

Dual effects of CGRP-antagonist on allergic contact dermatitis in human skin

JOANNA WALLENGREN

Department of Dermatology, University Hospital, Lund, Sweden

There is increasing evidence of an interaction between the nervous and the immune systems. The aim of this study was to investigate the rôle of calcitonin gene-related peptide (CGRP) in the modulation of the elicitation of immediate and delayed, immunological and non-immunological reactions in human skin. CGRP (13 pmol and 39 pmol), and the CGRP-antagonist, CGRP_{18–37}, (50 pmol and 500 pmol) were injected intracutaneously prior to provocation tests. Patients with allergy to nickel were provoked with nickel sulfate epicutaneously, and the reactions were evaluated by a clinical scoring system (guidelines of the International Contact Dermatitis Research Group). Patients with allergy to birch pollen were provoked by a prick test with the allergen, and the volume of the weals was measured. The patients were also provoked with tuberculin (delayed immunologic reaction), benzalkonium chloride (irritant contact dermatitis), UV-light and benzoic acid (non-immunologic contact urticaria). The test reactions were estimated by planimetry. CGRP_{18–37} exerted dual effects on allergic contact dermatitis, causing potentiation at a dose of 500 pmol ($p=0.004$) and inhibition at a dose of 50 pmol ($p=0.012$). Other reactions were not significantly affected by the pretreatments. The results suggest that CGRP participates in delayed inflammatory reactions, but is not involved in immediate immunologic reactions.

Key words: contact dermatitis; contact allergy; CGRP; CGRP_{18–37}; neuropeptides; nickel; patch test; prick test. © Munksgaard, 2000.

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Abbreviations:

CGRP: calcitonin gene-related peptide; CGRP_{18–37}: calcitonin gene-related peptide-antagonist.

Accumulating evidence suggests an interplay between the nervous and immune systems. Nerve fibers in the skin provide chemical mediators, mainly neuropeptides, that enable blood vessels and other structures to respond to external stimuli. Vasodilatation and plasma extravasation initiate an inflammatory reaction in order to combat the intruders and remove them from the site of trauma. Increased concentrations of several neuropeptides have been shown in inflamed skin, indicating the significance of neurogenic inflammation (1, 2).

The communication between the nervous system and the immune system is supposed to engage nerve-derived mediators, such as neuropeptides, and also cell-derived agents, such as cytokines and prostaglandins. 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, the ability to synthesize and release such peptides (3–7).

Capsaicin, the active agent in hot pepper of the genus *Capsicum*, has proved a useful tool in the investigation of neurogenic inflammation. A single topical application of capsaicin causes a flare and weal reaction due to the release of neuropeptides from C-fibers in the skin (8). Repeated topical administration of capsaicin in human skin induces tachyphylaxis and abolished axon reflexes. The axon reflex recovers after 8 weeks, indicating that there are no chronic effects (9). Capsaicin has been used therapeutically to suppress localised pain and itch (10).

The rôle of neuropeptides in contact dermatitis has been studied using capsaicin, administered systemically in laboratory animals and topically in human subjects. Pretreatment with capsaicin in guinea pigs and mice enhances delayed-type allergic reactions (11, 12). Topical administration of capsaicin enhances contact dermatitis and tuberculin reaction in human subjects (13).

Capsaicin pretreatment has been shown to abolish the flare response to prick tests in human sub-

jects (14). It also suppresses the symptoms of cold and heat urticaria (15). Capsaicin depletes C-fibers of their neurotransmitters, the end result being a potentiation of delayed allergic immunologic reactions and a reduction of immediate immunologic reactions.

It is important to define the bioactivity profiles of the different neuropeptides since peptide agonists or antagonists may be potentially useful as immunomodulatory drugs. Girolomoni & Tigelaar (16), for instance, found vasoactive intestinal peptide to inhibit capsaicin-induced enhancement of the contact hypersensitivity reaction. The inhibiting effect of vasoactive intestinal peptide on the symptoms of contact dermatitis was confirmed by Lundeborg et al. (17) in human skin.

The pathophysiological rôle of substance P may be explored with the help of substance P receptor antagonists, such as spantide. A single dose (800 pmol) of spantide inhibits allergic contact dermatitis, tuberculin reaction and contact urticaria (18, 19). The inhibiting capacity of spantide on delayed allergic reactions and an enhancing capacity of an SP agonist has recently been documented in mice (20).

