The Role of Natriuretic Peptides in Renovascular Hypertension and Its Correlation with the Evolution of Myocardial Hypertrophy

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SUMMARY

The interactions between pressure and volume overload that occur in hypertension lead to different patterns of cardiac hypertrophy and to increase in natriuretic peptides (NPs). The profiles of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) synthesis and secretion have been investigated in models of hypertension. However, the different evolution of these profiles during the acute and chronic periods of pressure overload-induced cardiac hypertrophy is still unknown. For this reason, we studied one-kidney, one clip model using Sprague-Dawley rats at weeks 2, 4, 6 and 12 and correlated the evolution of these profiles with cardiac hypertrophy and hypertension.

We observed a positive correlation between blood pressure elevation and the degree of cardiac hypertrophy, with a time-dependent increase in both parameters from week 2. Levels of BNP expression showed an early increase after 2 weeks of treatment while ANP increased significantly after 6 weeks. Yet, the increase in ANP expression was gradual, allowing its correlation with hypertrophy and hypertension.

The NP expression has a differential response in the early stages of the development of hypertrophy induced by the renovascular model, with an early increase in BNP expression. Once hypertrophy develops, BNP expression is no longer specific and the increase of both NPs depends on and correlates with the degree of cardiac hypertrophy.

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Key words

Atrial Natriuretic Factor - Cardiomegaly - Renovascular Hypertension

Abbreviations

1K1C 1 kidney, 1 clip
ANP Atrial natriuretic peptide
mRNA Messenger ribonucleic acid
BNP B-type natriuretic peptide
CNP C-Type natriuretic peptide
RAW Right atrial weight
LAW Left atrial weight
BW Body weight

BACKGROUND

Hypertension may produce different patterns of ventricular remodelling as a result of the pathophysiological processes triggered by the interaction between pressure and volume loads. (1-3) In the early stages of cardiac hypertrophy, cardiomyocytes growth of is an adaptive response to increased functional demands of the heart. (4) Transition to pathological hypertrophy involves structural and functional changes, resulting in the predominance of the fetal gene programming with reactivation of the cardiac fetal phenotype. Thus, the endocrine and paracrine function of the heart increases the production of natriuretic peptides (NPs). (5, 6)

The family of NPs is constituted by three peptide hormones, atrial natriuretic peptide (ANP), type-B natriuretic peptide (BNP) and type-C natriuretic peptide (CNP). (7-9) These peptides reduce blood pressure as they produce diuresis, natriuresis and vasodilation. The also have anti-inflammatory effects and inhibit fibrosis and the hypertrophic growth of the myocardium. (10-12)

Renovascular (RV) hypertension increases left ventricular afterload with concomitant neurohumoral activation, producing cardiac hypertrophy and remodelling. In 1 kidney, 1 clip (1K-1C) Goldblatt model of renovascular hypertension, activation of the renin-
angiotensin-aldosterone system is rapidly followed by sodium and water retention and complemented by activation of the sympathetic nervous system. (13) Although this activation is primarily focused on preserving contractile function, chronic sympathetic hyperactivity increases vascular resistance and promotes arrhythmias and ventricular remodelling. (14)

The synthesis and secretion of NPs have been widely studied in several models of hypertension, such as DOCA-Salt model, aortic banding, 2 kidneys, 1clip model and 1K1C model. (15-23) In addition, NPs have been proposed as biomarkers of hemodynamic overload, severity and development in different cardiomyopathies. (23-26) However, the expression of NPs during the chronic evolution of the hypertensive process in the 1K1C pressure overload model has not been studied yet.

MATERIAL AND METHODS

Animals and surgical procedures
Male Sprague Dawley rats weighing 180 - 200 g were used. Animals were housed in a temperature controlled environment (21 ± 2 °C), illuminated with a 12:12 hours light-dark cycle (light from 7:00 am to 07:00 pm). They were fed standard diet and water at will. Animal care was in accordance with the international regulations recommended by the Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACyTAL). We studied animals with RV hypertension at 2 (RV2), 4 (RV4), 6 (RV6) and 12 (RV12) weeks after partial occlusion of the right renal artery with a 0.28-mm clip and contralateral nephrectomy. All animals had their respective control (sham) groups, Sh2, Sh4, Sh6 and Sh12, which underwent sham surgery with opening, closure in planes and manipulation of the renal artery without clip placement.

