Intramyocardial Adrenergic Activation in Chagasic Cardiomyopathy and Coronary Artery Disease

Luciano Nastari, Felix Jose Alvarez Ramires, Vera Maria Cury Salemi, Barbara Maria Ianni, Fábio Fernandes, Célia Maria Strunz, Edmundo Arteaga, Charles Mady

Instituto do Coração (InCor) da Faculdade de Medicina da Universidade de São Paulo, São Paulo - Brazil

Abstract

Background: Myocardial norepinephrine is altered in left ventricular impairment. In patients with Chagas' cardiomyopathy (CC), this issue has not been addressed.

Objective: To determine the level of myocardial norepinephrine in patients with CC and compare it in patients with coronary artery disease, and to relate myocardial norepinephrine to left ventricular ejection fraction (LVEF).

Methods: We studied 39 patients with CC, divided into group 1: 21 individuals with normal LVEF and group 2: 18 individuals with decreased LVEF. Seventeen patients with coronary artery disease were divided into group 3: 12 individuals with normal LVEF and group 4: 5 individuals with decreased LVEF. Two-dimensional echocardiography was used to measure LVEF. Myocardial norepinephrine was determined by high-performance liquid chromatography.

Results: Myocardial norepinephrine in CC with and without ventricular dysfunction was 1.3±1.3 and 6.1±4.2 pg/μg noncollagen protein, respectively (p<0.0001); in coronary artery disease with and without ventricular dysfunction, it was 3.3±3.0 and 9.8±4.2 pg/μg noncollagen protein, respectively (p<0.0001). A positive correlation was found between LVEF and myocardial norepinephrine concentration in the patients with Chagas' cardiomyopathy (p<0.01, r = 0.57) and also in those with coronary artery disease (p<0.01, r=0.69). A significant difference was demonstrated between norepinephrine concentrations in patients with normal LVEF (groups 1 and 3; p = 0.0182), but no difference was found in patients with decreased LVEF (groups 2 and 4; p = 0.1467).

Conclusion: In patients with Chagas' cardiomyopathy and normal global ejection fraction there is an early cardiac denervation, when compared to coronary artery disease patients. (Arq Bras Cardiol 2011; 96(2): 99-106)

Keywords: Cardiomyopathy; norepinephrine; ventricular dysfunction, left; coronary artery disease; heart failure.

Introduction

The neurohumoral adaptive response plays a key role in the complex system involved in heart failure pathophysiology. Nevertheless, it may also have a detrimental effect, which, if modulated, may result in better treatment of the cardiac dysfunction[1].

The pathways involved in the sympathetic activation occurring in heart failure are not well known. Chronic sympathetic activation contributes to the persistent increase in norepinephrine plasma levels and is associated with increased mortality[2]. Some studies on heart failure using animal models have demonstrated that catecholamines are higher in the right side compared with the left side of the heart and that the highest accumulation is found in the right atrium[3-8]. However, the norepinephrine distribution within the same heart chamber walls is uniform, and evidence exists that it is stored in the adrenergic neurons. Therefore, its concentration reflects the density of sympathetic innervation[9,10].

In 1983, Kawai et al[11] was the first to measure norepinephrine in samples of human myocardial tissue obtained by endomyocardial biopsy[11]. Subsequently, other studies showed the relation between left ventricular ejection fraction (LVEF) and the intramyocardial norepinephrine concentration in dilated valvular, ischemic and hypertrophic cardiomyopathy[12-15]. Chronic Chagas' cardiomyopathy is characterized by an intense reduction in the heart neuronal population, as documented in necroscopic studies of chagasic patients who died of heart failure[16]. Patients with Chagas' cardiomyopathy were studied by Correa-Araujo et al [17] and norepinephrine determination was carried out in myocardial tissue obtained from the autopsies. Currently, norepinephrine is known to undergo fast degradation outside the body, and the immediate cooling of the heart tissue is required for the proper determination of norepinephrine content[4]. Also, the catecholamine concentrations were expressed as micrograms per gram of myocardial tissue in this study, and, therefore, there was no correction in the determination of noncollagen proteins[17].

