from sensory nerves, activated by an axon reflex, releases in turn histamine from mast cells, and this histamine release produces vasodilatation which in skin is observed as a flare. No parallel exists between the ability of peptides to induce wheal or plasma extravasation and their ability to release histamine (17, 20). This too is consistent with the observation that local anaesthetic prevents flare but not wheal responses induced by peptides (20). Thus, flare is neurogenic and appears to involve substance P-induced histamine release from mast cells whereas wheal appears largely to be a direct effect of substance P on vascular permeability without a neurogenic component.

It must be pointed out, however, that this model is not universally true and in lung tissue, for example, the bronchoconstrictor and increased vascular permeability effects of substance P both appear to be mediated directly by substance P with little or no contribution from histamine released by mast cells (22, 47).

Peptide antagonists. Some of the most powerful evidence for the involvement of a putative neu-

rotransmitter in a physiological process comes from the use of antagonists. A considerable effort has been expended on the search for substance P antagonists with relatively little success. The antagonists that have been produced are substituted substance P - like peptides which have low affinity and poor selectivity (29, 58). The P and E type classification (35) of substance P receptors has not been supported by the antagonist studies, and it has not been uncommon to find that the affinity of the antagonist for the substance P receptors varies depending on which particular agonist has been used (58). Many of the antagonists of substance P developed in smooth muscle were subsequently found to be agonists at the substance P receptor in human skin and on rat mast cells (17, 21). Nevertheless, some antagonists have provided useful information, [D-Pro⁴, D-Trp^{7, 9, 10}] SP₄₋₁₁ (SPA) antagonises in a competitive manner the histamine release induced by substance P from rat mast cells (55). This same antagonist also inhibits the flare, but not the wheal, induced by the intradermal injection of substance P into human skin (57). This evidence again supports the conclusion that flare is mediated by substance P-induced histamine release from the mast cells in the skin.

The substance P antagonist [D-Arg¹, D-Pro², D-Trp^{7, 9}, Leu¹¹] SP₁₋₁₁ has been shown to prevent heat-induced oedema in rat skin (50) and also the increased vascular permeability that occurs in airways stimulated with cigarette smoke (46). Furthermore, this same substance P antagonist inhibits the non-cholinergic component of bronchial smooth muscle contraction induced by stimulation of the vagus nerve and also the increased vascular permeability of the airways that follows vagal stimulation (49). Trigeminal nerve stimulation produces inflammatory changes in the eye and this same substance P antagonist also blocks these responses (51). A different antagonist of substance P, [D-Pro², D-Phe⁷, D-Trp⁹] SP₁₋₁₁ has been shown to antagonize the vasodilatation of tooth pulp which occurs on stimulating, antidromically the sensory nerve to the tooth (60). Thus, the studies with antagonists add further weight to the evidence in favour of the hypothesis that

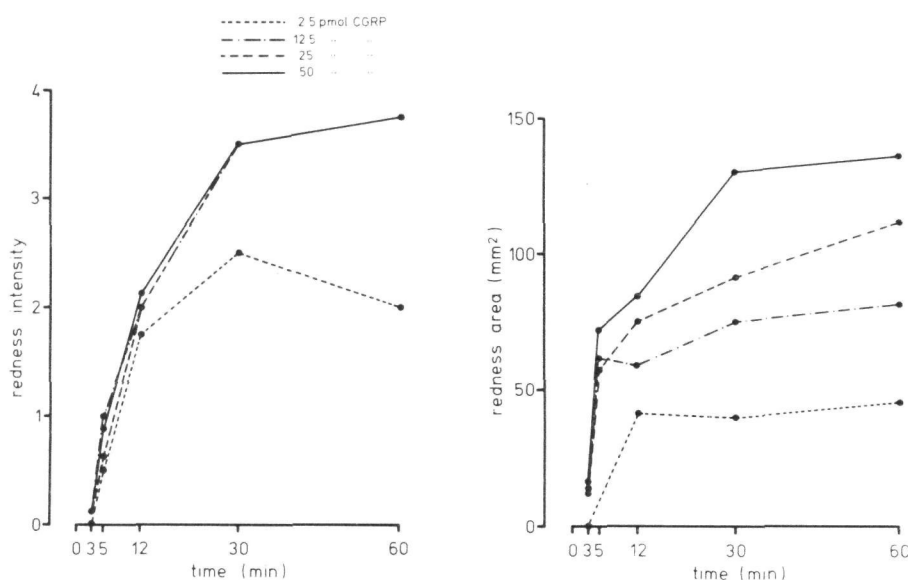


Fig. 5. Time-course of the erythema response to CGRP injected intradermally into human skin in the amounts shown. Area of redness was calculated from the average diameter of the response. Redness intensity was assessed on an arbitrary scale of 0 to 4. ($n = 4$).

inflammation may be generated by the release of substance P from sensory nerve endings stimulated antidromically through axon-reflex.

