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SCIENCE DIRECT
Regulatory Peptides 133 (2006) 134 – 138
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Apelin: A new plasma marker of cardiopulmonary disease

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Received 5 June 2005; received in revised form 6 September 2005; accepted 28 September 2005
Available online 2 November 2005

Abstract

Objectives: Dyspnea is a major symptom of both parenchymal lung disease and chronic heart failure. Underlying cardiac dysfunction can be assessed by measurement of cardiac-derived B-type natriuretic peptide or its precursor in plasma. However, no specific endocrine marker of the lung parenchyma has so far been identified. We therefore examined whether plasma concentrations of apelin, a novel inotropic hormone, is affected in patients with chronic parenchymal lung disease without cardiac dysfunction.
Methods and results: Patients with severe chronic parenchymal lung disease and normal cardiac function ($n=53$), idiopathic pulmonary hypertension with increased right ventricular pressure ($n=10$), and patients with severe left ventricular systolic dysfunction ($n=22$) were enrolled. Plasma apelin-36 and proBNP concentrations were measured with radioimmunoassays. While proBNP plasma concentrations were unaffected in chronic parenchymal lung disease patients compared to normal subjects, the apelin-36 concentration was reduced 3.3-fold (median 35 pmol/l (0–162 pmol/l) vs. 117 pmol/l (55–232 pmol/l), $P<0.001$). Moreover, the apelin-36 concentration was decreased in chronic heart failure patients (2.1-fold, $P<0.01$) and in patients with idiopathic pulmonary hypertension (4.0-fold, $P<0.001$). In contrast, the proBNP concentration was highly increased in both chronic heart failure and idiopathic pulmonary hypertension patients.
Conclusion: Plasma concentrations of apelin-36, a novel inotropic peptide, are decreased in patients with chronic parenchymal lung disease and preserved cardiac function. Combined measurement of apelin-36 and proBNP may be a new diagnostic approach in distinguishing pulmonary from cardiovascular causes of dyspnea.

Keywords: Apelin; BNP; Dyspnea; Heart failure; proBNP

1. Introduction

Dyspnea is a major symptom of both parenchymal lung disease and congestive heart failure. Examination of patients with dyspnea therefore includes evaluation of pulmonary as well as cardiac function by chest X-ray, microbiological screening, measurement of the ventilatory status, and echocardiographic assessment of cardiac function. Measurement of the cardiac-derived B-type natriuretic peptide (BNP) or its precursor (proBNP) has been used as markers of cardiovascular causes of dyspnea, where increased plasma concentrations are associated with congestive heart failure, pulmonary embolism, or pulmonary vascular disease [1]. In contrast, plasma BNP and proBNP concentrations seem to be unaffected in patients with strictly parenchymal lung disease [2]. Until now, however, there have been no specific endocrine markers to assess the lung parenchyma.

Apelin is a novel peptide identified as the endogenous ligand to the APJ (angiotensin receptor-like 1) receptor [3]. Although apelin initially was identified in cow stomach, both apelin and the APJ receptor are expressed in cardiac tissue [4,5]. Recent reports have furthermore established that apelin exerts a positive inotropic effect on both normal hearts and in heart failure after myocardial infarction [4,6]. While this inotropic effect suggests an important biological role in endocrine regulation of heart function, the potential clinical use of apelin measurement in human plasma is still unknown, as few studies have reported both increased and decreased

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0167-0115/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2005.09.032

concentrations in heart failure patients [5,7]. Interestingly, apelin mRNA and peptide are predominantly expressed in normal mammary and lung tissue from rat [8]. We therefore hypothesized that apelin concentrations in plasma may be affected in patients with chronic parenchymal lung disease without cardiac dysfunction.

2. Methods

2.1. Patients

Venous plasma was obtained from patients with chronic parenchymal lung disease (chronic obstructive pulmonary disease, fibrosis, or emphysema, $n=53$) or idiopathic pulmonary hypertension ($n=10$) referred for evaluation prior to lung transplantation. Notably, patients considered clinically unfit for transplantation were not included. Left and right heart catheterization was performed and the hemodynamic status was determined. Inclusion criteria were normal left ventricular systolic function on echocardiography, no significant coronary artery disease on angiography, and no overt renal dysfunction. In addition, chronic heart failure patients on standard treatment undergoing routine cardiac catheterization were included ($n=22$). Control subjects without clinical signs and symptoms of pulmonary or cardiovascular disease were also recruited (14 females and 12 men, median age 67 years (range 57–77 years, $n=26$). The control subjects did not receive medical treatment, and all patients and control subjects gave written informed consent for participation. The study protocol was approved by the local ethics committee (KF 01-307/99 and 01-231/99).