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide encoded by a gene that also encodes calcitonin (21). It is stored in C-fibre neurones and exerts effects on the heart, pancreas, and gastrointestinal system, as well as serving as an important regulator of vascular tone (22).

SP and CGRP are often co-localized in the same C-fibers, although many such fibres contain CGRP only (2, 23). CGRP is the most abundant neuropeptide in human skin (2). Upon nerve stimulation, both SP and CGRP will be released concomitantly. CGRP has been found to inhibit the antigen-presenting capacity of the Langerhans cell and to suppress the induction phase of the delayed hypersensitivity reaction in mice (24). The rôle of CGRP in the elicitation phase of the delayed hypersensitivity reaction in human skin have not yet been investigated.

The aim of the present study was to investigate the role of CGRP in established delayed hypersensitivity reaction, immediate immunologic reaction, irritant contact dermatitis and non-immunologic contact urticaria.

Materials and Methods

The study series comprised 25 female patients with nickel contact allergy (aged 22–75 years, mean age 47 ± 10 years) and 8 patients (3 men, 5 women) with birch-pollen-induced allergic rhinitis (aged 24–58 years, mean age 39 ± 14 years). Informed consent was obtained in all cases. The study was

approved by the Ethics Committee of Lund University Medical Faculty.

All pretreatments took the form of intracutaneous injections of 0.05 ml of the various test substances. Synthetic CGRP (Peninsula Lab Europe Ltd) was used at doses of 13 pmol, based on dose-response curves from previous experiments (25). The CGRP-antagonist, CGRP_{/8–37/} (Peninsula Lab Europe Ltd) was used at a dose of 50 pmol. In experiments with allergic delayed contact dermatitis, additional doses of CGRP, 39 pmol and CGRP_{/8–37/} 500 pmol were used in a pilot study.

Control injections with physiological saline were performed on each occasion.

Allergic contact dermatitis

Allergic contact dermatitis was induced by patch test (Al-test[®]) with nickel sulfate 0.2%, 0.4% and/or 0.8% in water in 19 nickel-allergic patients. The testing was performed with varying concentration of nickel sulfate. Physiological saline was injected in each experiment. The patch tests were applied on normal skin of the back for 48 h and read after a further 24 h. The response was estimated, using a scoring system according to the guidelines of the International Contact Dermatitis Research Group (26). According to this system, erythema is denoted by +, infiltration by ++, and vesicular or bullous reaction by ++++. This scoring system provides information on the intensity of the reaction as well as the size. An area of the response measured by planimetry may be the same in a ++ and +++ reaction, while the intensity of the reaction is different.

Tuberculin reaction

The tuberculin reaction was tested on the volar aspect of the forearm by intracutaneous injection of 0.1 ml of 2TU PPD (Statens Serum Institut, Copenhagen, Denmark) in 18 subjects. The response area was estimated planimetrically after 72 h.

Irritant contact dermatitis

Benzalkonium chloride (1%) in water solution was applied to the skin of the back using Al-tests[®] in 21 subjects. The compound was applied for 48 h and the reaction was examined after a further 24 h, the response area being estimated planimetrically.

Ultraviolet evoked reaction

8 subjects with skin type I or II were enrolled in the study and exposed to UVB. Erythema was induced on the back by exposure to 0.11 J/cm² of 1/2

of the back, and 0.13 J/cm^2 of the other half. The inflammatory response was evaluated after 24 h by the naked eye.

Allergic Type I reaction

8 subjects with allergy to birch pollen were tested. Prick tests were administered on the volar aspect of the lower arms 10 min and 1 h after the pretreatment with CGRP and CGRP_{18-37/}. Extract of birch pollen (Soluprick®, ALK, Denmark), was 'pricked' into the volar aspect of the lower arm of each patient. Evaluation vis-à-vis erythema and infiltration was made after 20 min. The volume of the weal reactions was calculated as the product of weal area and depth (skinfold thickness-baseline/2). The skinfold thickness was measured using a low tension spring-loaded caliper (Mitutoyo®, Japan). The baseline value in each experiment was measured as the positive prick test depth after pretreatment with saline at corresponding time-points.

Non-immunological contact urticaria (NICU)

7 subjects with allergy to birch pollen participated in the experiments with benzoic acid. 25 min after the pretreatments with CGRP and CGRP_{18-37/}, benzoic acid, 5% in pet. was applied to the skin epicutaneously by AL-tests®. The patch tests were removed after 45 min and the area of the reaction was estimated planimetrically.

