Gastrointestinal peptides in serum and synovial fluid from patients with inflammatory joint disease

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SUMMARY The concentrations of immunoreactive vasoactive intestinal polypeptide (ir-VIP), immunoreactive pancreatic polypeptide (ir-PP), ir-somatostatin, and ir-secretin were measured in serum and synovial fluid from patients suffering from various inflammatory joint diseases. One group of patients were not taking any medication, while another group received anti-inflammatory treatment at the time of sampling. High levels of ir-VIP in the synovial fluid were observed in the untreated group of patients, and the concentration of ir-VIP in the synovial fluid was significantly higher than in parallel serum samples. On the other hand, no significant differences in the concentrations of the other peptides were observed either between serum and synovial fluid or between the two groups of patients. It is suggested that VIP is released locally at the inflammatory site and that VIP may be of significance in inflammatory disorders.

Key word: inflammation.

Recently, vasoactive intestinal polypeptide (VIP) and somatostatin have been shown to be present in human leucocytes.¹ In addition, VIP has been found in mast cells,² and a local wheal and flare response after intradermal injection of VIP has been reported.³ Furthermore, high levels of circulating pancreatic polypeptide (PP) have been found in some inflammatory diseases,⁴ and increased serum secretin concentrations have been reported after treatment with anti-inflammatory drugs.⁵

In the present study we have measured the concentration of immunoreactive VIP (ir-VIP), immunoreactive PP (ir-PP), immunoreactive somatostatin (ir-somatostatin), and immunoreactive secretin (ir-secretin) in serum and synovial fluid from patients suffering from various inflammatory joint diseases, to look firstly, for the presence of these peptides, and secondly, for possible differences in the concentration of the various peptides between treated and untreated patients and between patients and healthy controls.

Materials and methods

Seventy six patients (28 women and 48 men) aged 20

to 77 years who were admitted for various inflammatory joint diseases to the Department of Rheumatology at the University Hospital of Tromsø, Norway were included in the study. Twenty eight patients, mainly with reactive arthritis or Reiter's syndrome, were not taking any medication, while 48 patients were treated with non-steroidal anti-inflammatory drugs alone (33 patients) or in combination with remission inducing drugs (gold, chloroquine, penicillamine, or azathioprine) (n=12), or low dose corticosteroids (n=3) at the time of sampling. Twenty six healthy students aged 20 to 35 years served as controls.

Blood for analysis of serum concentrations of ir-VIP, ir-PP, ir-somatostatin, and ir-secretin was drawn from the antecubital vein, centrifuged, immediately frozen, and stored at -20°C until analysed.

Forty three fluid samples for analysis of the same peptides were collected from 39 patients at the time of clinically indicated diagnostic or therapeutic arthrocentesis of the knee joint. In four of these patients the concentration of ir-VIP was measured before and after intra-articular injection of corticosteroids (20 mg triamcinolone) into the affected knee joint. After centrifugation at 3000 rpm for 15 minutes the supernatants were immediately frozen and kept at -20°C until analysed. In 26 patients

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Table 1 Concentrations of ir-VIP, ir-PP, ir-somatostatin, and ir-secretin in serum from healthy controls and from treated and untreated groups of patients with inflammatory joint disease

	Control group			Treated group			Untreated group		
	n	Median	Range	n	Median	Range	n	Median	Range
VIP (pmol/l)	26	5.0	0-13.5	29	6.5	1-20	22	5.8	0-22
PP (pmol)	26	14.0	5-45	27	20.0	2-200	22	13.5	4-105
Somatostatin (pmol/l)	26	23.5	10-60	26	33.5	0-70	9	37.0	0-80
Secretin (pmol/l)	26	10.0	5-17	23	11.0	6-18	8	10.25	7-22

parallel samples of serum and synovial fluid were collected.

The concentration of the various peptides in serum and in synovial fluid was determined by radioimmunoassay methods previously described.⁶⁻⁹

The statistical analyses were performed by the Student's *t* test and by a two tailed Wilcoxon's paired rank test.

Results

No significant differences for serum levels of ir-VIP, ir-PP, and ir-somatostatin were found between patients and controls. On the other hand, ir-secretin levels were significantly higher ($p < 0.05$) in drug treated patients (Table 1).

No significant differences were seen in the concentration of ir-PP or ir-somatostatin either between serum and synovial fluid or between the two groups of patients (Tables 1, 2, and 3).

The concentration of ir-secretin in serum was significantly higher than in the synovial fluid (Table 3), while no differences in the level of ir-secretin in serum (Table 1) or in synovial fluid (Table 2) were observed between untreated and treated patients.

The concentration of ir-VIP in the synovial fluid was significantly higher than in the serum (Table 3). Furthermore, the level of ir-VIP in the synovial fluid from patients receiving no medication was higher

Table 2 Concentrations of ir-VIP, ir-PP, ir-somatostatin, and ir-secretin in synovial fluid from treated and untreated groups of patients with inflammatory joint disease

	Treated group			Untreated group		
	n	Median	Range	n	Median	Range
VIP (pmol/l)	29	10.0	2.5-52	8	88.5*	17-170
PP (pmol/l)	29	15.0	4-90	7	7.0	5-57
Somatostatin (pmol/l)	21	40.0	0-140	7	55.0	0-90
Secretin (pmol/l)	18	3.25	0-14	5	4.0	3-6

*The asterisk indicates a significant difference between treated and untreated groups of patients ($p < 0.05$).

