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

CAROLINA S. CERRUDO[1], SUSANA CAVALLERO[1], MARTÍN RODRÍGUEZ FERMEPIN[1], CECILIA M. HERTIG[2], BELISARIO E. FERNÁNDEZ[1]

Received: 08/27/2009
Accepted: 02/23/2010

Address for reprints:
Carolina S. Cerrudo
Facultad de Farmacia y Bioquímica
Cátedra de Fisiopatología
Junín 956 - 5º Piso
(C1113AAD) Buenos Aires, Argentina
Phone/fax number: 4964-8268
e-mail: cerrudocarolina@yahoo.com.ar

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.

Rev Argent Cardiol 2010;78:339-345.

Key words > Atrial Natriuretic Factor - Cardiomegaly - Renovascular Hypertension

Abbreviations >

1K1C	1 kidney, 1 clip	**HW**	Heart weight
ANP	Atrial natriuretic peptide	**LWW**	Lung wet weight
mRNA	Messenger ribonucleic acid	**NP**	Natriuretic peptide
BNP	B-type natriuretic peptide	**SBP**	Systolic blood pressure
CNP	C-Type natriuretic peptide	**LDW**	Lung dry weight
RAW	Right atrial weight	**RVW**	Right ventricular weight
LAW	Left atrial weight	**LVW**	Left ventricular weight
BW	Body weight	**RV**	Renovascular

BACKGROUND

Hypertension may produce different patterns of ventricular remodelling as a result of the pathophysiological processes triggered by the interaction between pressure and volume overloads. (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-

[1] Chair of Pathophysiology, INFIBIOC (*Instituto de Fisiopatología y Bioquímica Clínica*), School of Pharmacy and Biochemistry, University of Buenos Aires, CONICET
[2] *Instituto de Investigaciones en Ingeniería Genética y Biología Molecular* (INGEBI) - CONICET, Argentina

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 bk 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 $p < 0.05$ 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 (LVW/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; Sh12: 100.0 ± 3.2; 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

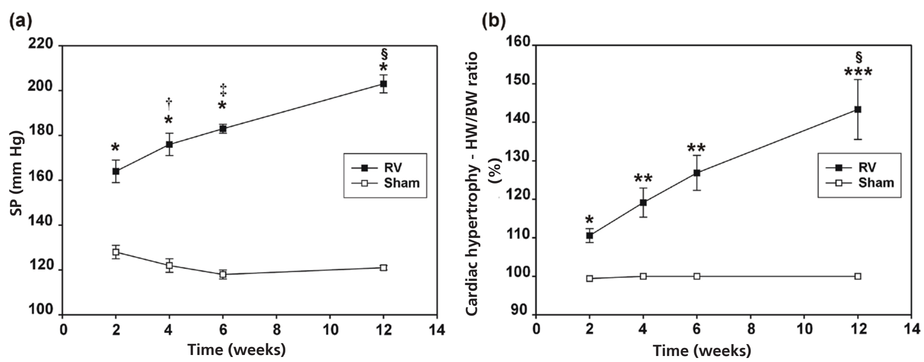

Fig 1. Changes in systolic pressure (SP) (pannel a) and cardiac hypertrophy, determined by heart weight/ body weight ratio (HW/ BW) (pannel b) according to treatment time. N = 149 Pannel a: values are expressed as mean ±SEM, * p < 0.001 versus the corresponding value in the sham groups; † p < 0.05 versus RV2; ‡ p < 0.001 versus RV2; § p < 0-01 versus RV6. Pannel b: values expressed as percentage of the corresponding value in the sham groups, mean ± SEM. * p < 0.05 versus Sh2. ** p < 0.01 versus Sh4 and Sh6. *** p < 0.001 versus Sh12. § p < 0.05 versus RV6.