Mailing address: Luciano Nastari •
Rua Doutor Diogo de Faria, 1077 – 62 - Vila Clementino – 04037003 - São Paulo, SP – Brasil
E-mail: lnastari@cardiol.br, luciano-nastari@uol.com.br
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This is the first systematic study performed *in vivo* aiming to identify, quantify, and correlate the presence of myocardial tissue norepinephrine with the LVEF in Chagas' cardiomyopathy patients and to compare it with coronary artery disease patients.

Material and methods

Patient selection

Thirty-nine consecutive patients with Chagas' cardiomyopathy whose diagnosis was established based on the clinical picture and positive serum reactions (immunofluorescence and ELISA), and 21 consecutive patients with coronary artery disease who underwent myocardial bypass surgery were evaluated.

The inclusion criteria were as follows: age ≥18 years, absence of pregnancy or breast-feeding, and the patient's willingness and ability to provide informed consent.

The exclusion criteria were arterial hypertension (blood pressure >140/90 mmHg), diabetes mellitus, renal failure (serum creatinine >1.6 mg/dL), chronic pulmonary disease, liver failure, autoimmune disease, collagen vascular disease, and neoplasia.

This investigation conforms to the principles outlined in the Declaration of Helsinki. The Ethics Committee approved the study and all patients signed a written consent form: protocol number 231/96.

Selected groups

The selected patients were divided into 4 groups as follows:

Group 1 - 21 patients with Chagas' disease with electrocardiographic changes, with no symptoms of heart failure and LVEF ≥55%.

Group 2 - 18 patients with Chagas' cardiomyopathy, functional class II and III congestive heart failure (NYHA), undergoing conventional treatment with diuretics, digitalis, and angiotensin-converting enzyme inhibitors, and LVEF <55%.

Group 3 - 12 patients with coronary artery disease and LVEF≥55% who had undergone myocardial bypass surgery.

Group 4 – 9 patients with coronary artery disease and LVEF <55%, who had undergone myocardial bypass surgery. Of the 9 patients from this group, 4 were excluded, as intravenous vasoactive drugs (dobutamine and/or norepinephrine) had been used during the surgery, which could compromise the myocardial norepinephrine measurements.

Methodology

All patients underwent peripheral venous blood specimen collection to determine specific serum reactions for Chagas' disease (immunofluorescence and ELISA) and a 12-lead electrocardiogram. Two-dimensional echocardiography with M-Mode and Doppler study was performed in all patients. The ejection fraction was calculated using the Teichholz method. High-performance liquid chromatography was used to assess plasma norepinephrine. Myocardial tissue norepinephrine levels were measured as described below.

Quantification of myocardial tissue norepinephrine

For the Chagas' cardiomyopathy group, the fragments were obtained by endomyocardial biopsy of the right ventricle through the right internal jugular vein (Mason's technique)[18], being immediately placed in stoppered polypropylene microtubes (*Eppendorf*). Soon after collection, specimens were placed in a specific styrofoam container with ice and immediately taken to the laboratory. In patients with coronary artery disease, the biopsies were obtained during the intraoperative period after the opening of the right atrium with access to the right ventricle, and fragments were taken and processed as previously described. Norepinephrine, epinephrine, dopamine, and 3,4-dihydroxy benzylamine standard solutions were prepared at a concentration of 200 µg/mL in 0.1N acetic acid. These solutions were stored at -20°C in amber vials for up to one month and thawed only at the time of the tests. The tissue was thawed in an ice bath and underwent mechanical maceration after the addition of 350 µL 0,1M perchloric acid[6]. After addition of the standard solution, these were centrifuged for removal of protein precipitates and cell debris. These precipitates were later used for the determination of noncollagen proteins (NCP). The catecholamines found in the supernatants (±400 µL) were purified by absorption in alumina. A portion of the filtrate (25 µL) was injected into a high-pressure chromatographic system with an electrochemical detector that determined each catecholamine concentration, and this result (pg/mL) was then expressed in relation to the amount of noncollagen proteins in that fragment (pg/µg NCP). (Figure 1)

Determination of noncollagen proteins

The methodology described by Lilienthal et al[3] was used for the determination of noncollagen proteins. Proteins contained in the supernatant were electrophotometrically determined by Lowry et al method[4]. (Figure 2)

Statistical analysis

The groups were compared regarding the variables obtained from biopsies and echocardiograms by the analysis of variance with normal distribution and by the Kruskal-Wallis test for those variables with unknown distribution.

