

Neuropeptide levels in Dercum's disease (adiposis dolorosa)

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SUMMARY

Dercum's disease (adiposis dolorosa) is characterised by adiposity and chronic pain in the adipose tissue. It has been proposed that conditions encompassing chronic pain have altered concentrations of neuropeptides involved in pain transmission. The aim of this investigation was to examine whether patients with Dercum's disease have abnormal concentrations of different neuropeptides. In cerebrospinal fluid (CSF) and in plasma (P) from 53 patients with Dercum's disease substance P-like immunoreactivity (SP-LI), neuropeptide Y-like immunoreactivity (NPY-LI), β -endorphin-like immunoreactivity (β -END-LI), calcitonin gene-related peptide-like immunoreactivity (CGRP-LI), met-enkephalin-like immunoreactivity (m-ENK-LI), vasoactive intestinal polypeptide-like immunoreactivity (VIP-LI), somatostatin (SOM-LI), γ_2 -melanocyte-stimulating hormone-like immunoreactivity (γ_2 -MSH-LI), and dynorphin-like immunoreactivity (DYN-LI) were measured. Three of the substances were also measured in a control group. The CSF concentration of SP was statistically significantly lower in the Dercum group than in the control group, whereas NPY-LI and β -END-LI were borderline statistically significantly lower and higher, respectively, in Dercum patients compared to controls. Compared with reference values, CSF-MSH-LI levels were slightly elevated and CSF-NPY-LI levels were slightly lowered in the Dercum group. The other substances in both CSF and plasma were within the reference values with a high degree of statistical significance. In conclusion, altered levels of neuropeptides that have previously been seen in different pain conditions cannot clearly be demonstrated in Dercum's disease.

Key words: Dercum's disease, adiposis dolorosa, neuropeptides, chronic pain.

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INTRODUCTION

Dercum's disease (adiposis dolorosa) is characterised by pronounced pain in the adipose tissue and a number of associated symptoms. The pain is chronic (>3 months), symmetrical, and is often disabling and resistant to traditional analgesics (1, 2). The aetiology of Dercum's disease is unknown, but an inflammatory component has been proposed (2, 3). An inflammation leads to the release of neuropeptides that give rise to pain. It has been suggested that chronic pain syndroms, for instance fibromyalgia (4), encompass abnormal concentrations of central nervous system neuropeptides involved in pain transmission.

The aim of this investigation was to examine whether patients with Dercum's disease have abnormal concentrations of different neuropeptides, substances related to pain, in cerebrospinal fluid (CSF) and plasma (P).

MATERIAL AND METHODS

Patients and controls

A total of 53 patients fulfilling the clinical criteria of Dercum's disease were recruited and referred to the Department of Plastic and Reconstructive Surgery in Malmö by one of the authors. We defined Dercum's disease as adiposity and chronic pain (>3 months) in the adipose tissue (1).

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Diagnosis was based on the medical history evaluated from a standardised questionnaire and a systematic physical examination on three separate visits. The purpose of the questionnaire and the examination was to exclude other explanations than Dercum's disease to the patients' symptoms.

The included subjects did not have any disease, other than Dercum's disease, involving inflammation or pain. Moreover, none of the patients had any psychiatric diagnosis, such as major depression or schizophrenia.

The patients were referred to the Department of Plastic Surgery to be included in a study on the effect of liposuction on the pain experienced by the patients with Dercum's disease. The first 53 consecutively referred patients were later operated on with liposuction, and the following 58 women with Dercum's disease were recruited as controls to be able to evaluate the effect of liposuction. The result of that study has been reported elsewhere (5). The tests in this study were performed in connection with the liposuction.

Controls were selected among women, with no acute or chronic pain, that were to be operated on with abdominoplasty at the same department. In total, 40 women with BMI and age in a similar span as the Dercum patients were recruited for this purpose.

Ethics

The study was approved by the Ethics of Human Investigation Committee at Lund University (LU 236-89 and LU 422-91). All participants gave their informed consent to participate. The procedures followed were in accordance with the Declaration of Helsinki of 1964, and following revisions.

