CLINICAL STUDY

Adrenomedullin, a new peptide, in patients with insulinoma

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Abstract

Background: It has been demonstrated that adrenomedullin, a newly discovered peptide, affects the release of insulin from pancreatic islets cells, suggesting a role in the insulin-regulating system.

Objective: To investigate whether adrenomedullin secretion is modified in patients with insulin-secreting islet cell tumours.

Design: The study was performed in nine patients with surgically treated insulinoma. Circulating adrenomedullin was assayed using a specific radioimmunoassay and its localization and distribution in the tumour were determined by means of immunohistochemistry.

Results: Adrenomedullin concentrations were significantly greater in patients with insulinoma (6.6 ± 3.2 fmol/ml) than in controls (2.1 ± 1.1 fmol/ml). In six patients monitored before and after surgery, plasma adrenomedullin decreased from 6.3 ± 2.9 fmol/ml to 3.0 ± 1.6 fmol/ml. Immunoreactive adrenomedullin was localized exclusively in the tumour cells, whereas stroma, surrounding pancreas parenchyma and major ducts were negative for the peptide.

Conclusions: Our findings indicate that circulating adrenomedullin is increased in insulinoma and that this increase is related to the neoplastic phenotype.

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Introduction

Adrenomedullin is a potent hypotensive peptide originally discovered in extracts of human phaeochromocytoma (1). Adrenomedullin-immunoreactive cells are widely distributed in human tissues, including the endocrine and neuroendocrine adrenal medulla cells, the pancreatic islets, placenta, uterus, anterior pituitary gland and the gastrointestinal neuroendocrine system (2, 3). Adrenomedullin itself is secreted by endothelial cells and is present in plasma (4). It has been shown to regulate the production and secretion of some hormones (5, 6) and to modulate cellular proliferation and differentiation (7, 8).

Exogenous adrenomedullin inhibited the secretion of insulin from isolated rat pancreatic islets and the monoclonal adrenomedullin antibody was able to increase insulin release from isolated pancreatic islets, in the absence of exogenous adrenomedullin (9), suggesting that this peptide may be implicated in the insulin regulatory system.

Although histopathological studies have demonstrated the presence of adrenomedullin in some human neuroendocrine tumours (10, 11), there are no data available on the presence of adrenomedullin in insulinomas or on the behaviour of circulating adrenomedullin in patients with insulinoma.

The purpose of this study was to examine plasma concentrations of adrenomedullin in patients with insulinoma in comparison with healthy subjects, and to determine the tissue distribution of immunoreactive adrenomedullin in insulinoma neoplastic cells.

Subjects and methods

Study participants

Nine patients with insulinoma (four men and five women; mean age 48 ± 12 years) and 15 healthy subjects (seven men and eight women; mean age 44 ± 11 years) were studied.

In patients with insulinoma, the diagnosis of insulin-secreting islet cell tumour was based on the development of symptomatic hypoglycaemia (blood glucose ≤2.5 mmol/l) with inappropriate insulin concentrations during prolonged fasting (72 h). Once the biochemical diagnosis was confirmed, localization procedures were performed. Tumours were localized by means of abdominal ultrasonography (n = 4), computed tomography (n = 9), scintigraphy with
Subjects and methods

Sample collection

At the time of samples collection, patients and controls stayed in the recumbent position for at least 30 min. Samples were taken between 0800 and 0900 h, from the antecubital vein. Five millilitres of blood were collected in polystyrene tubes containing EDTA (1 mg/ml) and aprotinin (5000 KIU/ml) and placed on ice. The blood samples were then centrifuged at 3000 g for 15 min. The plasma was immediately frozen and stored in polypropylene tubes at −80 °C until required for analysis. In six patients with insulinoma, plasma adrenomedullin concentrations were measured before and after (approximately 4 weeks) tumour resection.

This study was approved by the local ethics committee and informed consent was obtained from all participants.