The relationship between norepinephrine obtained from biopsies with the LVEF was separately studied by means of simple linear regression models in the groups of patients with Chagas' cardiomyopathy and with coronary artery disease; $p \leq 5\%$ was considered statistically significant. Statistical analyses were performed using SAS version 6.11.

Results

Group 1 comprised 21 patients with a mean age of 48.7±8.8 years; 17 were female patients (80.9%). Group 2 comprised 18 patients with a mean age of 50.9±11.7 years; 14 were male patients (77.8%). Group 3 comprised 12 patients with a mean age of 61.9±7.5 years; 9 were male patients (75%). Group 4 comprised 5 patients with a mean age of 61.7±14.1 years; 4 were male patients (80%). The patients in the coronary artery disease groups were older than the

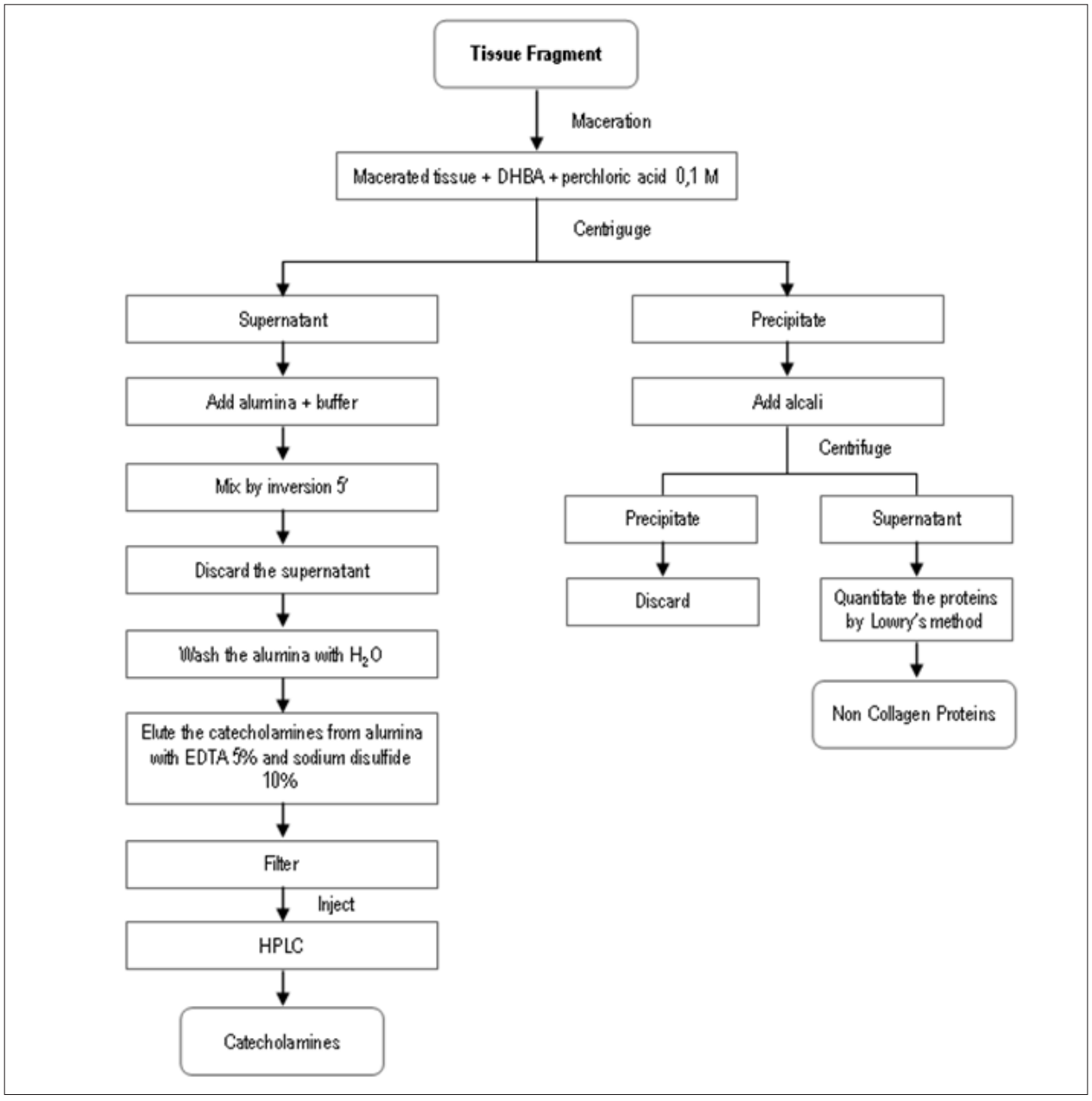

Figure 1 - Extraction, separation, identification and quantification of the tissue catecholamines; DHBA - 3,4-dihydroxibenzylamine; EDTA - ethylenediamine tetraacetic acid; HPLC - high performance/pressure liquid chromatography.

ones in the Chagas` cardiomyopathy groups. There was a significant predominance of females in group 1 and of males in groups 2, 3, and 4.

Serology for Chagas' disease

All patients in groups 1 and 2 had positive immunofluorescence and ELISA tests for Chagas' disease, and patients in groups 3 and 4 had negative Chagas' disease tests.

Electrocardiography

Electrocardiograms were abnormal for all patients in groups 1 and 2, with a predominance of right bundle-branch block (58.9 %), and left anterior hemiblock (51.3%) findings.

Echocardiography

Left ventricular diastolic diameters (LVDD, cm), comparing groups 1 to 2 and 3 to 4, were 4.9±0.5 and 6.8±0.7