Determination of systolic pressure
Tail-cuff systolic blood pressure (BS) was determined in conscious rats after 2, 4, 6 and 12 weeks of treatment and recorded on a polygraph Grass 7B between 9:00 am and 01:00 pm, after 3 days of exercise training.

Plasma and tissue sample processing
After 2, 4, 6, and 12 weeks, the inferior vena cava was punctured and blood samples were collected in plastic tubes containing 15% w/v EDTA to obtain plasma. A solution of KCl 1M was injected through the same via to induce diastolic arrest. The hearts were excised and washed with phosphate-buffered (pH = 7.4) saline solution, rinsed and weighed. The cardiac chambers were dissected, weighed and stored at -70°C until being analyzed. The interventricular septum and interatrial septum were included within the left ventricle and left atrium, respectively.

The lungs were excised and washed with phosphate-buffered saline solution, rinsed and weighed. Representative fragments of lung tissue were obtained, weighed (wet lung weight, W) and stored at -70°C. Then, the fragments were rinsed at 86 °C until constant weight was reached (dry lung weight, D). The wet-to-dry lung weight (W/D) ratio was indicative of pulmonary edema. (27)

Body (BW), heart (HW), left ventricle (LVW), right ventricle (RVW), left atrial (LAW) and right atrial (RAW) weights were determined to estimate cardiac hypertrophy. Thus, we determined the HW/BW, LVW/BW, RVW/BW, LAW/BW and RAW/BW ratios as indicative of cardiac hypertrophy and cardiac chambers hypertrophy, respectively.

RNA extraction and Northern blot analysis
RNA was isolated from atrium and ventricular samples by Trizol (Invitrogen, Carlsbad, California, USA) reagent and analyzed using the Northern blot protocol. (16) The following probes were used: 1) a 600 bp HindIII/BamHI fragment containing rat ANP cDNA, 2) a 600 bp HindIII/XbaI fragment containing mice BNP cDNA, and 3) a 1.2 kb EcoRI fragment containing human GAPDH cDNA. The signal intensities of ANP and BNP were normalized to that of mRNA GAPDH.

Extraction and radioimmunoassay BNP in plasma samples
Plasma BNP was extracted using the method described by Sarda et al. (28, 29) A BNP-45 (Rat) kit (Phoenix Pharmaceuticals, Inc. Burlingame, CA, USA) was used for radioimmunoassay (RIA).

Statistical Analysis
Results are expressed as mean values ± standard error of the mean (SEM). The t test was used to compare the mean values between the sham groups and RV groups at different weeks after treatment. Single-factor ANOVA followed by Tukey-Kramer post-test was used to compare the different experimental groups (RV groups in different weeks), using software GraphPad Instat® (GraphPad Software Inc., San Diego, California, USA). Correlations were analyzed using Pearson’s linear correlation coefficient. A p value < 0.05 was considered statistically significant.

RESULTS

Time course of hypertension and cardiac hypertrophy
SP was greater in the experimental groups vs. the sham groups since 2 weeks after surgery (160 mm Hg), and this increase was time-dependent in the RV groups, reaching 200 mmHg after 12 weeks (Figure A). Cardiac hypertrophy, indicated by the HW/BW ratio, had a similar behavior (Figure 1B).

Table 1 shows the coefficients of heart and cardiac chambers hypertrophy. Left ventricular hypertrophy (LW/BW) was noted from week 2, remained stable between weeks 4 to 6, and presented a significant increase in week 12. Increase in RVW/BW ratio was later, showing a significant raise in weeks 6 and 12, with similar values in both periods. Atrial remodelling was evident at week 4; increase in the LAW/BW ratio was time-dependent, while RAW/BW ratio increased only at week 12.

There were no significant differences in the W/D ratio (data not included) in the sham groups or experimental groups; thus, pulmonary edema and heart failure were ruled out in all groups.

