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Plasma Concentrations and Urinary Excretion of Regulatory Peptides in Patients with Urticaria Pigmentosa

Klas Nordlind and Elvar Theodorsson

Department of Dermatology, Academic Hospital, Uppsala, and Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden

ABSTRACT

Patients with urticaria pigmentosa were investigated during symptom-free interval regarding plasma concentrations and urinary excretion of immunoreactive regulatory peptides: calcitonin gene-related peptide (CGRP), gastrin, neurokinin A (NKA), neuropeptide Y (NPY), somatostatin (SOM), substance P (SP) and vasoactive intestinal peptide (VIP). The plasma concentrations of these peptides, except for CGRP, were below the detection limit. The urinary excretion of the regulatory peptides were not higher in the patient group than in the controls, but in individual patients there was high urinary excretion of SP and VIP. A lower urinary excretion of CGRP was found in the patient group in addition to a tendency to a lower plasma concentration.

INTRODUCTION

In mastocytosis there is a pathologic accumulation of mast cells in various tissues of the body. In most cases the mast cell increase occurs in the skin and the disease is then called urticaria pigmentosa. Common symptoms are pruritus, flushing and whealing, which often develop after strenuous work, sweating, or warm and cold baths, but may also occur spontaneously. Mammalian normal and tumor mast cells have been shown to contain several biologically active substances, including sulfated glycosaminoglycans, histamine, melanin, dopamine, serotonin, arachidonic acid metabolites (for refs see 18, 24) and also VIP

(9). In a previous immunohistochemical investigation (18), somatostatin-like immuno-reactivity was found in skin lesions of patients presenting with urticaria pigmentosa. Some regulatory peptides such as CGRP, opioid peptides, SOM, SP and VIP have been shown to be distributed in neurons in sensory ganglia and/or autonomic ganglia, as well as in peripheral nerve fibers (for refs see e.g. 17). The role of the peptides is not clear, but they may have an inflammatory or trophic effect, as well as functioning as neuromediators by electrophysiological criteria (16).

There is morphologic contact between mast cells and sensory nerves (see e.g. 23), and neuropeptides such as SP (8, 21, 23), VIP and SOM (8) have been shown to release histamine from human cutaneous mast cells.

An increased expression of some regulatory peptides in diseased skin has been reported, e.g. CGRP in prurigo nodularis (1,34), NPY in atopic dermatitis (25), SP in psoriasis (23), atopic dermatitis (22) and prurigo nodularis (1), VIP in eczema (2,21), psoriasis (2,27) and in lichen sclerosus et atrophicus (19), SP and VIP in psoriasis (13) and in bullous and inflammatory skin disease (35), SOM and avian pancreatic polypeptide in diabetic lipodystrophic skin (20), and CGRP, SP and VIP in dermographism and urticaria (36).

As a marker of disease activity in urticaria pigmentosa, the excretion of metabolites of the biogenic amine histamine has been investigated (24). Biogenic amines and regulatory peptides may be colocalized in different tissues. Regulatory peptides are degraded by specific enzymes to biologically inactive metabolites, which may be excreted in the urine.

There have been few reports about urinary excretion of regulatory peptides in normal and pathologic conditions (3,11,30). There might be a possibility of detecting a minor increase in the peptide turnover by analyzing urine samples collected over a 24-h period, since peptide metabolites might be excreted by the kidneys, and antisera as a rule bind to a small part of the peptide, which may be present in the metabolites.

In the present investigation patients with urticaria pigmentosa were

investigated during a symptom-free interval with regard to the plasma concentrations and urinary excretion of various regulatory peptides, namely CGRP, gastrin, NKA, NPY, SOM, SP and VIP, in order to find out whether these patients showed an increased level and excretion of neuropeptides.

MATERIAL AND METHODS

Patients. Fifteen patients with a mean age of 36 years (range 3-73 years) were admitted to the Department of Dermatology for investigation of urticaria pigmentosa generally affecting the extremities and the trunk. Twenty-six healthy subjects with a mean age of 38 years (5-60 years) were used as control persons.

Plasma samples from six patients and seven control subjects were analyzed.

Urine samples were taken over a 24-h period from 8-15 patients and from 18-26 of the control persons and aliquots of 50 ml were extracted using SepPak (Waters) (31).

Measurement of regulatory neuropeptides. Regulatory peptides were measured by means of competitive radioimmunoassays.

CGRP-like immunoreactivity (CGRP-LI) was assayed using antiserum CGRP8 raised in a rabbit against conjugated rat CGRP. HPLC-purified ^{125}I-Histidyl rat CGRP was used as radioligand and rat CGRP as standard. The detection limit of the assay for rat CGRP is 9 pmol/L and the cross-reactivity of the assay to SP, NKA, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, NPY and calcitonin was less than 0.01%. The cross-reactivities to human CGRP alpha and beta were 93% and 24%, respectively, and to rat CGRP alpha and beta, 100% and 120%, respectively. The intra- and interassay coefficients of variation were 8% and 14%, respectively. Gastrin-LI was assayed with antiserum 4562 (kindly provided by Professor Jens Rehfeld, Rigshospitalet, Copenhagen, Denmark). This antiserum was raised against human non-sulfated sequence 2-17 of gastrin-17. Setting the immunoreactivity for non-sulfated gastrin-17 to 100%, the immuno-reactivity for non-sulfated gastrin-34 is 64%. The antiserum has considerably lower cross-