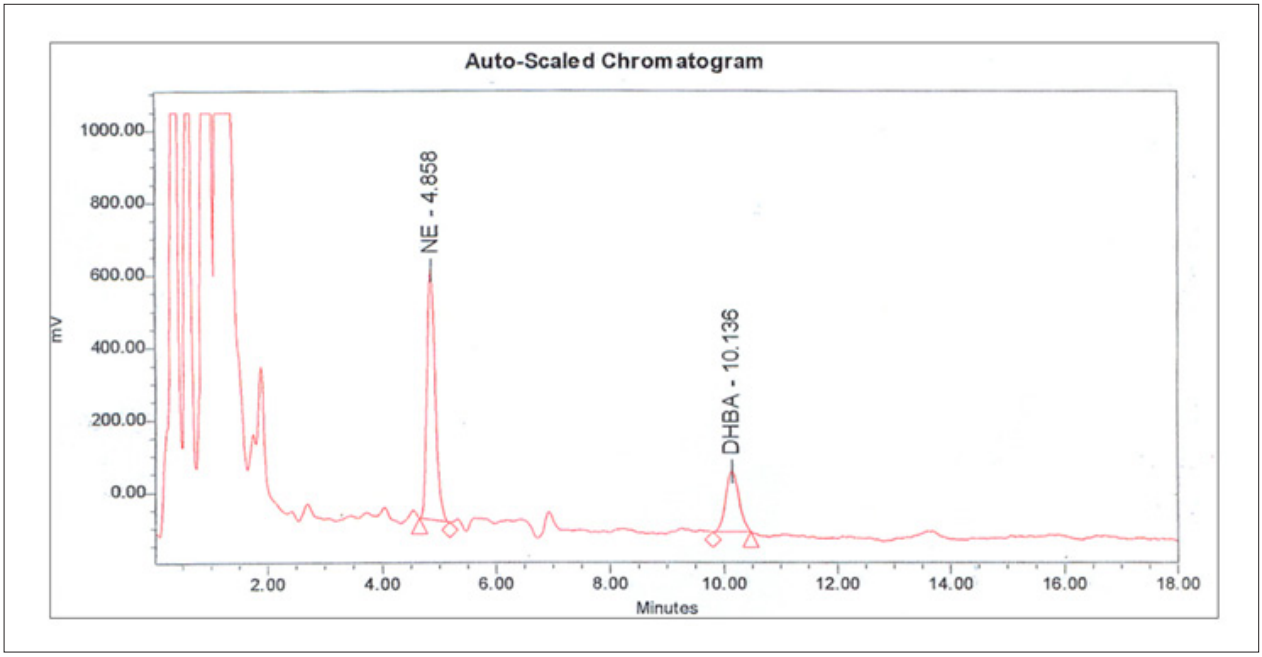

Figure 2 - Chromatogram of the catecholamines extracted from myocardial tissue fragment.; NE - norepinephrine; DHBA - 3,4-dihydroxibenzylamine.

(p<0.001); 4.9±0.3 and 6.6±0.8 (p<0.001), respectively. There was no statistical difference when comparing groups 1 to 3 and 2 to 4.

Left ventricular systolic diameters (LVSD, cm), when comparing groups 1 to 2 and 3 to 4, were 3.2±0.3 and 5.8±0.8 (p<0.001), and 3.1±0.3 and 5.6±0.9 (p<0.001), respectively. When comparing groups 1 to 3 and 2 to 4, no statistical difference was found.

The LVEF, when comparing groups 1 to 2 and 3 to 4, were 63.9±4.1 and 31.3±8.8 (p<0.001) and 64.4±5.2 and 31.6±6.3 (p<0.001), respectively. When comparing groups 1 to 3 and 2 to 4, no statistical difference was found.

Norepinephrine

Myocardial norepinephrine concentration was 6.1±4.2 pg/µg of NCP and 1.3±1.3 pg/µg of NCP (p<0.0001) in groups 1 and 2 (Figure 3), and 9.8±4.2 pg/µg of NCP and 3.3±3.0 pg/µg of NCP (p<0.0001) in groups 3 and 4 (Figure 4).