Table 1. Hypertrophy of the different cardiac chambers

	Cardiac chambers hypertrophy (%)			
Groups	LVW/BW	RVW/BW	LAW/BW	RAW/BW
Sh2	100	100	100	100
RV2	119 ± 3 *	100 ± 4 *	109 ± 9 *	98 ± 5 *
Sh4	100	100	100	100
RV4	127 ± 4 *	104 ± 9 *	125 ± 8 *	99 ± 6 *
Sh6	100	100	100	100
RV6	127 ± 5 &	131 ± 10 &	134 ± 10 &	102 ± 5 &
SH12	100	100	100	100
RV12	146 ± 12 §	130 ± 7 §	153 ± 10 §	133 ± 6 §
n	**4-8**	**4-8**	**8-16**	**8-16**

Values expressed as percentage of the corresponding value in the sham groups, mean ± SEM LVW/BW: Left ventricular weight/body weight. RVW/BW: Right ventricular weight/body weight. LAW/BW: Left atrial weight/body weight. RAW/BW: Right atrial weight/body weight. * p < 0.05 versus Sh2. # p < 0.05 versus Sh4. & p < 0.05 versus Sh6. § p < 0.05 and §§ p < 0.001 versus Sh12.

hypertrophy index and SBP values ($p = 0.0027$, $r = 0.9973$ and $r2 = 0.9946$; Figure 4 A) and ANP expression in the left ventricle ($p = 0.0048$, $r = 0.9522$ and $r2 = 0.9067$; Figure 4 B) during the studied periods. There was also a positive correlation between the latter and left ventricular hypertrophy index ($p = 0.0050$, $r = 0.8698$ and $r2 = 0.7566$). As it can be noted, there was a gradual, time-dependent increase in blood pressure levels, NPs expression and development of cardiac hypertrophy.

Finally, BNP plasma levels showed a positive correlation with BNP mRNA expression in the left ventricle ($p = 0.0004$, $r = 0.942922$ and $r2 = 0.8890$; Figure 3 B).

DISCUSSION

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 presented a significant increase in week 12. Conversely, ANP presented a gradual increase from week 4, reached significant values at 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

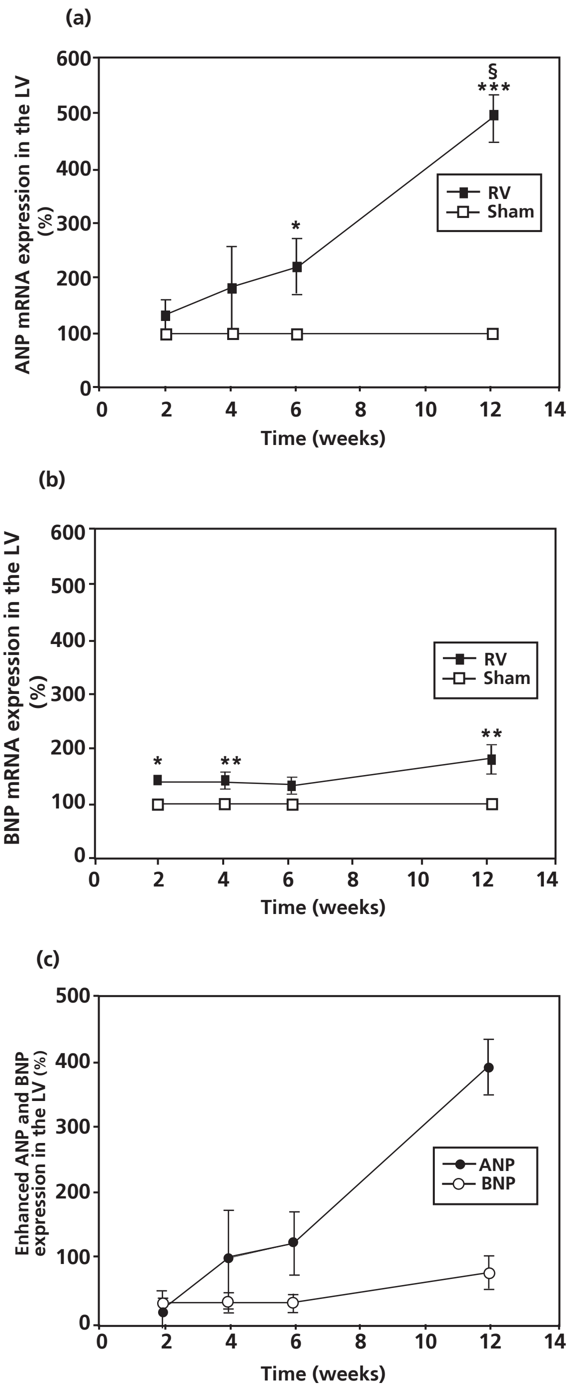

Fig. 1. Natriuretic peptide expression in the left ventricle (LV). ANP mRNA expression in the LV (pannel a), BNP mRNA expression in the LV (pannel b) and percentage of increase in ANP and BNP mRNA expression in relation to treatment time (pannel c). All values expressed as percentage of the corresponding value in the sham groups, mean ± SEM, n = 5.8 * $p < 0.05$, ** $p < 0.01$ y *** $p < 0.001$ versus sham. § $p < 0.001$ versus RV6.

in groups RV6 and RV12.