Table 3 Concentrations of ir-VIP, ir-PP, ir-somatostatin, and ir-secretin in parallel samples of serum and synovial fluid from patients with inflammatory joint disease

	Serum		Synovial fluid	
	Median	Range	Median	Range
VIP (pmol/l) (n=21)	5.5	0-22	17.0*	1-170
PP (pmol/l) (n=20)	20.0	2-200	18.5	4-90
Somatostatin (pmol/l) (n=19)	37.0	22-90	55.0	0-140
Secretin (pmol/l) (n=10)	15.0*	6-32	4.75	2-14

*The asterisks indicate significant differences between serum and synovial fluid ($p < 0.05$).

Table 4 Concentrations of ir-VIP in synovial fluid from four patients with inflammatory joint disease before and after injection of corticosteroids into the affected knee joint

	VIP (pmol/l)			
	Patient 1	Patient 2	Patient 3	Patient 4
Before corticosteroids	122	106	31	54
After corticosteroids	10	47	9.5	21

than that from the treated group of patients (Table 2). On the other hand, no significant differences were seen between the serum values of ir-VIP in the two groups (Table 1). Finally, a fall in the concentration of ir-VIP in the synovial fluid was observed in the four patients studied after injection of corticosteroids into the affected knee joint (Table 4).

Discussion

The most significant results in the present study were firstly, the high levels of ir-VIP seen in the synovial fluid from patients receiving no treatment at the sampling time compared with the treated group of patients with inflammatory joint disease, and secondly, that the concentration of ir-VIP in the

synovial fluid fell drastically after intra-articular injection of corticosteroids into the affected knee joint in the four patients studied.

VIP is considered to be a neurotransmitter in peptidergic autonomic nerve endings and VIP-ergic neurones have been found in several organ systems,¹⁰ but a possible relationship to joint structures has, to our knowledge, not yet been studied. Therefore, we do not know whether the high concentration of ir-VIP in the synovial fluid is caused by local release from VIP-ergic nerve endings. Another and perhaps more intriguing possibility, however, is that VIP may be released from leucocytes in the synovial fluid since ir-VIP is present in human polymorphonuclear and mononuclear leucocytes.¹ Furthermore, since the concentration of ir-VIP in the synovial fluid was significantly higher than in the serum, and no significant differences were found in circulating ir-VIP between treated and untreated patients, it is most likely that VIP is released locally at the site of inflammation, perhaps by inflammatory cells. The effect of intra-articular corticosteroids on the concentration of ir-VIP in the synovial fluid may suggest a relationship between ir-VIP and the degree of inflammation.

The pathophysiological significance of the present findings is not known. Among the leucocytes, specific VIP receptors have been found on the mononuclear cells only,¹¹ and it is possible that VIP, in addition to a possible neuronal release, may be released from polymorphonuclear leucocytes and in turn bind to receptors on the lymphocytes to modify the function of these cells. This probably occurs via activation of the adenylate cyclase-cyclic AMP system since VIP is known to stimulate adenylate cyclase and hence the production of cyclic AMP in the lymphocytes.^{11 12} Furthermore, vasodilatation is one of the most prominent effects of VIP,¹³ and therefore VIP may also be of significance in the mechanism of local vasodilatation at the inflammatory site.

Recently, we have found high levels of circulating VIP in experimentally induced endotoxaemia in pigs,¹⁴ and the present findings strongly suggest that VIP may be of significance in inflammatory disorders as well. Further support for this suggestion is the wheal and flare response seen after intradermal injection of VIP³ and that VIP, in addition to its presence in polymorphonuclear and mononuclear white blood cells,¹ also has been found in mast cells,² all cell types that are involved in joint inflammation.

In the present study no significant differences in the serum levels of ir-VIP, ir-PP, or ir-somatostatin were noted between controls and treated or untreated groups of patients. Significantly higher levels

of ir-secretin were found, however, in the serum of drug treated patients, probably reflecting the effect of anti-inflammatory treatment as previously suggested for secretin.⁵

The level of ir-secretin in the serum was significantly higher than that in the synovial fluid, while only a slight but not significant increase in serum ir-PP was seen. Both peptides are produced in special endocrine cells and are considered to be circulating hormones.^{15 16} Therefore their existence in the synovial fluid can most probably be ascribed to diffusion from the blood.

In contrast, somatostatin has in addition to its possible hormonal role also been suggested as a putative neurotransmitter substance,¹⁷ and recently somatostatin has also been shown to be present in leucocytes.¹ Thus the amount of ir-somatostatin in the synovial fluid, which was slightly but not significantly higher than in the serum, can be ascribed both to diffusion from the blood and to a possible neuronal release and to release of somatostatin from leucocytes in the synovial fluid.

The role of somatostatin in the synovial fluid is not known. Since somatostatin is known to have inhibitory effects on the release of several regulatory peptides, including VIP,¹⁸ it may also have an inhibitory effect on the release of VIP in the synovial fluid. This, however, needs to be studied further.

In conclusion, we have found increased levels of ir-VIP in the synovial fluid from untreated patients with inflammatory joint disease and a level of ir-VIP in the synovial fluid higher than that in the serum. In addition, intra-articular injection of corticosteroids in affected knee joints seems to suppress the level of ir-VIP in the synovial fluid. The results suggest that VIP is involved in joint inflammation.

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