A positive and significant correlation was found between LVEF and myocardial norepinephrine concentrations in the group of patients with Chagas' cardiomyopathy (p<0.01, r = 0.57) and also in those with coronary artery disease (p<0.01, r=0.69) (Figure 5 and Figure 6).

The 95% confidence interval and mean norepinephrine concentrations and the standard deviation were determined in the 4 groups.

A significant difference was demonstrated between norepinephrine concentrations in patients with normal LVEF (groups 1 and 3; p = 0.0182), but no difference was found in patients with impaired LVEF (groups 2 and 4; p = 0.1467). When we specifically analyzed the patients from groups 2 and 4 with LVEF< 35%, regarding tissue norepinephrine

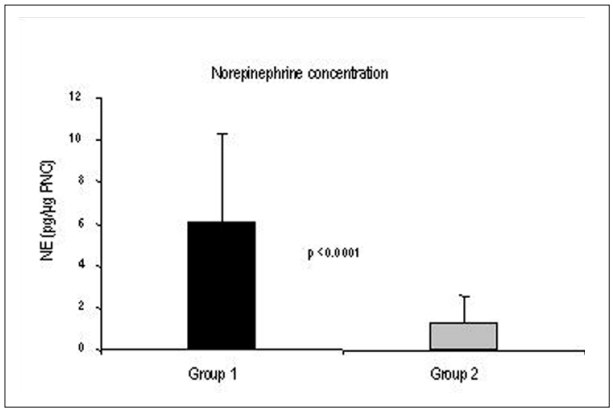

Figure 3 - Myocardial norepinephrine concentrations in groups 1 and 2.

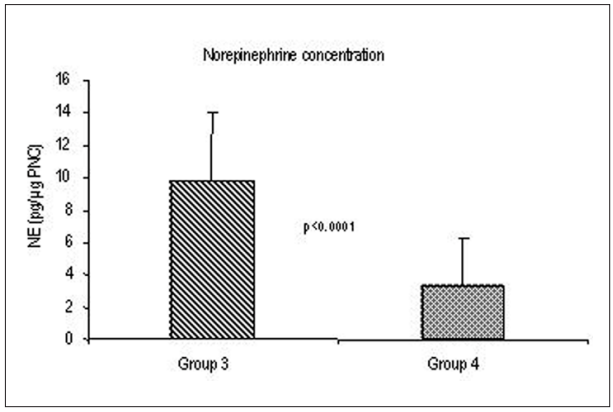

Figure 4 - Norepinephrine concentrations in the myocardial tissue of patients in groups 3 and 4.

