

# Natriuretic Peptides Synthesis and Secretion Profiles During the Evolution of Cardiac Hypertrophy in DOCA-Salt Hypertensive Rats

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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 ANP and 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 DOCA-salt treated Sprague-Dawley rats at weeks 2, 4, 6 and 12 and correlated the evolution of these profiles with cardiac hypertrophy and hypertension.

Cardiac hypertrophy had a positive correlation with ANP expression in the left ventricle and with ANP plasma levels. BNP expression increased after 4 weeks of treatment while BNP increased significantly after 6 weeks. In addition, BNP plasma levels increased only in the group treated for 12 weeks, while ANP plasma levels increased from week 2.

NP secretion has a differential response in the early stages of the development of cardiac hypertrophy induced by the DOCA-salt model, with early increase in ANP. As ANP levels were exceeded those of BNP in all the DOCA-salt groups, ANP might be considered a more specific marker of volume overload.

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**Key words >** Atrial Natriuretic Peptides - ANP - Cardiac Hypertrophy

## Abbreviations >

<b>RANP</b>	Atrial natriuretic peptide	<b>BW</b>	Body weight
<b>mRNA</b>	Messenger ribonucleic acid	<b>HW</b>	Heart weight
<b>BNP</b>	B-type natriuretic peptide	<b>NP</b>	Natriuretic peptide
<b>CNP</b>	C-type natriuretic peptide	<b>SP</b>	Systolic pressure
<b>DOCA</b>	Desoxycorticosterone acetate	<b>RVW</b>	Left ventricular weight
<b>DS</b>	DOCA-salt	<b>LVW</b>	Left ventricular weight
<b>RAW</b>	Right atrium weight	<b>RIA</b>	Radioimmunoassay
<b>LAW</b>	Left atrium weight	<b>RV</b>	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 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. They also have anti-inflammatory effects and inhibit fibrosis and the hypertrophic growth of the myocardium. (10-12)

The administration of desoxycorticosterone acetate (DOCA), in combination with a high salt diet and unilateral nephrectomy induces low-renin

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hypertension producing cardiac hypertrophy and remodeling, endothelial dysfunction, proteinuria and glomerulosclerosis. (13) The hypertension that develops is characterized by volume expansion and increased cardiac output, with subsequent volume overload. (14)

The synthesis and secretion of NPs have been widely studied in several models of hypertension, such as the DOCA-salt (DS) model, aortic coarctation and the renovascular models of hypertension: 2 kidneys, 1 clip model and 1 kidney, 1 clip model. (15-23) In addition, NPs have been proposed as biomarkers of hemodynamic overload, severity and outcomes in different cardiomyopathies. (23-26) However, the profiles of expression, synthesis and cardiac secretion of NPs over the chronic evolution of the hypertensive process in the DS volume overload model have not been clearly established yet.

The goal of the present study was to characterize the chronology of secretion and the variation of ANP and BNP mRNA expression in this model, correlating the evolution of these profiles with the development of hypertension and cardiac hypertrophy.

## 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 ( $21 \pm 2^\circ\text{C}$ ), illuminated with a 12:12 hours light-dark cycle (light from 07:00 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).

The rats subjected to left nephrectomy were studied with DS hypertension model at 2 (DS2), 4 (DS4), 6 (DS6) and 12 (DS12) weeks of treatment. They received weekly injections of deoxycorticosterone acetate (DOCA, 30 mg/kg) and were supplied with 1% NaCl in the drinking water. All animals had their respective control (sham) groups, Sh2, Sh4, Sh6 and Sh12 which underwent sham surgery with opening, closure in planes and received vehicle and water.

### Determination of systolic pressure

Tail-cuff systolic blood pressure (SP) was determined in conscious rats after 2, 4, 6 and 12 weeks of treatment ( $n = 10-19$ ), and recorded on a polygraph Grass 7B between 9:00 am and 01:00 pm, after 3 days of 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 ( $\text{pH} = 7.4$ ) saline solution, rinsed and weighed. The cardiac chambers were dissected, weighed and stored at  $-70^\circ\text{C}$  until being analyzed. The interventricular septum and interatrial septum were included within the left ventricle and left atrium, respectively ( $n = 10-19$ ).

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 and 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 ( $n = 4-8$ ) and ventricular ( $n = 4-8$ ) 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 ANP and BNP were extracted using the method described by Sarda et al. (27, 28) ( $n = 4-8$ ). (28, 29) ANP and BNP-45 (Rat) kits (Phoenix Pharmaceuticals, Inc. Burlingame, CA, USA) were used for radioimmunoassay (RIA).