Statistical analysis and presentation of results

The Wilcoxon signed-rank test was used for statistical analysis of intra-individual differences in response. Exact 2-sided *p*-values were computed in each case. No adjustment for multiplicity has been made.

Box-whisker plots were used to illustrate the differences in response when comparing pretreatment with a test substance and physiological saline. Boxes contain the 50% 'most central values,' the upper limit corresponding to the 75th percentile and the lower limit to the 25th percentile. The upper whisker extends to the largest value not exceeding the 75th percentile plus $1.5 \times$ of the height of the box, while the lower whisker extends to the smallest value not exceeding the 25th percentile minus $1.5 \times$ of the height of the box. Other values are shown as outliers. The magnitude of allergic nickel reactions is given in arbitrary units $\pm$ SEM.

Results

Injection of CGRP doses of 13 or 39 pmol into the skin produced a bright erythema. Within 5 min,

this was replaced by a slowly progressing dark erythema surrounded by a pallid edge. There was no itch and no distinct weal; instead, there was diffuse

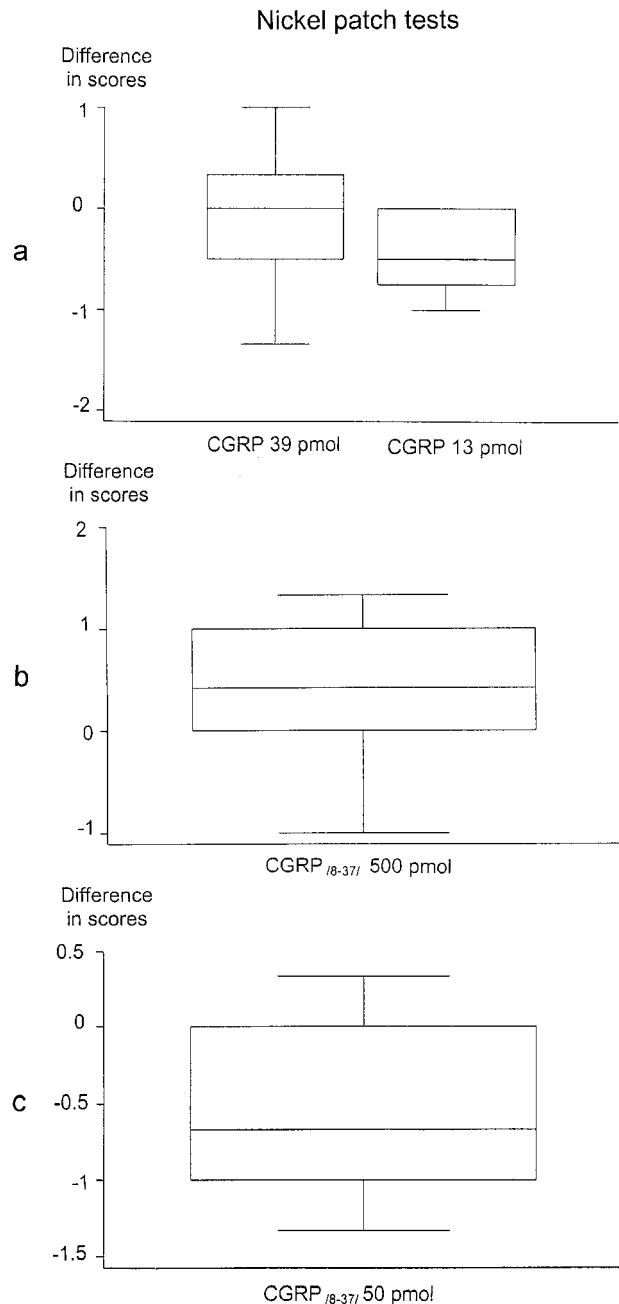


Fig. 1. The effect of CGRP and CGRP-antagonist (CGRP_{18-37/}) on allergic contact dermatitis from nickel. Responses to challenge with nickel sulfate (0.2%, 0.4%, 0.8%). The figure shows the distribution of the differences in scores between the area pretreated with physiological saline and that treated with CGRP at doses of 39 pmol and of 13 pmol ($n=8$) (a) and with CGRP_{18-37/} at doses of 500 pmol ($n=11$) (b) and 50 pmol ($n=11$) (c), respectively. The boxes represent $1/2$ of all responses, and the horizontal middle lines denote the median values. The mean difference was used when 3 concentrations of nickel sulfate were administered to the same subject.

induration of about the same outline as the erythema. The reaction persisted for several hours, still being present during the 90 min of experiments with immediate type reactions (Type I and NICU). The CGRP-antagonist, CGRP_{/8-37/}, used at a dose of 50 or 500 pmol, induced a faint, indurated erythema which occurred after 5 min and disappeared within 30 min.