According to these results, we may suggest that left ventricular hypertrophy develops until

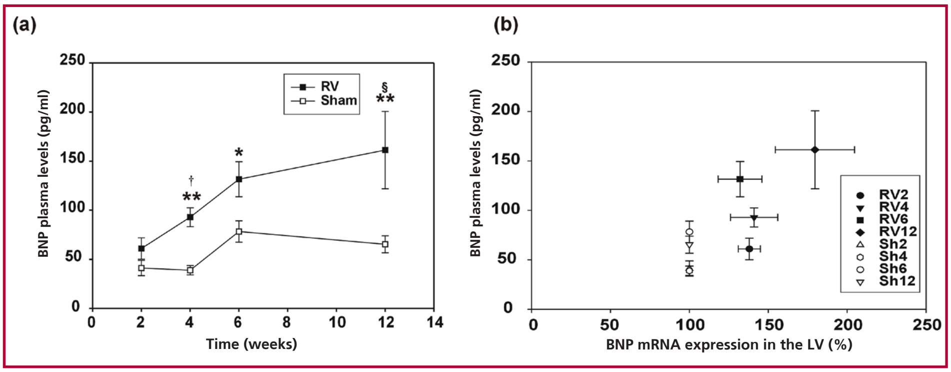

Fig 3. BNP plasma levels in relation to treatment time (pannel a) and correlation between BNP plasma levels (in %) and BNP mRNA expression in the LV (pannel b). Panel a: values are expressed as mean ±SEM, n = 6-13. * $p < 0.05$, ** $p < 0.001$ versus sham. † $p < 0.05$ versus RV2; § $p < 0.05$ versus RV4. Pannel b: each dot in the diagram represents mean ±SEM for each group.

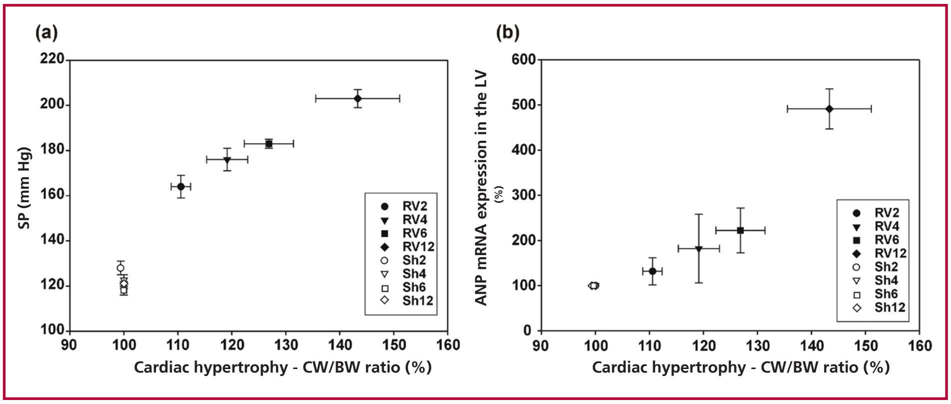

Fig 4. Correlation between systolic pressure and cardiac hypertrophy (pannel a) and correlation between ANP expression in the LV and cardiac hypertrophy (pannel b). Each dot in the diagram represents mean ±SEM for each group.

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 agian 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 cardíaca 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 períodos agudos y crónicos de la hipertrofia cardíaca producida por sobrecarga de presión. Por este motivo estudiamos ratas Sprague-Dawley con el modelo 1 riñón-1 clip a las 2, 4, 6 y 12 semanas, correlacionando la evolución de dichos perfiles con la hipertrofia cardíaca y la hipertensión arterial. Observamos una correlación positiva entre la elevación de la presión arterial y el grado de hipertrofia cardíaca, 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 sucorrelación con la hipertrofia y la hipertensión. En estadios 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 cardíaca.

