Synovial expression of vasoactive intestinal peptide in polymyalgia rheumatica

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Abstract

Objective

Polymyalgia rheumatica (PMR) is an inflammatory disease that tipically affects elderly people. Its clinical hallmark is the severity of pain in the shoulder and pelvic girdle. Mild to moderate synoivitis and/or bursitis of the joints involved has been described. Neuropeptides are involved in nociception and modulation of inflammatory reaction. To evaluate whether neuropeptides have a role in PMR pathophysiology, we studied the expression of substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and somatostatin (SOM) in shoulder synovial tissues of PMR patients.

Methods

Synovial expression of neuropeptides was investigated by immunohistochemical analysis, in two groups of PMR patients: the first one at the onset of disease and the second one after corticosteroid treatment, and in other joint diseases, rheumatoid arthritis (RA) and osteoarthritis (OA).

Results

The only significant expression of VIP was found in PMR and, to a lesser extent, in RA synovial tissue. In PMR, we observed VIP immunostaining both in the lining layer and in the sublining area. In patients on corticosteroid treatment VIP lining layer expression was not significantly different while VIP positive cells in the sublining area were almost absent.

Conclusion

Local VIP production in PMR synovial tissue might contribute to the typical musculoskeletal discomfort and it may have a role in the immunomodulation of synovial inflammation.

Key words

Polymyalgia rheumatica, vasoactive intestinal peptide, neuropeptides, synovial membrane.

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This work was supported by grants from Ricerca Corrente I.O.R and MIUR University of Bologna.

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Received on August 29, 2005; accepted in revised form on May 23, 2006.

Introduction

Polymyalgia rheumatica (PMR) is a common rheumatic disorder of unknown etiology (1) characterized by some peculiar and puzzling features: a) it occurs only in elderly people, b) the pronounced and diffuse muscoloskeletal discomfort, mainly localized in the neck and shoulder and pelvic girdle, is not matched by demonstrable muscle injury; c) mild to moderate shoulder joint synovitis and/or bursitis (2, 3) does not correlate with the diffusion and intensity of pain; d) in addition, systemic symptoms indicate a widespread intense inflammatory reaction (1); e) it may be associated with giant cell arteritis (GCA) which affects large supra-aortic arteries.

Articular joints are innervated by nociceptive fibers that can release neuropeptides with proinflammatory activity, on the other hand, inflammatory reaction could induce the production of neuropeptides. In this interplay there are factors that have a role both as neuropeptides and immune mediators, and changes in neuropeptide-immunomediator interactions might be implicated in the pathogenesis of rheumatic diseases (4-6).

Based on these findings, showing that neuropeptides are important mediators involved in the modulation of inflammation and nociception, together with the evidence that in PMR the muscles were not the source of symptoms, but the aching appears likely to be related to synovitis/bursitis, we studied the expression of substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and somatostatin (SOM) in shoulder synovial tissues obtained from PMR patients at the onset of disease and in patients after corticosteroid treatment. We also compared the synovial expression of these neuropeptides in other joint diseases, namely rheumatoid arthritis (RA) and osteoarthritis (OA).

Patients and methods

Patients

We evaluated 14 PMR patients: nine patients at the onset of disease (7 women, 2 men; mean age 71 years, range: 56-81 years) and five patients during corticosteroid therapy (4 women, 1 man; mean age: 68 years, range: 64 – 80 years). All PMR patients were diagnosed according to the criteria of Healey (7). In the nine patients at the onset of disease, shoulder synovial biopsy was obtained at diagnosis (before steroid therapy). In this group no associated giant-cell arteritis (GCA) was observed. In the five corticosteroid-treated patients, arthroscopic synovial biopsy was obtained after PMR had been diagnosed (mean time lapse after diagnosis: 13 months; range 1-36 months), when the disease was in clinical remission and ESR was in the normal range. Four patients were being treated with prednisone (mean dosage 13 mg/day; range: 5-25 mg/day) at the time of biopsy and one patient with PMR and associated GCA was treated with 50 mg/day prednisone.