Adrenomedullin measurement

Plasma adrenomedullin was measured by means of a specific RIA, as described previously (12, 13). Briefly, 2 ml sample was applied to conditioned Sep-Pak C18 columns (Millipore Corp. Waters Chromatography, Milford, MA, USA), and the column was sequentially washed with 5 ml of 20% acetonitrile in 0.1% trifluoroacetic acid. The absorbed material was eluted with 4 ml of 50% acetonitrile, and the eluate was lyophilized. After lyophilization, samples were dissolved in 50 mmol/L phosphate buffer (pH 7.4) and adrenomedullin was measured in plasma by RIA using a commercial Kit (Phoenix Pharmaceuticals Inc., Mountain View, CA, USA) with rabbit polyclonal antibody raised against human adrenomedullin 1–52. The antibody cross-reacts 100% with human adrenomedullin; no cross-reactivity was reported with rat adrenomedullin, endothelin-1 or atrial natriuretic peptide. The intra- and- interassay coefficients of variance were 5.1% and 12% respectively.

Immunohistochemical analysis

The indirect avidin–biotin complex (ABC) immunoperoxidase assay was performed on dewaxed and rehydrated 2-μm sections of formalyn-fixed, paraffin-embedded tissues obtained from the Department of Experimental Medicine and Pathology of the University of Rome ‘La Sapienza’, using a commercially available Vectastain ABC Kit (Vector Lab., Burlingame, CA, USA) as reported elsewhere (3). Section were incubated with polyclonal antibody raised in rabbits against purified human adrenomedullin 1–52 (Peninsula Lab. Inc., CA, USA) at a dilution of 1:150. A negative control was obtained in pancreatic tissue incubated with non-immune rabbit serum, antibody dilution buffer, or the primary antibody pre-adsorbed with an excess of human adrenomedullin (1 μmol/l). Cells were considered positively stained when a brown granulation of the cytoplasm was revealed at low-power magnification.

Statistical analysis

All data are given as mean ± s.d. Statistical calculation was performed using PRIMER software (14). Comparison between groups was performed by Kruskal–Wallis one way ANOVA. To compare adrenomedullin concentrations before and after surgery, one-way repeated measures ANOVA was used. The relationship between the plasma concentration of adrenomedullin and clinical and biochemical parameters was estimated by linear regression and correlation analysis.

Results

Clinical and laboratory data of the patients with insulinoma and healthy subjects are reported in Table 1. None of the patients had high blood pressure; however, two had a positive family history of hypertension. All patients with insulinoma were successfully treated by surgery.

As shown in Fig. 1, plasma adrenomedullin concentrations (mean ± s.d.) in patients with insulinoma (6.6 ± 3.2 fmol/ml) were significantly greater than those in controls (2.1 ± 1.1 fmol/ml; P < 0.001). In the six patients with insulinoma assayed before and after surgery, plasma adrenomedullin concentrations decreased from 6.3 ± 2.9 fmol/ml to 3.0 ± 1.6 fmol/ml (P < 0.01). No correlation was found between plasma adrenomedullin and all clinical and

| Table 1 Clinical and laboratory characteristics of all patients with insulinoma at diagnosis and normal subjects (controls). Values are number or means ± s.d. |
|-----------------|-----------------|
| **Controls** (n = 15) | **Insulinoma** (n = 9) |
| Sex (M/F) | 7/8 | 4/5 |
| Age (years) | 44 ± 11 | 48 ± 12 |
| Body mass index (kg/m²) | 24 ± 1 | 30.5 ± 9.5* |
| Systolic blood pressure (mmHg) | 120 ± 8 | 122 ± 13 |
| Diastolic blood pressure (mmHg) | 78 ± 6 | 81 ± 10 |
| Heart rate (beats/min) | 65 ± 3 | 66 ± 4 |
| Fasting blood glucose (mmol/l) | 4.7 ± 0.5 | 2.7 ± 0.7* |
| Fasting plasma insulin (pmol/l) | 71 ± 14 | 408 ± 383* |

* P < 0.001.
biochemical parameters examined, including arterial blood pressure values.