Allergic contact dermatitis

19 patients with nickel contact allergy underwent provocation with nickel sulfate in water at 3 concentrations: 0.2%, 0.4% and 0.8%. For each test subject, the mean value from 3 experiments at each concentration was used for statistical evaluation. CGRP, 39 pmol, did not affect response to nickel, the mean reaction score $\pm$ SEM being 2.33 ± 0.15 versus 2.41 ± 0.15 after pretreatment with physiological saline. CGRP, 13 pmol, seemed to suppress the response, the mean reaction score being 1.98 ± 0.20 versus 2.41 ± 0.15 after pretreatment with physiological saline ($p=0.063$, $n=8$) (Fig. 1a). CGRP_{/8-37/}, 500 pmol, augmented the response, The mean reaction score being 2.35 ± 0.13 versus 1.95 ± 0.16 after pretreatment with physiological saline ($p=0.004$; $n=11$) (Fig. 1b, 2). CGRP_{/8-37/}, 50 pmol, inhibited the response, The mean score reaction being 1.24 ± 0.16 versus 1.78 ± 0.18 after pretreatment with physiological saline ($p=0.012$, $n=11$) (Fig. 1c, 2). The data on individual patients are shown in Table 1.

Irritant contact dermatitis

Benzalkonium chloride induced a sharply demarcated homogeneous erythema, sometimes with oedema. None of the pretreatments changed the



Fig. 2. Responses to challenge with nickel sulfate after pretreatment with α -CGRP_{/8-37/} at doses of 500 pmol and of 50 pmol and physiological saline.

outcome, the mean of the reaction area being 1.51 ± 0.50 cm² after CGRP, and 1.56 ± 0.25 cm² after CGRP_{/8-37/}, versus 1.37 ± 0.22 cm² after pretreatment with physiological saline.

Tuberculin reactions

All patients with positive tuberculin responses were included in the data analysis ($n=20$). None of the pretreatments influenced the reaction to tuberculin, the mean reaction area being 2.92 ± 0.56 cm² after CGRP, and 3.2 ± 0.50 cm² after CGRP_{/8-37/}, versus 3.4 ± 0.54 cm² after pretreatment with physiological saline.

UVB reactions

After 24 h, UVB induced a patchy erythema. These reactions were unaffected by all pretreatments.

Allergic Type I reaction

All subjects responded to the allergen with weals. Administered 1 h prior to the prick testing, the reaction volume after pretreatment with CGRP was 82.5 ± 28.5 mm³ and with CGRP_{/8-37/} was 82.1 ± 28.9 mm³ versus 112.3 ± 49.7 mm³ after pretreatment with physiological saline. None of these differences was significant. Administered 10 min prior to prick testing, the reaction volume was unchanged by CGRP (221.9 ± 65.1 mm³), and by CGRP_{/8-37/} (294.1 ± 87.3 mm³), versus pretreatment with physiological saline (199.2 ± 66.6 mm³).

Non-immunological contact urticaria (NICU)

All subjects manifested response to benzoic acid, all but 1 developing an erythema, and the remaining subject an additional infiltration. All pretreatments were without effect, the mean response area being 32.4 ± 6.05 cm² after CGRP, and 17.8 ± 7.6 cm² after CGRP_{/8-37/}, versus 14.7 ± 4.7 cm² after pretreatment with physiological saline.