Synovial biopsies were also obtained from seven patients (4 women, 3 men; mean age: 60 years, range: 52-71 years) with OA diagnosed on clinical, laboratory and radiological findings (8), and five patients with RA, meeting the criteria of the American College of Rheumatology formerly the American Rheumatism Association (9) (3 women, 2 men; mean age: 54 years, range: 48-62 years). Two synovial biopsies were obtained during knee arthroscopies in all but one RA patient in whom shoulder arthroscopy was performed and one shoulder synovial biopsy was obtained. Informed consent from all patients and approval by the ethical committee of the involved hospitals were obtained.

Immunohistochemistry

All biopsy samples were snap-frozen in liquid nitrogen and stored at –80°C. Serial sections cut with a cryostat, 10 μm thick, were air dried, fixed in acetone at 4°C for 10 minutes and stored at –80°C until analyzed. Rehydrated sections were immunostained with: rabbit polyclonal antibody to human SP (1:500) (Serotec Ltd, Oxford, UK), rabbit polyclonal antibody to human SOM (1:400) (Serotec Ltd.), mouse monoclonal antibody to human VIP (VIP55) (1:500) (10), mouse monoclonal antibody to human CGRP (CGRP4901) (1:200), monoclonal mouse anti-human Neuron

Specific Enolase (NSE) (1:500) (Dako, Glostrup, Denmark) and polyclonal rabbit-anti human S100 (1:10,000) (Dako). Antibody binding was detected using EnVision™ system (Dako). All reactions were developed using a new fuchsin substrate solution (Dako) as previously described (2). Control experiments were performed according to the above-described procedure, with the omission of the primary antibodies. Specific staining control was carried out using an isotype immunoglobulin subclass at the same concentration as the specific monoclonal antibody.
To assess the specificity of anti-VIP staining, we performed VIP antigen preabsorption as described in Ref. 10.

Quantification by image analysis
Morphometric analysis of immunohistochemical stained serial sections was performed using Cytometrica software (Byk Gulden, Milan, Italy) as previously described (2). Results were expressed as the percentage of positive cells and number of positive cells/10^4 μm^2 of tissue surface area.

Double immunofluorescence
Immunofluorescent staining was performed by incubating sections with monoclonal antibodies anti VIP (VIP55) and with the secondary antisera TRITC-conjugated rabbit anti-mouse (1:500) (Dako; Glostrup, Denmark) at room temperature for 1 hour. Finally, the sections were incubated with mouse monoclonal FITC-conjugated, anti-CD3 (anti-pan-T lymphocytes) (Dako; Glostrup, Denmark) or anti-CD68 (anti tissue macrophage) (Dako; Glostrup, Denmark) antibodies, diluted 1:50, at room temperature for 1 hour. Specificity control was assessed by performing only the VIP staining and only the CD3 and CD68 staining.

Statistical analysis
The Kruskal-Wallis test was used to perform multiple comparisons of unpaired data and the non-parametric Mann-Whitney U test was used to compare variables between groups. The Statistica for Windows package was used to perform statistical analysis (Statsoft Inc., Tulsa, OK).

Results

The expression of SP, SOM and CGRP was almost absent in PMR synovial tissue and it was negative in all OA cases. Among untreated PMR cases, only one patient was positive for SP, one for SOM, and one for CGRP. All positive cases showed low percentages of

Table I. VIP synovial expression in patients with active untreated polymyalgia rheumatica (NT PMR), corticosteroid treated polymyalgia rheumatica (T PMR), rheumatoid arthritis (RA) and osteoarthritis (OA).