2.2. Plasma analysis

Plasma proBNP concentrations were measured by a processing-independent radioimmunoanalysis [9]. The proBNP concentrations in some of the patients have been reported previously [2].This assay quantitates the total concentration of prohormone products after a pre-analytical enzymatic step. Plasma is briefly incubated with trypsin, which cleaves proBNP after an arginyl residue in position 21. Intact proBNP and its N-terminal fragment(s) are thereby cleaved into the same analyte (proBNP 1–21) and measured with a conventional radioimmunoassay specific for the N-terminal decapeptide. Assay imprecision (within-run) is 12% at 13 pmol/l and 5% at 130 pmol/l, and between-run 20% at 16 pmol/l and 8% at 70 pmol/l. The analytical detection limit is 0.2 pmol/l [9]. Plasma apelin-36 concentrations were measured with a commercial kit (Phoenix peptides, Karlsruhe, Germany). According to the manufacturer, this radioimmunoassay is specific for apelin-36 with no cross-reactivity to apelin-16 or apelin-13. The intra- and inter-assay imprecision is 3.8% and 12.5%, respectively, with a detection limit of 4.8 pmol/l. All plasma samples were analyzed in duplicate. Plasma concentrations were compared between groups with the Kruskall–Wallis test followed by Dunn's multiple comparison test.

2.3. Immunocytochemistry

Lung tissue was collected from parenchymal lung disease patients undergoing pulmonary transplantation ($n=4$). In addition, mouse stomachs were obtained from wild-type mice (control tissue). Tissues biopsies were immersed and fixed overnight in 4% paraformaldehyde in buffered sodium phosphate (pH 7). Six micrometer paraffin sections were cut, mounted on superfrosted glass slides, and baked overnight at 60 °C. Antigen retrieval was performed by boiling the slides in a microwave oven for 3 min at 800 W followed by 3×5 min at 400 W in a 10 mM Tris-buffered EGTA solution at pH 9.0. Preblocking was performed using 10% bovine serum in Tris-buffer saline (pH 7) for 20 min. Tissue sections were then incubated with a new apelin antiserum (no. 04019, dilution 1:4000) overnight at 4 °C. This antiserum was raised against the evolutionary preserved C-terminal decapeptide of human apelin-36. The primary antiserum was detected with Alexa 488 conjugated donkey–anti rabbit Fab-fragments in a 1:1000 dilution (Molecular Probes Inc., Eugene, OR). Coverslips were mounted with aquamount (Dako, Glostrup, Denmark). The sections were examined with a Zeiss LSM510 confocal microscope (Zeiss, Oberkochen, Germany).

3. Results

The patient characteristics including hemodynamic findings from the invasive examination are shown in Table 1. Ventilatory examination further corroborated the severely reduced respiratory function in the chronic parenchymal lung disease patients (median forced expiratory volume (FEV_1): 29% of

Table 1
Patient characteristics

	Chronic lung disease	Chronic heart failure	Idiopathic pulmonary hypertension
Number of patients (females/males)	53 (30/23)	22 (5/17)	10 (7/3)
Age (years)	56 (39–68)	53 (26–61)	46 (24–65)
Serum creatinine (μmol/l)	75 (49–178)	106 (72–162)	94 (80–118)
Left ventricular ejection fraction (%)	65 (60–70)	20 (10–30)	60 (55–65)
Cardiac index (l/min m^2)	2.8 (1.5–4.7)	2.4 (1.3–3.4)	2.1 (1.5–4.0)
Mean pulmonary artery pressure (mm Hg)	20 (11–40)	29 (17–50)	56 (50–99)
Pulmonary vascular resistance (Wood units)	2.4 (1.0–5.0)	2.0 (0.8–5.4)	10 (5.0–17.0)
Mean pulmonary capillary wedge pressure (mm Hg)	10 (5–22)	22 (8–36)	8 (5–12)

Values are listed as medians with ranges in parentheses.