Palabras clave > Factor natriurético auricular - Cardiomegalia - Hipertensión renovascular

BIBLIOGRAPHY

1. Devereux RB, Roman MJ, Ganau A, de Simone G, Okin PM, Kligfield P. Cardiac and arterial hypertrophy and atherosclerosis in hypertension. Hypertension 1994;23:802-9.

2. Muiesan ML, Salvetti M, Monteduro C, Bonzi B, Paini A, Viola S, et al. Left ventricular concentric geometry during treatment adversely affects cardiovascular prognosis in hypertensive patients. Hypertension 2004;43:731-8.

3. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006;367:356-67.

4. Khan MG. Encyclopedia of heart diseases. 2005. Chapter: Hypertrophy of the heart. p. 493-99.

5. Chien KR, Zhu H, Knowlton KU, Miller-Hance W, van-Bilsen M, O'Brien TX, et al. Transcriptional regulation during cardiac growth and development. Annu Rev Physiol 1993;55:77-95.

6. Yokota N, Bruneau BG, Fernández BE, Kuroski de Bold ML, Piazza LA, et al. Dissociation of cardiac hypertrophy, myosin heavy chain isoform expression and natriuretic peptide production in DOCA-salt rats. Am J Hypertens 1995;8:301-10.

7. Brown LA, Rutherford RA, Nunez DJ, Wharton J, Lowe DG, Wilkins MR. Downregulation of natriuretic peptide C-receptor protein in the hypertrophied ventricle of the aortovenocaval fistula rat. Cardiovasc Res 1997;36:363-71.

8. Silberbach M, Roberts CT. Natriuretic peptide signalling: molecular and cellular pathways to growth regulation. Cell Signal 2001;13:221-31.

9. Pandey KN. Biology of natriuretic peptides and their receptors. Peptides 2005;26:901-32.

10. de Bold AJ, Bruneau BG, Kuroski de Bold ML. Mechanical and neuroendocrine regulation of the endocrine heart. Cardiovasc Res 1996;31:7-18.

11. Gardner DG. Natriuretic peptides: markers or modulators of cardiac hypertrophy? Trends Endocrinol Metab 2003;14:411-6.

12. McGrath MF, de Bold ML, de Bold AJ. The endocrine function of the heart. Trends Endocrinol Metab 2005;16:469-77.

13. O'Sullivan JB, Black MJ, Bertram JF, et al. Cardiovascular hypertrophy in one-kidney, one clip renal hypertensive rats: a role for angiotensin II? J Hypertens 1994;12:1163-70.

14. Leenen FH. Cardiovascular consequences of sympathetic hyperactivity. Can J Cardiol 1999;15:2A-7A.

15. Ogawa T, Linz W, Stevenson M, Bruneau BG, Kuroski de Bold ML, Chen JH, et al. Evidence for load-dependent and load-independent determinants of cardiac natriuretic peptide production. Circulation 1996;93:2059-67.

16. Su X, Brower G, Janicki JS, Chen YF, Oparil S, Dell'Italia LJ. Differential expression of natriuretic peptides and their receptors in volume overload cardiac hypertrophy in the rat. J Mol Cell Cardiol 1999;31:1927-36.

17. Dobrzynski E, Wang C, Chao J, Chao L. Adrenomedullin gene delivery attenuates hypertension, cardiac remodeling, and renal injury in deoxycorticosterone acetate-salt hypertensive rats. Hypertension 2000;36:995-1001.

18. Bianciotti LG, de Bold AJ. Modulation of cardiac natriuretic peptide gene expression following endothelin type A receptor blockade in renovascular hypertension. Cardiovasc Res 2001;49:808-16.

19. Morgan T, Aubert JF, Brunner H. Interaction between sodium intake, angiotensin II, and blood pressure as a cause of cardiac hypertrophy. Am J Hypertens 2001;14:914-20.

20. Bianciotti LG, de Bold AJ. Natriuretic peptide gene expression in DOCA-salt hypertension after blockade of type B endothelin receptor. Am J Physiol Heart Circ Physiol 2002;282:H1127-34.