Sex/Age	Disease	Lining Layer		Sublining area	
		Pos. cells (%)	Pos. cells/10^4 μm^2	Pos. Cells (%)	Pos. cells/10^4 μm^2
1 F/74	NT PMR	27	17	11	7
2 M/76	NT PMR	32	12	17	5
3 F/69	NT PMR	0	0	4	2
4 F/79	NT PMR	23	9	5	2
5 F/56	NT PMR	22	5	13	4
6 F/71	NT PMR	nv*	nv*	7	2
7 M/60	NT PMR	12	3	15	5
8 F/81	NT PMR	37	13	22	4
9 F/73	NT PMR	25	6	20	2
1 F/67	T PMR	18	9	0	0
2 M/80	T PMR	35	12	0	0
3 F/64	T PMR	36	19	0	0
4 F/65	T PMR	0	0	0	0
5 F/66	T PMR	12	10	0	0
1 F/50	RA	0	0	22	7
2 F/58	RA	0	0	12	5
3 M/48	RA	0	0	28	7
4 F/51	RA	0	0	10	3
5 M/62	RA	0	0	0	0
1 M/71	OA	37	9	0	0
2 F/52	OA	0	0	0	0
3 F/60	OA	0	0	0	0
4 M/61	OA	0	0	0	0
5 F/54	OA	0	0	11	3
6 M/67	OA	43	23	0	0
7 F/58	OA	0	0	0	0

*nv: not valuable.

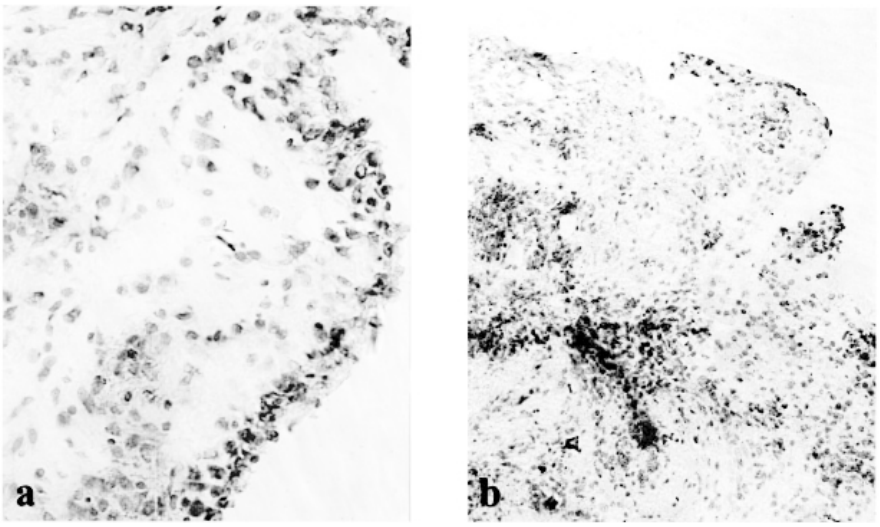

Fig. 1. Representative immunohistochemical pictures showing staining of vasoactive intestinal peptide (VIP) in frozen synovial tissues from a patient with polymyalgia rheumatica (PMR) (a) and from a patient with rheumatoid arthritis (RA) (b). In PMR (a), VIP expression is shown both in the lining layer and in sublining area; in RA (b), VIP immunostaining is localized only in the sublining area. Original magnification x 320 in a); x 125 in b).

stained cells (< 10%). In RA, SP expression was detectable in 4 out of 5 cases, while 3 patients were positive for SOM and 1 patient was positive for CGRP expression. In all cases the number of positive cells was lower than 10%.

Results obtained on synovial biopsies immunostained for VIP are reported in Table I. Compared to untreated PMR, VIP expression in the lining layer of RA patients was virtually absent ($p < 0.01$ compared to VIP expression in the lining layer of PMR biopsies). In contrast, a moderate expression of VIP was found in the sublining area of RA biopsies (Fig. 1).

Double immunofluorescence analysis of sections of synovial biopsies showed that only rare CD3 positive cells were VIP immunoreactive, while more CD68 positive macrophage were also VIP positive. Using anti-CD3 and anti-CD68 monoclonal antibodies simultaneously we are able to evaluate that more than 80% of VIP immunopositive cells were not identified by either anti-CD3 or anti-CD68 (Fig 2).

In corticosteroid-treated, inactive PMR patients, VIP immunostaining was absent in the sublining area, whereas it remained unchanged in the lining layer of 4/5 patients (Table 1).

No evidence of any NSE or S-100 positive nerves was detected (data not shown).