Discussion

The rich and widespread distribution of CGRP in free sensory nerve fibers in dermis and epidermis, around blood vessels and hair follicles, creates good conditions for CGRP to participate in the defense of skin from trauma (25). Sensory C-fibers conduct the sensation of itch and pain from the skin to the CNS (27). CGRP released in the skin from C-fibers activated by an axon reflex exerts profound vascular effects during the following

Table 1. Nickel patch test results after pretreatment with CGRP and the CGRP-antagonist, CGRP₈₋₃₇/

Patient no.	Nickel sulfate conc (%)	Phys. saline	CGRP 39 pmol	CGRP 13 pmol	Patient no.	Nickel sulfate conc (%)	Phys. saline	CGRP ₈₋₃₇ 500 pmol	CGRP ₈₋₃₇ 50 pmol
1	0.2	+	+	+	9	0.2	++	+++	++
	0.4	++	++	+++		0.4	+++	+++	+++
	0.8	++	++	+++		0.8	+++	+++	+
2	0.2	+	+	+	10	0.2	+++	+++	+++
	0.4	++	++	+++		0.4	+++	+++	++
	0.8	+++	+++	+++		0.8	+	+++	++
3	0.2	+++	+++	+++	11	0.2	+++	0	++
	0.4	+++	+++	+		0.4	+++	+++	++
	0.8	++	++	0		0.8	++	++	++
4	0.2	+++	+++	+++	12	0.2	++	+++	++
	0.4	+++	+++	+++		0.4	+	+++	+
	0.8	++	++	++		0.8	+	+++	++
5	0.2	+++	+++	+++	13	0.2	++	++	0
	0.4	+++	+++	+++		0.4	+	++	+
	0.8	+++	+++	+++		0.8	++	+	++
6	0.2	+++	+++	+	14	0.2	++	+++	+
	0.4	+	+	+		0.4	0	+	0
	0.8	+++	+++	+		0.8	0	++	0
7	0.2	+++	+++	++	15	0.2	+++	+++	++
	0.4	+++	+++	++		0.4	+	++	+
	0.8	++	++	+		0.8	+++	+++	+
8	0.2	++	++	+	16	0.2	++	+++	+
	0.4	+	+	+		0.4	++	++	+
	0.8	+	+	++		0.8	++	+	++
					17	0.2	+++	+++	0
						0.4	++	+++	++
						0.8	+++	+++	++
					18	0.2	0	+++	0
						0.4	++	+	0
						0.8	+	++	0
					19	0.2	+	++	+
						0.4	0	+	0
						0.8	0	+	0

hours. The vascular effects are dose-dependent. The erythema induced by CGRP is dependent on neural mechanisms and can be inhibited by local anaesthetics, while the wheal is mediated by the lymphatic system (25). CGRP is a potent chemoattractant and increases the infiltration of polymorphonuclear leucocytes (28, 29) and T-lymphocytes (30). These cells will arrive within minutes to hours. In the present experiments with Type I immunologic reaction, CGRP and CGRP₈₋₃₇, injected 10 min and 1 h, respectively, before the prick tests, did not influence the volume of the wheal reaction to birch pollen. A possible explanation is that CGRP effects are not mediated by histamine (25). This study does not confirm the results of Raud et al. (31) who found that CGRP inhibits histamine-induced weal.

In the present study, neither CGRP nor CGRP₈₋₃₇ affected UVB erythema observed with the naked eye 24 h after irradiation. Benrath et al.

(32) found, however, that CGRP₈₋₃₇ inhibits skin blood flow and oedema of rat skin following UV-irradiation, with maximum effectiveness 24 h after irradiation.

In the epidermis, CGRP-positive nerve fibers have been shown to be localized close to Langerhans cells and to interact with their function as antigen presenters (7, 33). Antigen presentation is a process that lasts for several hours. CGRP has been found to inhibit the antigen-presenting capacity of the Langerhans cell and to inhibit the induction phase of contact dermatitis (DNFB) in mice (24). These results are in contrast to the results of Gutwald et al. (34), who showed that CGRP enhances both sensitization and challenge phases of allergic contact dermatitis (oxazolone).

The results in this study do not explain these conflicting results, as the suppressive capacity of CGRP (13 pmol) on the challenge phase of delayed allergic contact dermatitis did not reach the level

of significance. An indirect indication that CGRP actually influences contact dermatitis is the effect of the CGRP antagonist CGRP_{/8-37/}. CGRP_{/8-37/} exerted dual effects on allergic contact dermatitis, potentiating it at a dose of 500 pmol and inhibiting it at a dose of 50 pmol (Fig. 1b, c). The question is whether the potentiating activity of CGRP_{/8-37/} at higher doses is immunological or due to a non-immunological inflammatory reaction. Although in the present study, CGRP_{/8-37/} per se induced a faint, transient erythema, it is possible that at a dose of 500 pmol, the CGRP fragment induces an inflammatory reaction, which is responsible for the augmentation of allergic contact dermatitis. A dual immunological effect has also been reported for vasoactive intestinal peptide with respect to its effect on leukocyte migration (35).