Radioimmunoassays

The examined neuropeptides in CSF were: substance P-like immunoreactivity (SP-LI), neuropeptide Y-like immunoreactivity (NPY-LI), β -endorphin-like immunoreactivity (β -END-LI), calcitonin gene-related peptide-like immunoreactivity (CGRP-LI), met-enkephalin-like immunoreactivity (m-ENK-LI), vasoactive intestinal polypep-

ptide-like immunoreactivity (VIP-LI), somatostatin (SOM-LI), γ_2 -melanocyte-stimulating hormone-like immunoreactivity (γ_2 -MSH-LI), and dynorphin-like immunoreactivity (DYN-LI).

The same substances in plasma were analyzed with the exception of DYN and γ_2 -MSH. The erythrocyte sedimentation rate (ESR) was also measured. All substances were measured in the Dercum group. The control group was used for analysis of three peptides in CSF, namely SP-LI, NPY-LI and β -END-LI.

Samples were collected after overnight fasting: CSF samples (12 mL) were taken peroperatively and blood samples at admittance to hospital before the surgery. No complications developed following the collection of the specimens.

The CSF samples were collected in unprepared plastic tubes and the blood samples, except that for analysis of SOM-LI, in EDTA tubes. SOM-LI samples were collected in sodium citrate tubes.

All samples were immediately cooled in ice water and centrifuged at 4°C. The plasma, as well as the supernatant of the CSF samples were divided into three portions and stored in plastic tubes at -20°C (SOM at -70°C) until assay.

Quantitative analysis of SP-LI, CGRP-LI, SOM-LI, β -END-LI and NPY-LI was performed using different immunoassay systems. For the radioimmunoassays of the various peptides the samples were analyzed in serial dilutions, optimized to the linear part of the standard curve and corrected for non-specific binding. Since cross-reactivity with other peptides or proteins, sharing immuno-determinants with the analyzed peptide, cannot be excluded it is appropriate to refer to like immunoreactivity, for example SP-LI, rather than to the respective peptide.

SP-LI was quantified using a rabbit antiserum (SP-2, provided by Dr.E. Brodin, Stockholm, Sweden) (6) in a final solution of 1:50,000 with (Tyr⁸)-SP as tracer. The SP-2 antiserum does not detect any known tachykinin besides SP (6). The detection limit was 0.5 pmol/L and interassay variation <8%. NPY-LI was analyzed as pre-

viously described (7). A rabbit antiserum raised against porcine NPY (provided by Dr P.C. Emson, Cambridge, UK) was used. The antiserum cross-reacts with peptide YY (PYY) to 33%, but not with C-terminal fragments of NPY and PYY. Detection limit is 11 pmol/L and the interassay coefficient of variation <7%. β -END-LI was quantified using a rabbit antiserum (K-7762, and antiserum 5422, provided by Dr D Marshak, Houston, Texas, USA) (8) raised against unconjugated synthetic human β -END. The antiserum was used in a final dilution of 1:25,000 and has negligible cross-reactivity against β -lipotropin (<1.5%). The detection limit in a direct assay was 10 pmol/l and the interassay coefficient of variation <10%. CGRP-LI was quantified using a rabbit antiserum (R-8429) (6) raised against synthetic rat CGRP. CGRP was conjugated to bovine serum albumin and used in a final dilution of 1:37 500. This allowed measurements of CGRP-like material with a minimum of 10 pmol/L. The interassay variation was 12%. ENK-LI was determined using a rabbit antiserum (5422, provided by Dr. K.H. Voigt, Ulm, Germany) (8) in a final dilution of 1:20,000 with a detection limit of 10 pmol/L. The antibody recognises the C-terminus of ENK and does not cross-react with leu-enkephalin, β -END and dynorphin (DYN). The interassay variation was <10% (8). VIP-LI was determined using a rabbit antiserum (code 7852, Milab, Malmö, Sweden). The antiserum was used in dilution of 1:60,000 and does not cross-react with the peptides histidine, isoleucine, secretin, or glucagon. The detection limit was 6 pmol/L and the interassay variation 8.5% (6). SOM-LI was determined using a rabbit antiserum (K18, Milab, Malmö, Sweden) in a final solution of 1:25,000.

The antiserum has not shown cross-reaction with any other known neuropeptide except cyclic SOM (100%), linear SOM (50%) and (Tyr¹)-SOM (100%). The detection limit was 6 pmol/l, expressed as SOM-IR 15-28 equivalents.