21. Capuano V, Ruchon Y, Antoine S, Sant MC, Renaud JF. Ventricular hypertrophy induced by mineralocorticoid treatment or aortic stenosis differentially regulates the expression of cardiac K+ channels in the rat. Mol Cell Biochem 2002;237:1-10.

22. Kvist S, Mulvany MJ. Contrasting regression of blood pressure and cardiovascular structure in declipped renovascular hypertensive rats. Hypertension 2003;41:540-5.

23. Cavallero S, González GE, Puyó AM, Rosón MI, Pérez S, Morales C, et al. Atrial natriuretic factor behaviour and myocyte hypertrophic profile in combined pressure and volume-induced cardiac hypertrophy. J Hypertens 2007;25:1940-50.

24. Scaglione J, Puyó AM, Dupuy HA, Postan M, Fernández BE. Behavior of atrial natriuretic factor in an experimental model of Trypanosoma cruzi infection in rats. J Parasitol 2001;87:923-6.

25. Ruskoaho H. Cardiac hormones as diagnostic tools in heart failure. Endocrine Rev 2003;24:341-56.

26. Puyó AM, Scaglione J, Auger S, Cavallero S, Postan M, Fernández BE. Natriuretic peptides as prognostic and diagnostic markers in Chagas' disease. Regul Pept 2005;128:203-10.

27. Briest W, Homagk L, Baba HA, Deten A, Rassler B, Tannapfel A, et al. Cardiac remodeling in erythropoietin-transgenic mice. Cell Physiol Biochem 2004;14:277-84.

28. Sarda IR, de Bold ML, de Bold AJ. Optimization of atrial natriuretic factor immunoassay. Clin Biochem 1989;22:11-15.

29. Puyó AM, Scaglione J, Auger S, Cavallero S, Donoso AS, Dupuy HA, et al. Atrial natriuretic factor as marker of myocardial compromise in Chagas' disease. Regul Pept 2002;105:139-43.

30. Baker KM, Chernin MI, Wixson SK, Aceto JF. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. Am J Physiol Heart Circ Physiol 1990;259:H324-32.

31. Fenoy FJ, Tornel J, Madrid MI, Lopez E, Garcia-Salom M. Effects of N omega-nitro-L-arginine and N-acetyl-L-cysteine on the reversal of one-kidney, one-clip hypertension. Am J Hypertens 1997;10:1208-15.

32. De Simone G, Devereux R, Camargo M, Volpe M, Wallerson D, Atlas S, et al. In vivo left ventricular anatomy in rats with two-kidney, one clip and one-kidney, one clip renovascular hypertension. J Hypertens 1992;10:725-32.

33. Matsubara H, Yamamoto J, Hirata Y, Mori Y, Oikawa S, Inada M. Changes of atrial natriuretic peptide and its messenger RNA with development and regression of cardiac hypertrophy in renovascular hypertensive rats. Circ Res 1990;66:176-84.

34. Cerrudo CS, Matorra F, Seropian I, Cavallero S, González GE, Morales C, Hertig CM, Gelpi RJ, Fernández BE. Caracterización hormonal y funcional de la hipertrofia de miocardio en modelos crónicos combinados de hipertensión arterial renovascular (RV) y DOCA-Sal (DS). Medicina 2008;68 (Suppl II):67.

35. Cerrudo CS, Matorra F, Rodriguez Fermepin M, Rey Deutsch AC, Saucedo SL, Cavallero S, González GE, Hertig CM, Gelpi RJ, Fernández BE. Hipertrofia de miocardio en modelos combinados de hipertensión arterial renovascular (RV) y DOCA-Sal (DS): funcionalidad ventricular y comportamiento hormonal. Medicina 2009;69(Suppl I):151-2.

36. An MR, Cheng Y, Kang D, Nam S, Lee J. Augmented expression of cardiac atrial natriuretic peptide system in hypertensive rats. J Korean Med Sci 1999;14:497-501.

Acknowledgments

The authors are grateful to Silvia Saucedo for her technical support.

This study was supported by grants from the University of Buenos Aires (UBACyT B607 and B014), CONICET (PIP 6161), and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT PICT 05-13775).