CGRP and neurokinin A. So far, particular emphasis has been laid upon substance P as the peptide mediator of neurogenic inflammation but as has already been pointed out, CGRP and neurokinin A coexist with substance P in sensory neurones (18). Furthermore, capsaicin may deplete these nerves of their content of these other peptides as well as substance P (63). In human skin, CGRP produces a wheal and flare response but is less potent than substance P in this respect (56). As with substance P, the flare induced by CGRP is abolished by pretreatment of the subject with a H_1 histamine antagonist but the wheal is not affected. The response of human skin to CGRP injected intradermally differs in an important respect from the response to substance P in that CGRP induces a slow onset intense erythema local to the site of injection which persists for several hours (6, 56). No such response is seen with substance P. Fig. 5 shows the time course of development of the area and intensity of the erythema which is still increasing as the wheal response to CGRP is disappearing. The erythema response to CGRP persists for at least 4 h after injection. In other tissue CGRP has been found

to be a potent vasodilator and whilst it has little or no action itself on vascular permeability (8), it acts synergistically to increase vascular permeability with other inflammatory mediators, such as C5a, leukotriene B_4 , platelet activating factor and bradykinin (5). In human skin, the mechanism by which CGRP produces the slow onset, prolonged erythema is not known but it is not neurogenic since local anaesthetic infiltration does not modify the response and it is not histamine-mediated since H_1 histamine antagonists fail to modify it (56). The erythema response to CGRP, 1 h after injection, is however, accompanied by a granulocyte infiltration into the skin, but it is unclear whether this is causally related to the vasodilatation or whether it is merely an unrelated response (56). Neurokinin A is also a vasodilator and causes increased vascular permeability. It is also a bronchoconstricting agent and in this respect is more potent than substance P (76).

Conclusion

There is very clear evidence for reflexes in sensory neurones playing a part in the generation of certain types of inflammation, and such reflexes clearly operate to cause vasodilatation and increased vascular permeability in both skin and

lung. The mediator of the axon reflex appears to be one or more neuropeptides and substance P is clearly a candidate as a mediator. In the skin, there is evidence that mast cells also play a part in axon-reflex, neurogenic inflammation but this does not appear to be the case in the lung. CGRP, because of the time course of the erythema that it induces, cannot be a mediator of the flare in the acute phase of the response of skin to injury. However, CGRP may well have a role in the vasodilatation associated with later phases of inflammation, such as the prolonged erythema that surrounds burns or healing wounds (77). The associated pain is good evidence of C fibre activity in these situations and axon reflexes might well be expected to release vasodilator substances.

Apart from the role of neuropeptides in the vascular effects of acute inflammation associated with allergy or injury, the question arises as to whether they mediate other effects. In the lung, vagal axon reflexes stimulated by mucosal irritation or by allergen-induced mast cell degranulation have the potential to release bronchoconstricting neuropeptides such as neurokinin A and substance P. Thus the spread of bronchospasm from the degranulation of a few mast cells in or on the bronchial mucosa may result from histamine-stimulated axon-reflex release of bronchoconstrictor peptides. It is interesting that there is a report in the literature that cromoglycate reduces the firing rate in the vagal fibres stimulated by capsaicin and hence part of the antiallergic activity of this drug may result from a suppression of axon reflex-induced bronchospasm (14).

Apart from the potential role for neuropeptides in mediating the changes in bronchial vascular permeability and smooth muscle contraction that occur in asthma, and the potential role for these peptides in mediating the responses to injury and allergens in skin, there are other inflammatory disease processes where they may have a functional role. The pars flaccida of the tympanic membrane contains many mast cells and also substance P-containing neurones, and there has been a suggestion that secretory otitis media may result from axon-reflex-induced changes in vascular permeability in the middle ear. Further-

more, evidence is now being presented which argues a role for neuropeptide release from peripheral sensory nerves in the manifestations of some types of experimental arthritis (11).

There can be no doubt that this new and exciting field has already changed our ways of looking at inflammation and allergy and it is likely that the new data on neuropeptides will stimulate new lines of research in the direction of identifying novel antiallergic and antiinflammatory agents.

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