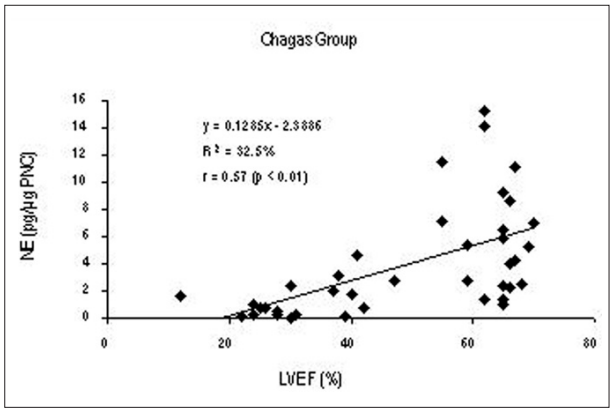

Figure 5 - Correlation between left ventricular ejection fraction and myocardial norepinephrine concentrations in the patients with Chagasic cardiomyopathy (groups 1 and 2).

concentration, no statistical difference was observed, either (p=0.29) (Figure 7).

Dopamine and epinephrine concentrations in myocardial tissue were reduced and for most of the specimens, no measurement was detected, thus making the statistical analysis of these data difficult.

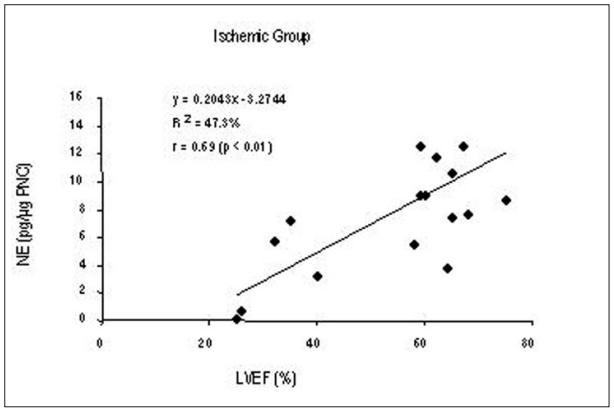

Figure 6 - Correlation between left ventricular ejection fraction and norepinephrine concentrations in patients with coronary artery disease (groups 3 and 4).

Myocardial norepinephrine concentrations showed a negative correlation with norepinephrine plasma concentrations in patients with Chagas' cardiomyopathy, regardless of normal or abnormal ventricular function (groups 1 and 2), (Figure 8).

Discussion

This is the first study carried out in patients with Chagas' cardiomyopathy with normal and abnormal LVEF, in which a positive correlation was observed between the LVEF and norepinephrine concentration in the myocardial tissue. Moreover, a positive correlation was found between the LVEF and norepinephrine concentration in the myocardial tissue in patients with coronary artery disease with normal and impaired left ventricular function. Similar results were described in the literature for ischemic cardiopathy patients when fragments were collected by endomyocardial biopsy of the right ventricle[13]. It is very difficult to study a control group consisting of healthy individuals for comparison with diseased groups due to ethical problems. However, the literature has shown some control groups consisting of volunteer prisoners and patients previously suspected of having myocarditis, hemochromatosis, and endomyocardial fibrosis who underwent endomyocardial biopsy, but whose biopsies were diagnosed as normal[6,12,14,19]. In these cases, the levels of myocardial norepinephrine were similar to those found in patients with coronary artery disease with normal left ventricular function.

Heart failure generates a decreased concentration of norepinephrine in the myocardial tissue regardless of its cause. Studies of patients with coronary artery disease, dilated or valvular cardiomyopathy, all with LVEF <35%, have not revealed significant differences in norepinephrine concentration in the myocardial tissue for these different cardiomyopathies. In our study, no significant differences were observed between the patients with Chagas' cardiomyopathy with impaired LVEF and the patients with coronary artery disease with the same ventricular dysfunction. It must be pointed out that the latter group comprised 5 patients only, due to the difficulty in obtaining fragments of tissue during the intraoperative period, as a result of hemodynamic instability showed by these patients.