Upon trauma, sensory C-fibers will be stimulated to release several neuropeptides, mainly CGRP and SP. The interaction between the 2 peptides will decide the final outcome of the reaction (36, 37). The concentration of the peptides, as well as their antagonists and their occupation of specific receptors on the immune cells, will influence the immunological reactions.

In the present study, neither CGRP nor CGRP_{/8-37/} affected the symptoms of irritant contact dermatitis. In the literature, conflicting results are found in relation to this issue. Gutwald et al. (34), found local administration of CGRP to enhance irritant contact dermatitis from croton oil in mice, while Clementi et al. (38) reported it to reduce irritant contact dermatitis from croton oil, arachidonic acid and phorbol esters.

To sum up, this study shows that CGRP participates in delayed inflammatory processes, like allergic contact dermatitis, but is not involved in immediate immunologic reactions. The concentration of a peptide seems to constitute a regulatory mechanism of its immunological effects, as the CGRP antagonist, CGRP_{/8-37/}, exerts opposite effects at different doses.

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References

1. Wallengren J, Ekman R, Möller M. Substance P and vasoactive intestinal peptide in bullous and inflammatory skin disease. *Acta Dermato-venereologica* 1986; 66: 23–28.

2. Wallengren J, Ekman R, Sundler F. Occurrence and distribution of neuropeptides in the human skin. *Acta Dermato-venereologica* 1987; 67: 185–192.
3. Ralevic V, Milner P, Hudlicka O, Kristeck F, Burnstock G. Substance P is released from endothelium of normal and capsaicin-treated rat hindlimb vasculature, in vivo, by increased flow. *Circ Res* 1990; 65: 1178–1189.
4. Aliakbar J, Sreedharan S P, Turck C W, Goetzl E J. Selective localization of VIP and substance P in human eosinophils. *Biochem Biophys Res Comm* 1987; 188: 1440–1445.
5. Besedovsky H O, Del Rey A. Immune-Neuro-endocrine interactions: facts and hypotheses. *Endocrine Review* 1996; 17: 64–102.
6. Concha M, Figueroa C D, Carosi I. Ultrastructural characteristics of the contact zones between langerhans cells and lymphocytes. *J Pathol* 1988; 156: 29–36.
7. Hosoi J, Murphy G F, Egan C L, Lerner E A, Grabbe S, Asahina A, Granstein R D. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature* 1993; 363: 159–163.
8. Fitzgerald M. Capsaicin and sensory neurons: a review. *Pain* 1983; 15: 109–130.
9. Wallengren J, Håkanson R. Effects of capsaicin, bradykinin and prostaglandin E₂ in the human skin. *Br J Dermatol* 1992; 126: 111–117.
10. Rumsfield J A, West D P. Topical capsaicin in dermatologic and peripheral pain disorders. *DICP, Ann Pharmacotherapy* 1991; 25: 381–387.
11. Wallengren J, Ekman R, Möller H. Capsaicin enhances allergic contact dermatitis in the guinea pig. *Contact Dermatitis* 1991; 24: 30–34.
12. Girolomoni G, Tigelaar R E. Capsaicin-sensitive primary sensory neurons are potent modulators of murine delayed-type hypersensitivity reactions. *J Immunol* 1990; 145: 1105–1112.
13. Wallengren J, Möller H. The effect on capsaicin on some experimental inflammations in human skin. *Acta Dermato-venereologica* 1986; 66: 375–80.
14. Lundblad L, Lundberg J M, Änggård A, Zetterström O. Capsaicin sensitive nerves and the cutaneous allergy reactions in man. *Allergy* 1987; 42: 20–25.
15. Toth-Kasa J, Jancso G, Obal F, Husz S, Simon N. Involvement of sensory nerve endings in cold and heat urticaria. *J Invest Dermatol* 1983; 80: 34–36.
16. Girolomoni G, Tigelaar R E. Peptidergic neurons and vasoactive intestinal peptide modulate experimental delayed-type hypersensitivity reactions. *Ann NY Acad Sci* 1992; 650: 9–12.
17. Lundberg L, Mutt V, Nordlind K. Inhibitory effect of vasoactive intestinal peptide on the challenge phase of allergic contact dermatitis in humans. *Acta Dermato-venereologica* 1999; 79: 178–182.
18. Wallengren J, Möller H. Some neuropeptides as modulators of experimental contact allergy. *Contact Dermatitis* 1988; 19: 351–354.
19. Wallengren J. Substance P antagonist inhibits immediate and delayed type cutaneous hypersensitivity reactions. *Br J Derm* 1991; 124: 324–328.
20. Nizzeke H, Kurimoto I, Streilein J W. A Substance P agonist acts as an adjuvant to promote hapten-specific skin immunity. *J Invest Dermatol* 1999; 112: 437–442.
21. Amara S G, Evans R M, Rosenfeld M G. Calcitonin/calcitonin gene-related peptide transcription unit: tissue-specific expression involves specific use of alternative polyadenylation sites. *Mol Cell Biol* 1984; 4: 2151–2160.
22. Zaidi M, Moonga B S, Bevis P J, Bascal Z A, Breimer L H. The calcitonin gene peptides: Biological and clinical relevance. *Crit Rev Clin Lab Sci* 1990; 28: 109–174.
23. Gibbins I L, Furness J B, Costa M, MacIntyre I, Hilly C