The interassay variation was <12% (9). γ_2 -MSH-LI was determined using a rabbit

antiserum in a final dilution of 1:75,000 (K-8032) (10). The antiserum does not show cross-reaction with related peptides such as γ_1 -MSH, γ_3 -MSH or other peptides containing the MSH sequence (α -MSH, β -MSH, ACTH 4-10). The detection limit for the assay was 5 pmol/L and the interassay variation <11%. DYN-LI was determined using a rabbit antiserum (K-8027) (11) in a final dilution of 1:12,500. The antiserum was directed against the C-terminal portion of dynorphin 1-13 and cross-reacted with dynorphin 1-9, 1-10, and 1-11 <3.3%. The detection limit was 20 pmol/L and the interassay coefficient of variation was <12% (11).

The erythrocyte sedimentation rate (ESR) was measured using the Westergren method, that is 4 parts blood are diluted with one part isotonic citric solution. The level of sedimentation is measured after one hour (12).

Statistical analysis

Values were given as medians and ranges. Histograms were drawn to examine the distribution of the measured factors. The histograms indicated that the factors were not normally distributed and hence medians and ranges were used, as well as non-parametric tests. Differences between cases and controls in CSF concentrations of three substances (SP-LI, NPY-LI, β -END-LI) were analysed using the Mann-Whitney test.

When comparisons were made between the patients' values and reference values for all factors, the patients' values and reference values were all logarithmed, to create values with a normal distribution, and tested against each other with the one sample *t*-test. In cases of reference intervals, the patient group's mean values were tested against the upper and lower reference values. A significant difference thus indicated that the patient group's mean values were within the reference values.

A non-significant P value indicates that the patient group's mean value was different from the reference values. A P value of <0.05 was considered to indicate a statistically significant difference.

■ RESULTS

Baseline characteristics are given in Table I. The CSF concentrations of SP-LI was statistically significantly lower in the Dercum group than in the control group, whereas NPY-LI and β -END-LI were borderline statistically significantly lower and

higher, respectively, in the two groups (Table II).

Compared with reference values, CSP-Sp-LI and P-Sp-LI were elevated, CSF- γ_2 -MSH-LI levels were slightly elevated and CSF-NPY-LI levels were slightly lowered in the Dercum group. The other substances in both CSF and plasma were within the reference values with a high degree of statistical significance (Table III).

Table I - Baseline characteristics; values are given as medians and ranges.

	Dercum (n=53)	Control (n=40)
Age (years)	50.5 (22-68)	50.0 (26-69)
Weight (kg)	94.6 (55-140)	90.7 (55-129)
BMI (kg/m ²)	34.6 (28-55)	34.2 (28-46)

■ DISCUSSION

Altered, mostly elevated, levels of neuropeptides that have previously been seen

Table II - Neuropeptides measured in both patients and controls.

Substance (pmol/L)	Group	n	Median (range)	Difference between the groups (P value)
CSF-SP-LI	Dercum Control	51	12 (6-45)	<0.001
		40	17(8-60)	
CSF-NPY-LI	Dercum Control	51	119 (44-266)	0.053
		40	130 (25-205)	
CSF-b-END-LI	Dercum Control	51	110 (81-178)	0.080
		40	107 (17-177)	

CSF, cerebrospinal fluid.

Table III - Neuropeptides measured in patients with Dercum's disease.

Substance (pmol/L)	n	Median (range)	Reference	Tested value (reference value before log)	Difference between log measured value and log reference value (P value)
CSF-SP-LI	51	12 (6-45)	<10	10	<0.001
CSF-NPY-LI	51	119 (44-266)	120-170	120; 170	0.354; 0.157
CSF-b-END-LI	51	110 (81-178)	80-110	80; 110	<0.001; <0.001
CSF-CGRP-LI	44	4 (4-7)	<10	10	<0.001
CSF-m-ENK-LI	45	99 (60-271)	30-150	30; 150	<0.001; <0.001
CSF-VIP-LI	44	16.5 (3-59)	<20	20	<0.001
CSF-SOM-LI	45	27 (14-78)	20-50	20; 50	<0.001; <0.001
CSF-g2-MSH-LI	18	27 (10-82)	<20	20	0.048
CSF-DYN-LI	45	24 (12-69)	20 - 50	20; 50	<0.001; <0.001
P-SP-LI	47	2 (1-7)	<4	4	0.005
P-NPY-LI	47	119 (60-278)	<130	130	<0.001
P-b-END-LI	47	38 (26-70)	30-45	30; 45	<0.001; <0.001
P-CGRP-LI	47	15 (10-80)	<40	40	<0.001
P-m-ENK-LI	47	20 (20-48)	<60	60	<0.001
P-VIP-LI	47	5 (3-15)	<20	20	<0.001
P-SOM-LI	47	6 (4-14)	<12	12	<0.001
ESR (mm)	48	11 (4-38)	<21	21	<0.001