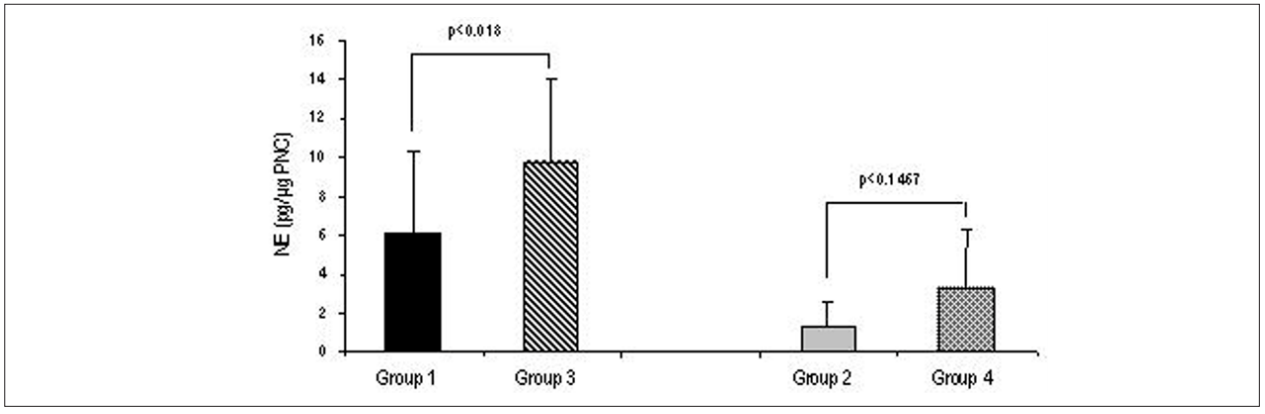

Figure 7 - Norepinephrine concentrations in patients with normal ventricular function (groups 1 and 3) and patients with impaired ventricular function (groups 2 and 4).

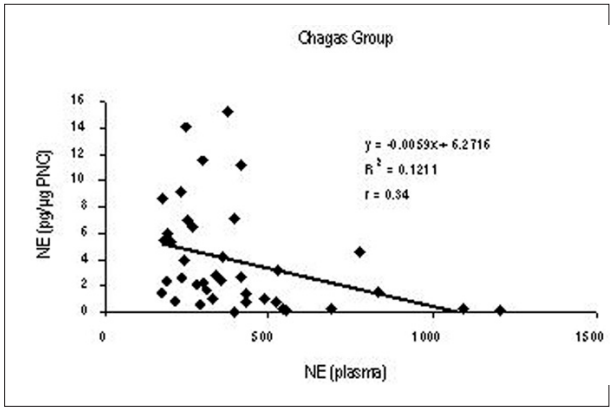

Figure 8 - Myocardial norepinephrine concentrations had a negative correlation with norepinephrine plasma concentrations in patients with Chagasic cardiomyopathy (groups 1 and 2).

However, in patients with normal LVEF, the norepinephrine concentration was significantly lower in those with Chagas' cardiomyopathy than in patients with coronary artery disease. Norepinephrine is stored in adrenergic neurons and its concentration, measured at a given site, reflects the sympathetic innervation density[8,10]. Decreased myocardial norepinephrine concentration affects all heart chambers proportionally[20]. Studies with more than two specimens of the myocardial tissue from the same patient were carried out and results of norepinephrine concentrations were compared. Kawai et al[11] found a variation ratio of just 7.6% between two samples of right ventricular biopsies in 6 patients. Regitz et al[14] studied 22 pairs of right ventricular biopsies and showed just 17.2% difference for the group with left ventricular dysfunction and 15.4% for the group with no dysfunction. Our study did not evaluate pairs of biopsies from patients with Chagas' cardiomyopathy because we had to use the 2 collected fragments in the same analysis to assess the tissue norepinephrine concentration. In 5 patients from the coronary artery disease and normal left ventricular function group, 2 samples were taken during the intraoperative period and the norepinephrine concentrations showed a 15.8% difference as previously described in the literature. Moreover, it has been shown that the collection of fragments of myocardial tissue in the intraoperative period of heart surgery did not change the norepinephrine concentration[21-24]; therefore, we decided to use intraoperative myocardial biopsy samples from patients with coronary artery disease. The cause of the decreased norepinephrine tissue in heart failure is not well defined, but factors such as decreased synthesis (as evidenced by the decreased tyrosine-hydroxylase activity[22,25]), change in neuronal reuptake[26], and excessive release with decreased storage after intense sympathetic stimulation should, together, determine the low norepinephrine concentration in the myocardium[26].