Cardiac chamber gene expression of ANP and BNP
The time course (2, 4, 6 and 12 weeks after surgery)
of ANP and BNP mRNA expression in the cardiac chambers was studied. ANP mRNA presented a time-dependent increase in the left ventricle, reaching statistically significant values at week 6 (Figure 2 A). Changes in BNP were moderate and occurred earlier, with significant increase in group RV2 that remained stable until week 6, rising again in week 12 (Figure 2 B). Increase in BNP mRNA expression at 6 and 12 weeks was lower compared to ANP mRNA expression (Figure 2 C).

In the right ventricle, ANP mRNA expression increased in the RV group only 12 weeks after treatment (Sh6: 100.0 ± 1.0; RV6: 100.0 ± 1.5; Sh12: 108.2 ± 7.2; RV12: 482.2 ± 55.3*; the values are expressed in percentages of the corresponding value in the sham groups, mean ± SEM, n = 5-8; *p < 0.001). However, enhanced BNP mRNA expression occurred earlier, at 6 weeks (Sh6: 100.0 ± 5.7; RV6: 137.9 ± 40.9; RV12: 200.7 ± 9.1*; the values are expressed in percentages of the corresponding value in the sham groups, mean ± SEM, n = 5-8; *p < 0.01). This behavior correlates with the increase in the RVW/BW ratio of groups RV6 and RV12, the only two groups which developed right ventricular hypertrophy.

Despite ANP and BNP mRNA expression increased significantly in both ventricles, we did not find any changes in the atria (data not shown).

Profile of BNP secretion in plasma
Plasma BNP levels increased significantly in the RV model since week 4 (Figure 3 A), and this raise was also time-dependent.

Correlations between systolic pressure, hypertrophy and NP expression
A positive correlation was observed between cardiac hypertrophy and BNP and ANP expression.
Plasma renin activity is normal in 1K1C model; the initial increase in systolic pressure is due to early fluid and sodium retention with a transient elevation in plasma volume which was followed by a return to normal levels, while hypertension in chronic stages is associated with elevated peripheral resistance. (30, 31)

The present study evaluated the time-course of the synthesis and secretion of NPs ANP and BNP in relation with the degree of cardiac hypertrophy secondary to pressure overload in the model of renovascular hypertension 1K1C. RV treatment induced a time-dependent elevation of SP that reached 200 mm Hg at 12 weeks. In addition, the degree of cardiac hypertrophy increased progressively and in parallel with the elevation of SP during the course of treatment. These results are consistent with the data found by Simone et al., (32) who studied rats after 8 weeks of RV treatment and observed a relation between blood pressure and left ventricular mass index.

Cardiac hypertrophy has been associated with increased synthesis and secretion of ANP in the ventricles. (33) In coincidence, we found a positive correlation between ventricular ANP expression, cardiac hypertrophy index and left ventricular hypertrophy index, demonstrated by greater cardiac hypertrophy and left ventricular ANP mRNA expression with longer treatment time.

BNP gene expression in both ventricles increases rapidly in response to hemodynamic overload, while increase in ANP expression appears in an advanced stage and has greater magnitude. Increased BNP expression in the left ventricle was noted from week 2, remained stable until week 6 and continued rising in week 12. In consequence, BNP expression did not correlate with the degree of hypertrophy and hypertension as ANP. In the right ventricle, BNP expression also increased earlier compared to ANP. BNP mRNA expression increased since week 6, while ANP mRNA did not rise until week 12. This pattern correlates with right ventricular hypertrophy present in groups RV6 and RV12.

According to these results, we may suggest that left ventricular hypertrophy develops until
it counteracts the afterload, with HW/BW ratio values that remain quite similar between weeks 4 and 6. During this period, left atrium enlargement begins, overloading of the pulmonary circulation. Thus, animals after 6 weeks of treatment show left and right ventricular hypertrophy, and left atrium enlargement. After 6 additional weeks (RV12), the left ventricle might not compensate for the increased afterload induced by renal artery stenosis, developing greater hypertrophy. We found neither pulmonary hypertension nor pulmonary congestion, as the W/D ratio, used to evaluate the presence of pulmonary edema, did not show any significant difference. The right ventricular hypertrophy remained during 6 weeks as a consequence of increased overload, leading to right atrial enlargement in the group RV12. After 12 weeks, we did not find clinical or morphometric signs of dilated cardiomyopathy as seen in the DOCA-salt model of hypertension. (34) Therefore, changes in ANP and BNP synthesis and secretion can only be related with the development of cardiac hypertrophy. Left ventricular systolic pressure, developed pressure of the left ventricle and +dP/dt were elevated in the experimental model and to a similar extent in the groups RV6 and RV12, with absence of significant differences between weeks 6 and 12. (35) These left ventricular functional variables were incidental to hypertrophy with absence of heart failure signs.