Grossly, the neoplastic masses measured from 0.5 to 1.2 cm (median 1 cm). On pure histological grounds, insulinomas showed modulated ribbons of elongated to round tumour cells with centrally or basally located nuclei. Nuclei revealed purely dispersed chromat in with inconspicuous nucleoli. Mitotic figures were absent. Cells had an abundant eosinophilic granular cytoplasm. Neoplastic islets were separated by a highly vascularized stroma. All tumours revealed strong immunostaining for adrenomedullin in the cytoplasm of the neoplastic cells (Fig. 2). Stroma and surrounding pancreas parenchyma and major ducts were negative for adrenomedullin.

**Discussion**

To our knowledge, this is the first study of plasma adrenomedullin concentrations and adrenomedullin immunostaining in patients with insulinoma. We found that circulating concentrations of adrenomedullin were significantly greater in patients with insulinoma compared with healthy individuals, and that concentrations decreased after surgical treatment. The mechanism of the increase in circulating adrenomedullin in patients with insulinoma is not clear. The increase in plasma adrenomedullin may be determined by excessive secretion by neoplastic pancreatic islet cells, as demonstrated for other tumours (15, 16). Recently, we reported that pituitary corticotrophin tumours secrete large amounts of adrenomedullin, indicating that adrenomedullin is also produced by endocrine tumours (17). This hypothesis is supported by the presence of adrenomedullin immunostaining in the insulinoma neoplastic cells, whereas normal pancreatic lobules and ducts were negative. Our findings are in accordance with those of others demonstrating the absence of immunoreactive adrenomedullin in the pancreatic islets in adults (17) and the presence of an adrenomedullin receptor system in insulin-producing cell lines in vitro (9). Moreover, although adrenomedullin-immunoreactive cells were not observed in the pancreatic islets in adult humans, in the developing pancreas of rat, adrenomedullin was co-localized with glucagon in the ducts and with C-peptide in primitive islets, in which almost all cells expressing insulin also contained adrenomedullin (19). These data suggest that endocrine cells of the pancreas have the capacity of producing adrenomedullin during development, and that this peptide may be related with the onset and progression of the differentiation process and could perform a regulatory function for insulin secretion.

We can not exclude that inappropriate adrenomedullin secretion found in patients with insulinoma may be induced in response to hyperinsulinaemia, in an attempt to reduce insulin concentrations. Indeed, studies in vitro have shown that adrenomedullin inhibits the release of insulin from cultured isolated islet cells in a dose-dependent manner and that mRNA

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**Figure 2** Immunohistochemical staining for adrenomedullin. Strong immunostaining is localized in the cytoplasm of pancreatic insulinoma cells, as indicated by the arrow (original magnification × 160).
for adrenomedullin receptors are homogeneously distributed throughout the islets (9). Thus increased circulating adrenomedullin may have a counter-regulatory significance and adrenomedullin immunostaining may simply reflect binding to specific receptors on the insulin-producing cells that are not responding to the negative feed-back. However, lack of correlation between insulin and adrenomedullin concentrations argues against this hypothesis.

Lastly, increased circulating adrenomedullin could also be endothelial or adrenal in origin, in response to hypoglycaemia. However, insulin-induced hypoglycaemia had no effect on plasma adrenomedullin concentrations, in contrast to the adrenaline response (16).

In conclusion, this study has demonstrated that patients with insulinoma have a remarkably increased concentration of circulating adrenomedullin compared with that in normal individuals, and that this increase is related to the neoplastic phenotype. The mechanism that gives rise to increased adrenomedullin and its relevance to the clinical features of insulinoma remain to be established and require further studies, in particular to validate the potential usefulness of adrenomedullin as an additional marker in the clinical monitoring of these patients.

References

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