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Calcium-regulating peptide hormones and blood electrolytic balance in chronic heart failure

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Abstract

Calcium-regulating system is important for the functional activity of myocardium. However, little is known about the role of this system in the pathogenesis of cardiovascular diseases.

Blood samples from the patients with chronic heart failure (CHF) caused by ischaemic disease (coronary artery disease) (NYHA class I–IV) were used to analyze the levels of calcium, inorganic phosphate, sodium, potassium, parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP). The heart beat rate and arterial blood pressure were chosen as additional tests for the functional status of cardiovascular system. The alteration of electrolytes homeostasis was found dependent on the severity of the pathology being maximally expressed in the NYHA class IV patients. Similar tendency was demonstrated for circulating PTH and PTHrP with the highest blood concentrations observed in patients of the NYHA class III and IV. The extent of these changes was found more pronounced in the female patients. It is suggested that the calcium-regulating hormonal system is involved in the pathogenesis of the ischaemic heart disease; however the sharp increase of PTH and PTHrP at the severe stages of pathology may play a compensatory role in maintaining the heart function.

Keywords: Parathyroid hormone; Parathyroid hormone-related protein; Electrolytes; Calcium; Heart ischaemic disease

1. Introduction

The importance of ionized calcium (Ca^{2+}) for the functional activity of heart is illustrated by its essential role in the synchronized cellular depolarization, pacemaker activity and subsequent activation of contractile proteins, i.e. in the cardiac excitation–contraction coupling [1–3]. Other important triggers of the cardiomyocyte excitation and cardiac electrical activity are Na and K ions [4–7]. Understandably, the disturbance of the Ca^{2+} and other electrolytes homeostasis may result in the development of severe pathologies including misbalance of the cardiovascular system. Among different factors regulating the homeostasis of these ions, the important role of endocrine, paracrine and autocrine signaling is widely accepted [8]. However, the involvement of such regulatory mechanisms in the pathogenesis of cardiovascular diseases remains largely undisclosed. A number of studies [9–11] indicate involvement of the major calcium-regulating factor, parathyroid hormone (PTH) in the regulation of heart contractile activity.

However, the regulation of Ca^{2+} homeostasis is not limited exclusively to the PTH signaling. Accumulating evidence suggests that a PTH-like factor, parathyroid hormone-related protein (PTHrP) can activate PTH/PTHrP receptor with the ensuing regulation of Ca^{2+} levels [12]. Calcium-regulating properties of PTHrP are exerted by the N-terminal fragment of the protein (amino acids 1–34), one of the three peptides produced by the prohormone convertases and the only one that bears strong

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homology to the corresponding peptide of PTH (see review [13] and references herein). Although PTHrP was originally identified in cancer cells as PTH-like factor responsible for the humoral hypercalciemia of malignancy, later it was shown that PTHrP is expressed in all tissues including the heart [13–15]. Physiological significance of this protein (especially the specific roles of the different proteolytic fragments) is still under discussion, however most of the data point to its role as a local regulator of cell proliferation, cell differentiation and cell death [16], (review [17] and references herein). The impact of PTHrP on the cardiac function is not clear. The existing data demonstrate PTHrP-dependent positive inotropic and chronotropic effects and its impact on coronary flow (review [18] and references herein). Most of the above cited results are produced in the animal and *in vitro* studies whereas not so much is known about the status of PTH/PTHrP system in cardiovascular pathologies. Current study is an attempt to shed more light on the possible involvement of calcium-regulating hormonal system including PTH and PTHrP and circulating electrolytes in the development of heart failure caused by the ischaemic disease (coronary artery disease).

2. Subjects and methods

2.1. Subjects

All subjects gave written informed consent to the experimental procedures which were approved by the Ethics Committee of the Yerevan State Medical University. The investigation conformed with the principles outlined in the Declaration of Helsinki.

Seventeen (17) female and twenty one (21) male patients, aged 44–70 years with a diagnosis of a chronic heart failure (CHF) caused exclusively by the ischaemic heart disease (including ischaemic cardiomyopathy (20 patients), stable and unstable angina (10 patients) and myocardial infarction (8 patients)), were divided into four groups according to the New York Heart Association (NYHA) functional classification (Table 1). The diagnosis of the ischaemic heart disease as well as CHF was based on the characteristic clinical signs, electrocardiography and echocardiography. Based on the latter the NYHA I group included patients with the left ventricular ejection fraction 51.43±0.56%, NYHA II, 44.17±0.66%, NYHA III, 37.2±0.55% and NYHA IV, 29.2±0.67%. Exclusion criteria included any history of kidney, liver and other organ disease. Sixteen age-matched (45–65 years), healthy volunteers (7 men and 9 women), with no history of heart or other diseases were recruited into the control group. Electrocardiograms of none of them showed any deviation from the norm.

Table 1
Description of control and patient groups

	Sex	Number	Age	Diagnosis	Class NYHA
Control	Female	9	45–54		
	Male	7	45–65		
Patients	Female	17	51–70	Cardiomyopathy (7)	I (3)
				Angina (8)	II (7)
				Myocardial infarction (2)	III (5)
					IV (2)
	Male	21	44–70	Cardiomyopathy (13)	I (4)
				Angina (2)	II (5)
				Myocardial infarction (6)	III (5)
					IV (7)

2.2. Methods

Blood specimens from each patient were collected immediately upon the admission to the clinic and before the commencement of the drug treatment. Venous blood was collected into two tubes in order to obtain simultaneously blood plasma (for PTHrP assay) and serum (electrolytes and PTH determination). Blood destined for the plasma isolation was collected into the Vacutaner 4.5 ml tubes containing EDTA. Tubes were gently rocked, blood transferred to the centrifuge tubes containing aprotinin (0.6 TIU/ml of blood). Plasma was collected after centrifugation at 1600 ×*g* for 15 min at 4 °C. In order to obtain serum blood was collected directly into the centrifuge tubes without EDTA and centrifuged under the same conditions. Plasma was stored at −70 °C and serum at −20 °C.

Total calcium and inorganic phosphate concentrations were determined spectrophotometrically in the serum using kits from LaChema (Czech Republic). Serum concentrations of the ionized calcium, sodium and potassium were determined by the ion-selective electrolyte analyzer (KONE, Finland). Circulating PTH levels were detected (intact PTH) by the enzyme immunoassay kit from DRG International (USA). PTHrP from plasma was extracted on the SEP-column, lyophilized and determined (peptide 1–34) by the enzyme immunoassay as to the manufacturer's recommendations (Phoenix Pharmaceuticals, USA).

Heart rate and arterial blood pressure (diastolic, DP and systolic, SP) were measured in all patients and volunteers from the control group.

Statistical analysis was carried out using non-parametric Kruskal–Wallis *H*-test and Dan's criteria (*Q* test) for multiple comparisons.

3. Results

3.1. Circulating electrolytes at different stages of chronic heart failure

Analysis of the levels of circulating electrolytes reveals no drastic changes in patients with a mild degree (NYHA class I) of disease except moderate hypokalemia (Table 2). Further aggravation of the disease leads to the decrease of circulating Na^+. Ionized Ca data demonstrate similar tendency, statistically insignificant in the patients of first three groups (NYHA class I, II, III) but more pronounced and statistically supported in the group with the most severe extent of disease (NYHA class IV). At the same time the phosphate levels remain stable in all of the studied groups.

Interestingly, no discernible association is seen between the parameters of systemic haemodynamics such as blood pressure