in different pain conditions cannot clearly be demonstrated in Dercum's disease.

Methodological issues

The operational criteria of Dercum's disease, used in this survey, are adiposity and chronic pain (>3 months) in the adipose tissue. However, the criteria have not been validated, and there is no clear consensus regarding which criteria should be used to make the diagnosis (13). In 1901, Roux and Vitaut (14) proposed four cardinal symptoms of Dercum's disease, namely:

1. multiple, painful, fatty masses;
2. generalised adiposity;
3. weakness and fatigability (asthenia);
4. psychiatric manifestation, including emotional instability, depression, epilepsy, confusion and dementia.

However, it can be discussed which are to be considered cardinal symptoms and which associated. It can be argued that the third cardinal symptom, weakness and fatigability, frequently is part of different psychiatric disorders, including depression (15). Furthermore, severe adiposity is associated with sleeping disorders (16), which could contribute to the weakness and fatigability experienced by patients with Dercum's disease.

As regards the fourth cardinal symptom, that is, psychiatric manifestation, modern research has revealed that pain is a common symptom in depression (17). Similarly, it has been demonstrated that there is a co-morbidity of chronic pain disease and psychiatric disorders (18).

Furthermore, an association between BMI and anxiety and personality disorders has been seen (19). Hence, the patients' pain as well as their adiposity could contribute to psychiatric manifestations in Dercum's disease. Following the complex issue of defining Dercum's disease, we used a "minimal definition", including only adiposity and pain. We believe that this may limit the problem of misclassification with regard to other inflammatory or psychiatric conditions. However, it is unclear how many of the patients, that fulfil criteria of Dercum's disease used in this study, also fulfil the criteria of, for instance, fibromy-

algia and other pain syndromes. In fact, there is a clear overlap in diagnostic criteria symptoms for Dercum's disease and other conditions (20). These syndromes are sometimes referred to as functional somatic syndromes and their aetiology is unclear. The functional somatic syndromes have a number of features in common, that is, no objectively observable abnormalities can be found in the patients, the syndromes often affect predominately females; they are negatively affected by stress, and demonstrate co-existing emotional disorder. Moreover, the syndromes have similar prognosis and respond to different treatments in a similar way (20). We noted wide ranges in all measured factors, which with a few exceptions, did not differ from the reference values.

One possible explanation for these findings is that there seems to be a close interrelation between Dercum's disease and other conditions encompassing adiposity accompanied with various states of pain.

As the prevalence of Dercum's disease is unknown, it is difficult to know how many patients could be eligible for inclusion in the study. However, the strength of the present study is that the same consultant diagnosed all the patients.

Nonetheless, a weakness is that only the patients examined by the same consultant had the possibility to be included in the study. It cannot be assessed if the patients seeking medical treatment differ from the patients that abstain from contact with physicians.

Nonetheless, it is likely that patients with pronounced pain seek medical care more readily than patients with little pain. Moreover, only patients interested in surgical treatment of their condition were referred to us. For these reasons, it cannot be judged if our sample was representative for Dercum's disease in a wider perspective. It is likely that the patients in the study actually experienced more pain than other Dercum patients and therefore the prospect of finding a characteristic alteration or profile in neuropeptides for Dercum's disease would seem apparent in our study group if such a profile exists.

A type I error (alfa-error) means that the null hypothesis is falsely rejected, giving a 'false positive' result. The risk of occurrence of a type I error increases with the number of comparisons conducted. However, in this study we saw considerably more statistically significant results than would be expected by chance alone.