- J, Girgis S. Colocalization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. *Neuroscience Letters* 1985; 57: 125–130.
24. Asahina A, Hosoi J, Beissert S, Stratigos A, Granstein R D. Inhibition of the induction of delayed-type contact hypersensitivity by calcitonin gene-related peptide. *J Immunol* 1995; 154: 3056–3061.
25. Wallengren J, Håkanson R. Effects of substance P, neurokinin A and calcitonin gene-related peptide in human skin and their involvement in sensory nerve-mediated responses. *Eur J Pharmacol* 1987; 143: 267–273.
26. Fregert S. *Manual of contact dermatitis*. 2nd edition. Copenhagen: Munksgaard, 1981.
27. Wallengren J. Pathophysiology of itch. *Eur J Derm* 1993; 3: 643–647.
28. Piotrowski W, Foreman J C. Some effects of calcitonin gene-related peptide in human skin and on histamine release. *Br J Dermatol* 1986; 114: 37–46.
29. Goebeler M, Henseleit U, Roth J, Sorg C. Substance P and calcitonin gene-related peptide modulate leucocyte infiltration to mouse skin during allergic contact dermatitis. *Arch Dermatol Res* 1994; 286: 341–346.
30. Foster C A, Mandak B, Kromer E, Rot A. Calcitonin Gene-related peptide is chemotactic for human T lymphocytes. *Ann NY Acad Sci* 1992; 657: 397–404.
31. Raud J, Lundeberg T, Brodda-Jansen G, Theodorsson E, Hedqvist P. Potent anti-inflammatory action of calcitonin gene-related peptide. *Biochem Biophys Res Commun* 1991; 180: 1429–1435.
32. Benrath J, Eschenfelder C, Zimmermann M, Gillardon F. Calcitonin gene-related peptide, substance P and nitric oxide are involved in cutaneous inflammation following ultraviolet irradiation. *Eur J Pharmacol* 1995; 293: 87–96.
33. Misery L. Langerhans cell in the neuro-immuno-cutaneous system. *J Neuroimmunol* 1998; 89: 83–87.
34. Gutwald J, Goebeler M, Sorg C. Neuropeptides enhance irritant and allergic contact dermatitis. *J Invest Dermatol* 1991; 96: 695–698.
35. Bondesson L, Nordlindh S, Gafvelin G, Theodorsson E, Mutt V. Dual effects of vasoactive intestinal peptide (VIP) on leucocyte migration. *Acta Physiol Scand* 1991; 141: 477–481.
36. Brain S D, Williams T J. Substance P regulates the vasodilator activity of calcitonin gene related peptide. *Nature* 1988; 335: 73–75.
37. Wallengren J, Wang Z-Y. Interactions between tachykinins and CGRP in human skin. *Acta Dermato-venereologica* 1993; 73: 259–261.
38. Clementi G, Amico-Roxas M, Caruso A et al. Effects of CGRP in different models of mouse ears inflammation. *Life Sci* 1994; 54: 119–124.

Address:

Joanna Wallengren
Department of Dermatology
University Hospital
SE-221 85 Lund
Sweden