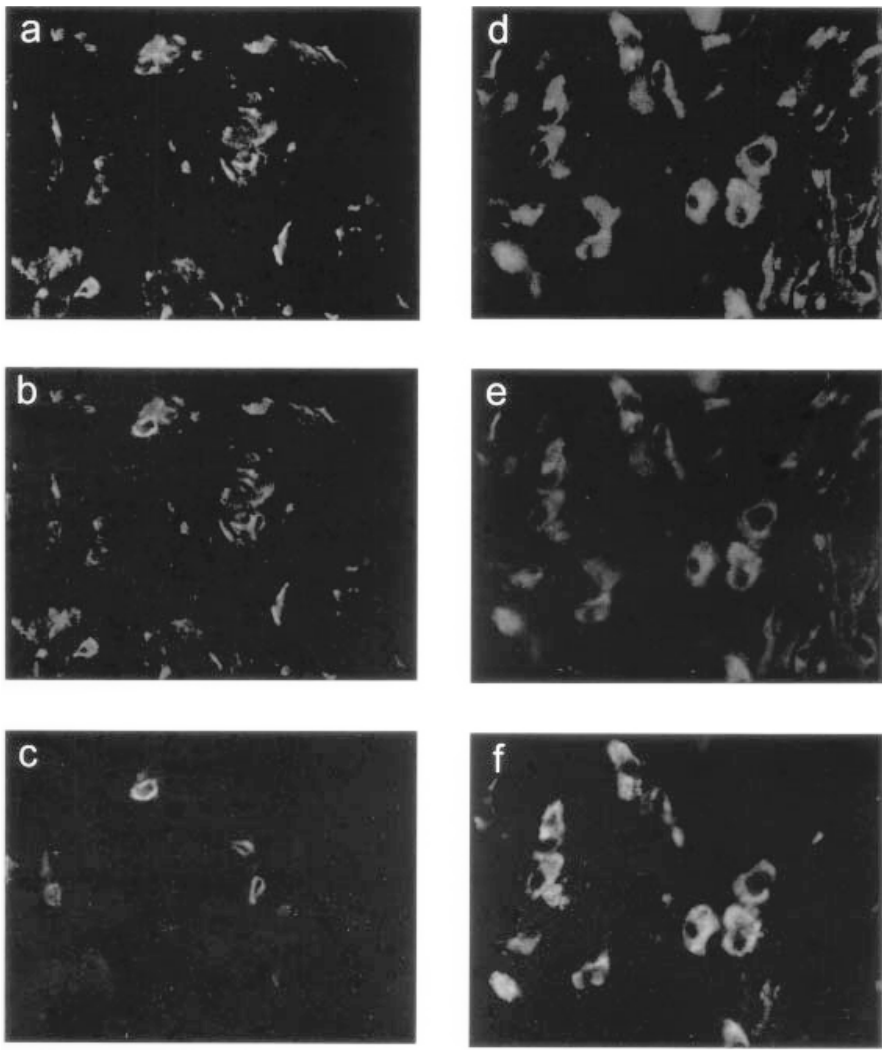

Fig. 2. Representative photomicrographs showing a section of synovial tissues from a patient with polymyalgia rheumatica stained by double immunofluorescence method. VIP immunoreactivity was detected using a TRITC-conjugated secondary antibody (a) (d) and anti-CD3 positive cells were identified by a FITC-conjugated monoclonal antibody (c). No CD3 positive cells were also VIP immunoreactive. Note VIP expressing cells located closely to CD3 positive cells (b). Anti-CD68 positive cells were identified by a FITC-conjugated monoclonal antibodies (f). VIP and CD68 co-localization is shown (e). In macrophage, VIP staining was located in central cytoplasmic area of the cells clearly separated from CD68 labeled peripheral rim (e). Original magnification x 400.

Discussion

VIP is a 28-amino acid neuropeptide expressed by several cell populations involved in innate and acquired immunity and by neurons of the central and peripheral nervous system (11). From a biological standpoint, VIP shows a wide array of actions including immunoregulatory functions (11).