Type II error (beta-error) means that the null hypothesis fails to be rejected and as a consequence an actual difference between populations is missed. A limitation of this study is that Dercum's disease is a rare condition, and as a consequence the number of patients recruited, and hence the statistical power, might be low. Another limitation of the present study is that we had no information on the use of over the counter analgesics. It is possible that such drugs could have affected the levels of neuropeptides, but to our knowledge the direction of such changes, with regard to the studied factors in this study, is not well known and this may have introduced a confounding effect. A strength of the study is that laboratory tests with low coefficients of variation were used. However, a weakness is that there are some missing values, as only three substances were measured in the control group. Unfortunately, during the time when the samples from the controls were analysed, only Sp-SP, Sp-NPY, and Sp- β -End could be analyzed, due to administrative reasons. Hence, all the substances were measured in Dercum patients but not in controls.

Findings and previous studies

An inflammatory aetiology has been proposed for Dercum's disease (2, 3). However, standard laboratory markers for inflammatory and autoimmune disease are commonly negative in the condition (1). Similarly, normal ESR levels were seen in the present study (Table III).

SP-LI is a neuromodulator present in several neural pathways associated with pain. Previous studies on patients with diseases involving chronic idiopathic pain and with conditions associated with pain such as depression (21) and fibromyalgia (4), have pointed out a clear elevation of SP-LI in

CSF. However, there are also studies on chronic pain and SP-LI levels that have suggested significantly lower levels in patients with chronic pain syndromes than in healthy controls, especially among patients with chronic neurogenic or idiopathic pain (22). This divergence may be explained by findings in animal models where it has been proposed that SP may be an important factor in the early response to pain, but insignificant in the maintenance of central sensitization (23). This could explain why our patients, that all have a long history of pain, only have moderately elevated SP-LI values.

It has been suggested that NPY-LI concentration is an indicator of sympathetic-neuronal output as it is a neurotransmitter released principally by sympathetic neurons (24). A few previous studies have demonstrated that patients with fibromyalgia have elevated plasma levels of NPY-LI (24, 25), even though the finding is more controversial than the increase in substance P, which has been confirmed by several studies. Martinez-Lavin et al. (24) have suggested that an autonomic nervous system dysfunction might be the crucial factor in the pathogenesis of fibromyalgia. In contrast to this, the subjects in this study have slightly decreased levels of NPY-LI in CSF compared to reference values (Table II) and statistically significantly lower levels than the controls ($P=0.048$).

β -END-LI is an endogenous opioid peptide derived from one of three endogenous opioid systems. It plays an important role in the mechanism of pain and an experimentally raised level of β -END alter the peripheral pain threshold (26). Furthermore, a small clinical study has suggested that β -END-LI could be decreased in certain chronic painful conditions and normal in others (27, 28). This is in accordance with the normal levels of β -END found in the Dercum patients. DYN-LI and m-ENK-LI are peptides from the other major endogenous opioid system. The normal levels of β -END, DYN and m-ENK suggest that there is no endorphin deficiency in Dercum's disease.

MSH-LI is one of the peptides derived from

melanocortins and it is released at different sites in both the central nervous system and the peripheral tissue. It has a variety of functions including immunomodulation, anti-inflammatory effects and facilitation of nerve regeneration following peripheral nerve injury. Furthermore, MSH-LI and related peptides seem to have a role in pain processing and the induction of analgesia (29). The median γ_2 -MSH-LI was slightly elevated among our subjects compared to reference values.

SOM-LI can be found in both the peripheral and the central nervous system and has several functions. Studies using experimental pain models, mainly regarding acute pain, have demonstrated that SOM-LI release can alter the experience of pain, and the release of other neuropeptides (30). There are few, if any, previous studies on SOM-LI in chronic pain, and our findings suggest the pain in Dercum's disease cannot be related to changes in SOM-LI.

In conclusion, altered, mostly elevated, levels of neuropeptides that have previously been seen in different pain conditions cannot clearly be demonstrated in Dercum's disease. The findings of this study indicate that neuropeptides cannot be used as diagnostic markers for Dercum's disease.

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Abbreviations

β -END	β -endorphin
CGRP	Calcitonin gene-related peptide
DYN	Dynorphin
LI	Like immunoreactivity
m-ENK	Met-enkephalin
NPY	Neuropeptide Y
VIP	Vasoactive intestinal polypeptide
SOM	Somatostatin
SP	Substance P
γ_2 -MSH	γ_2 -melanocyte-stimulating hormone

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