With the results currently available from necroscopic studies performed in several independent centers that analyzed human trypanosomiasis, the aggression to the autonomous nervous system is known to be present in Chagas' cardiomyopathy with a peculiar focal-nature characteristic, in an irregular and unforeseeable distribution[27,28]. The first anatomical and functional[29] studies identifying the role of the sympathetic system in the development of Chagas' disease were published in the early 1970s. Due to the fact that ^{123}I-meta-iodo-benzylguanidine (MIGB) scintigraphy can be quickly captured by the sympathetic nervous terminations of the heart, it is considered an important marker of adrenergic innervation in patients "in vivo" under normal and pathological conditions[30]. When studying patients with the indeterminate form of Chagas' disease and with Chagas' cardiomyopathy, Giorgi[31] found hypo-uptake areas compatible with myocardial fibrosis by perfusion scanning with sestamibi and sympathetic denervation areas by scanning with MIBG. The location of the areas with uptake changes with MIBG corresponded to those showing changes with sestamibi, both in the indeterminate and chronic forms of the disease. Simões et al[32] studied patients with Chagas' disease with normal and decreased LVEF by performing MIBG and ^{201}thallium segment uptake and found a strong association between the perfusion, innervation, and wall motility abnormalities, showing that major changes in sympathetic heart function occur at the ventricular level early in this pathology.

In 1989, Regitz et al[13] found a value of 10.3±3 pg/µg NCP in patients with no cardiopathy, whose fragment drawing was carried out through endomyocardial biopsy of the right ventricle. Our study found a similar concentration of 9.8±4.2 pg/µg NCP in patients with coronary artery disease with normal LVEF. When this group was compared with Chagas' cardiomyopathy patients with normal LVEF, a significantly lower norepinephrine concentration was found in the myocardium ($p < 0.0182$). Therefore, these data are in agreement with results obtained with the MIBG uptake studies. It should be pointed out that our study has also evidenced lower mean norepinephrine concentrations in patients with Chagas' cardiomyopathy with left ventricular dysfunction when compared to the group with coronary artery disease and ventricular dysfunction; however, no statistical significance was determined. This finding could be related to a higher presence of fibrosis found in this patient group[33] and/or by the neuronal destruction evidenced in Chagas' cardiomyopathy.[16]

Recent studies on the treatment of heart failure related to adrenergic system blocking show major advances in relation to decreased mortality and improvement in quality of life for that patient group. It must be pointed out that few studies included Chagas' disease patients.

In conclusion, norepinephrine concentration is decreased in Chagas' cardiomyopathy with left ventricular dysfunction as in other cardiomyopathies. Moreover, in patients with Chagas' disease and preserved left ventricular systolic function, the norepinephrine concentration is lower, compared to that in coronary artery disease patients. So, in patients with Chagas' cardiomyopathy and normal global ejection fraction there is an early cardiac denervation compared to coronary artery disease patients. Therefore, a specific, double-blind, placebo-controlled, randomized

study is necessary to determine the role of beta-blocking drugs in the treatment of chagasic patients.

Limitations of this study

Difficulty in including healthy individuals for endomyocardial biopsy in comparison with diseased groups due to ethical problems, as mentioned before.

Difficulty in enrolling enough patients with coronary artery disease and decreased LVEF that had not used vasoactive drugs (dobutamine and norepinephrine) in the intraoperative period, which should lead to alterations in the intramyocardial norepinephrine levels.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

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