The endocrine response of the heart to pressure or volume overload varies in relation to whether the challenge is acute, subacute or chronic. The acute response to cardiomyocyte stretch results in enhanced secretion of ANP (exocytosis) stored in granules. ANP release following stretch is made at the expense of a depletable NP cytoplasmatic pool with no apparent effect on synthesis in the atrium. (11) In chronic hypertensive stage, increased ANP mRNA expression is more important in the ventricles than in the atria (18, 20, 36). ANP is secreted via a constitutive pathway. Thus, we find enhanced PN mRNA expression in the ventricles but not in the atria.

In the model studied, cardiac hypertrophy and left ventricular hypertrophy had a common pattern: both were time-dependent with a time-window between 4 to 6 weeks in which parameters remained stable. BNP expression increased earlier in both ventricles, compared to ANP. However, as ANP expression is closely related to cardiac hypertrophy, when ANP expression values increase as a consequence of significant hypertrophy of both ventricles they are higher than those of BNP. Therefore, BNP might respond selectively to pressure overload, while ANP might be more related to cardiac hypertrophy. When we compare these results with those previously reported (23) studying RV model, DOCA-salt model (where volume overload predominates) and the combination of both models in inverse sequence, we find that DOCA-Salt model did not increase BNP mRNA, while ANP expression increased early at 2 weeks after treatment and showed a significant increase ay...
4 weeks. Therefore, the response of NP expression in early stages of antihypertensive treatment would depend on the therapy used: enhanced BNP expression in the RV model and ANP in the DOCA-Salt model. In chronic stages, NP expression does no longer depend on the type of treatment but on the degree of cardiac hypertrophy.

Finally, we observed that BNP plasma levels increased by week 4, remained stable until week 6 and increased again thereafter until week 12. Plasma BNP profile has a similar pattern to that of BNP mRNA expression in the left ventricle. Increased BNP plasma levels at 2 weeks is indicative of enhanced synthesis which is significant at 4 weeks. The evolution profiles of synthesis and endocrine secretion is similar.

RESUMEN
Participación de los péptidos natriuréticos en la hipertensión renovascular y su correlación con la evolución de la hipertrofia de miocardio
Durante la hipertensión arterial, las interacciones entre las sobrecargas de presión y volumen conducen a diferentes patrones de hipertrofia cardiaca y a un aumento de los péptidos natriuréticos (PN). Los perfiles de síntesis y secreción de ANP y BNP se han investigado en modelos de hipertensión arterial. Sin embargo, aún no se ha estudiado su evolución diferencial durante periodos agudos y crónicos de la hipertrofia cardiaca producida por sobrecarga de presión. Por este motivo estudiamos ratas Sprague-Dawley con el modelo 1 ríoñón-1 clip a las 2, 4, 6 y 12 semanas, correlacionando la evolución de dichos perfiles con la hipertrofia cardiaca y la hipertensión arterial. Observamos una correlación positiva entre la elevación de la presión arterial y el grado de hipertrofia cardiaca, presentando ambos parámetros un incremento dependiente del tiempo a partir de las 2 semanas. La expresión del BNP mostró un aumento precoz a las 2 semanas de tratamiento, mientras que el ANP se incrementó significativamente a las 6 semanas. No obstante, la expresión del ANP aumentó en forma gradual, lo que permitió su correlación con la hipertrofia y la hipertensión. En estudios tempranos del desarrollo de la hipertrofia producida por el modelo renovascular, la expresión de los PN respondería en forma diferencial, incrementándose en forma precoz el BNP. Con la evolución de la hipertrofia, la expresión del BNP deja de ser específica y el aumento de ambos PN pasa a depender y a correlacionarse con el grado de evolución de la hipertrofia cardiaca.

Palabras clave > Factor natriurético auricular - Cardiomegalia - Hipertensión renovascular

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