reactivity to sulfated than to non-sulfated gastrins. Thus the ratio of sulfated to non-sulfated gastrin-34 immunoreactivity is 0.23 (28). The detection limit of the assay was 3.5 pmol/L. The intra- and interassay coefficients were 6% and 11%, respectively. NKA-LI was assayed with antiserum K12, which reacts with NKA (100%), NKA(3-10) (48%), NKA(4-10) (45%), neurokinin B (26%), neuropeptide K (61%) and eledoisin (30%), but not with SP (32). The detection limit of the assay was 12 pmol/L. The intra- and interassay coefficients of variation were 7% and 12%, respectively. NPY-LI was assayed with antiserum N1, which cross-reacts 0.1% with avian pancreatic polypeptide, but not with other peptides (33). The detection limit of the assay was 11 pmol/L. The intra- and interassay coefficients of variation were 7% and 12%, respectively. SOM-LI was measured by a competitive immunoassay based on a monoclonal antiserum (Novo Clone SOM-2-28, Novo Bio Labs, Denmark). The detection limit of the assay was 2.3 pmol/L. The intra- and interassay coefficients were 6% and 9%, respectively. SP-LI was assayed with antiserum SP2 (6), which reacts with SP and SP sulfoxide, but not with other tachykinins. The detection limit was 10 pmol/L. The intra- and interassay coefficients of variation were 7% and 11%, respectively. VIP-LI was measured using antiserum 5603-7, kindly provided by Professor Jan Fahrenkrug (15). The detection limit of the assay was 3 pmol/L. The intra- and interassay coefficients of variation were 7% and 11%, respectively.

Statistics. The median and interquartile ranges were used as measures of central tendency and variation, respectively, throughout the study. They will be expressed as follows: median: upper quartile-lower quartile. The Mann-Whitney U test was used for statistical evaluation of results.

RESULTS

Plasma. The plasma concentrations of gastrin, NKA, NPY, SOM, SP, and VIP were below the detection limits of the assays both in the patient and in the control group. The difference in CGRP between patients and controls was not statistically significant (22+4 and 31+10 (mean+SD) respectively).

Urine. In the patient group none of the peptides showed a significantly increased urinary excretion (Fig. 1), but in four patients high excretion values of SP (10, 30, 32 and 74 pmol/day) and in three patients a high excretion of VIP (6, 9 and 13 pmol/day), were observed. In two of these patients, without other clinical symptoms, both these peptides showed elevated values.

A significantly ($p<0.01$) decreased urinary excretion of CGRP was found in the group of patients (median and quartiles 14.7:8.9-24) compared with the control group (median and quartiles 35:32-37).

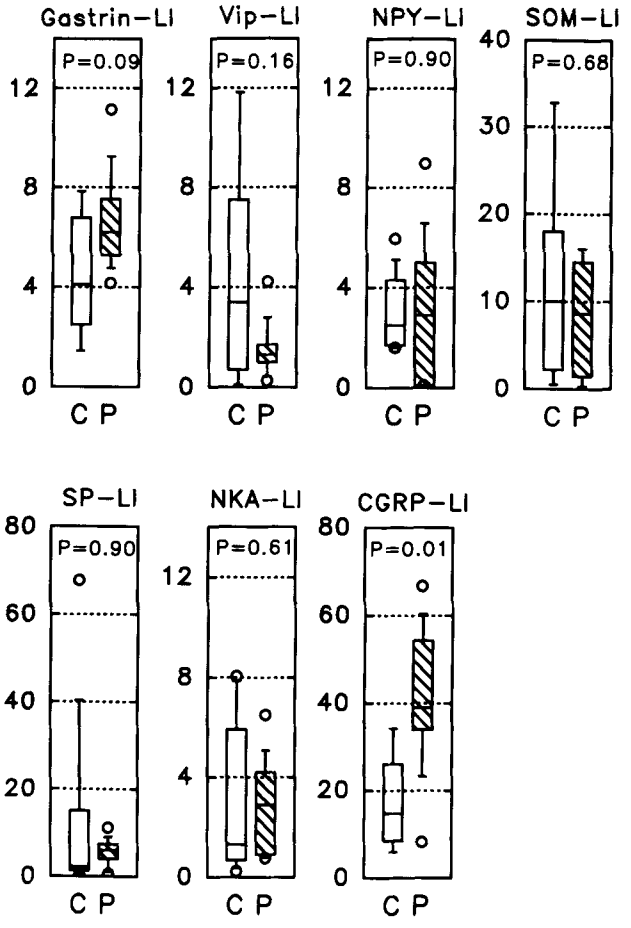

Fig. 1. Box plots of the urinary excretion (pmol/24 h) of immunoreactive regulatory peptidesin patients with urticaria pigmentosa (open boxes) and control subjects (hatched boxes).

DISCUSSION

An increased production of regulatory peptides has been observed in a wide range of endocrine and nonendocrine tumors. For instance VIP hypersecretion may be a feature of tumors such as pheochromocytoma and hepatocellular carcinoma (7,29), and an elevated concentration of this peptide has been found in the plasma in a case of solitary cutaneous mastocytoma (37).

Most regulatory peptides have a very short half-life in the blood due to rapid degradation (10,14), produced by peptidases. Moreover, mast cells contain proteases that degrade SP and VIP (22). There may also be an indirect mechanism of degradation as an increased level of SP may lead to a decreased level of CGRP as a result of an effect on mast cells and release of proteases degrading CGRP (4,5).

The absence of an increase in urinary excretion of the peptides in this group of patients with urticaria pigmentosa might be due to a short turnover time in the plasma as well as to metabolization of the peptides in the kidneys, and does not rule out the possibility that there may be some increase in the excretion but not sufficiently high to overrule the metabolization rate. As regards CGRP, this peptide might be especially prone to this effect, giving a decreased urinary excretion in the patient group compared with the controls.

Our results might also explain the relative lack of symptoms shown by patients with urticaria pigmentosa, i.e., secreted peptides such as VIP, may undergo rapid degradation.

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Address for reprints:
Klas Nordlind
Department of Dermatology
University Hospital
S-751 85 Uppsala