Pain is the main symptom of PMR, and in patients with active disease it is so intense that it prevents their daily living activity (1). Inflammatory mediators, such as histamine, serotonin, bradykinin, may directly sensitize nociceptive fibers and induce pain sensation. In an experimental model of pain induced by peripheral nerve fiber injury, it has been shown that nociceptive fibers containing VIP change their phenotype (i.e. modifying gene expression) (12) with a dramatic increase in expression of this neuropeptide (12, 13). The role of VIP in pain transmission is also supported by the results of a study performed on two groups of patients who had undergone appendicectomy due to acute appendicitis, with and without histological signs of acute inflammation (14). The increased number of VIP immunoreactive fibers in patients without signs of acute inflammation is interesting because of the analogy with PMR patients, where the pronounced and diffuse proximal musculoskeletal discomfort may be only partly due to joint synovitis or bursitis. Therefore, our results suggest that VIP might be involved in the mechanisms underlying the abnormal pain perception typical of PMR.

In addition to the pain-mediator role, VIP regulates numerous functions and mechanisms involved in immunity and tissue injury, with a prevailing down-modulation activity on inflammatory mechanisms (11). The important implications of this anti-inflammatory activity in rheumatic diseases have recently been highlighted by both *in vitro* and *in vivo* studies (5, 15). Therefore, an intriguing suggestion emerging from

this work is that VIP may be involved in mechanisms subserving the modulation of local synovial inflammation. One of the immunomodulatory effects of VIP is related to its ability to induce a shift of CD4+ T lymphocyte phenothype from Th1 to Th2 type (16). Th2 cells produce a cytokine pattern (i.e.: IL-4, IL-5, IL-10) involved in the down-regulation of the cellular immune response; furthermore these cells characteristically do not secrete IFNγ. VIP production and consequent inhibition of IFNγ production in PMR is also consistent with the findings of Weyand *et al*. (17) on IFNγ expression in temporal artery biopsies. IFNγ transcripts were found in biopsies from patients with giant cell arteritis, while temporal artery biopsies from PMR patients without GCA showed no IFNγ transcripts. With regards to this, a recent study aimed at determining the influence of IFNγ gene microsatellite polymorphisms in patients with biopsy-proven GCA and isolated PMR showed that functional polymorphisms of this gene modulated the clinical expression of these conditions (18).

Recently, the ability of VIP to induce tolerogenic dendritic cells with a capacity to generate regulatory T cells (with a distinctive pattern of cytokine production: IL-10 and TGFβ, but not IL-2 or IFNγ) has been reported (19) (20). On these bases, since GCA local inflammation is a dominant event, while in PMR a relevant systemic involvement is associated with aborted vasculitis, thus supporting a role for a specific immune tolerance mechanisms (21), it would be interesting to investigate VIP expression in GCA and PMR artery biopsy. Its possible different expression might be related to a distinct immunotolerance modulation and could explain the different cytokine profile characterizing GCA and PMR, that correlate with different clinical outcomes.

Our data with neuron specific enolase and S-100 did not reveal any evidence of nerve fibers or bundles supplying the synovial tissue. This is probably due to the fact that we analysed small biopsies of the shoulder, which may represent a limiting factor to the identification of nerves in the synovia. Fibers may be located in a deeper layer of this tissue not commonly available during biopsy procedure.

In this work, we reported that in PMR patients treated with corticosteroids, VIP synovial expression is not present in the sublining area, while its expression in the lining layer persists in a comparable manner to that found in untreated patients. Corticosteroids, down-regulating inflammatory infiltration in synovial sublining area (2), might deplete VIP positive cells in this area, while its production by lining layer cells (fibroblast-type synoviocytes), whose number is unaffected by corticosteroid treatment, is maintained even during corticosteroid therapy.

In conclusion, local VIP production in PMR synovial tissue might contribute to the typical musculoskeletal discomfort of this disease and it may have a role in controlling synovial immunoinflammation in PMR patients.

Acknowledgments

We thank Mrs. Helen Wong and Dr. Catia Sternini (Center of Ulcer Research and Education, UCLA School of Medicine, Los Angeles, CA) for providing anti-VIP mouse monoclonal antibody. We also thank Mrs. Graziella Salmi for editorial assistance, Mr. Luciano Pizzi for technical assistance, and Mr Keith Smith for linguistic support.

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