### Statistical Analysis

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

## RESULTS

### Time course of hypertension and cardiac hypertrophy

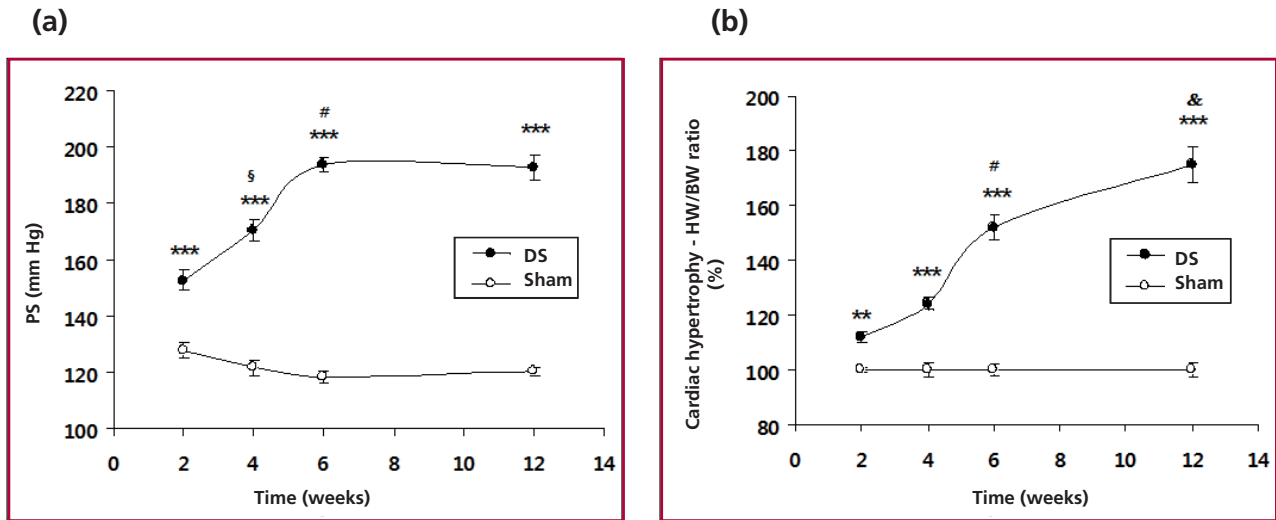
Systolic pressure was greater in the experimental groups vs. the sham groups 2 weeks after surgery (153 mm Hg), showing variations until week 6 and presenting stable values between week 6 and 12 (Figure 1 a).

Although all the DS groups developed cardiac hypertrophy after 2 weeks of treatment (evaluated by the HW/BW ratio), a time-dependent increase was observed from week 4 (Figure 1 b).

Left ventricular hypertrophy evaluated by the LVW/BW ratio was noted from week 2, remained stable between weeks 4 and 6, and presented a significant increase in week 12 (Table 1). Increase in RVW/BW ratio was later, showing a significant raise in weeks 6 and 12, with similar values in both periods. Left atrium remodeling was evident at week 4 (LAW/BW ratio) while right atrium remodeling was noted at week 6 (RAW/BW ratio). Both indices were time-dependent (Table 1).

### ANP and BNP mRNA expression in the left ventricle

ANP mRNA presented a moderate increase in the left ventricle from week 2, reaching statistically significant values at week 4, in which the increase was time-dependent (Figure 2 a). Changes in BNP expression



**Fig. 1.** Changes in systolic pressure (SP) (a) and cardiac hypertrophy, determined by heart weight/body weight ratio (HW/BW) (panel b) according to treatment time (n = 10-19). Panel a: values are expressed as mean ± SEM. Panel b: values expressed as percentage of the corresponding value in the sham group. \* p < 0.01 and \*\*\* p < 0.001 vs. sham; § p < 0.05 vs. DS2; # p < 0.05 vs. DS4; & p < 0.05 vs. DS6.

were moderate and occurred later, with statistical significant increase only in groups DS6 and DS12; these changes were greater in week 12 (Figure 2 b).

**Profile of ANP and BNP secretion in plasma**

Plasma BNP levels increased significantly in the DS model only at week 12 (Figure 3 a). However, plasma ANP levels increased earlier from week 2 (Figure 3 b).

**Correlations between systolic pressure, hypertrophy and NP expression**

A positive correlation was observed between cardiac hypertrophy and ANP expression in the left ventricle (r: 0.9923; p < 0.0008; Figure 4 a) and between cardiac hypertrophy index and ANP plasma levels (r: 0.9716; p < 0.0057; Figure 4 b). Yet, the development of cardiac hypertrophy did not correlate with BNP expression and BNP plasma levels (Figure 4 c and d).

**DISCUSSION**

The present study evaluated the time-course of the synthesis and secretion of the NPs ANP and BNP in relation with the degree of cardiac hypertrophy secondary to volume overload in the model of DS hypertension. DOCA-salt treatment induced a rapid, time-dependent elevation of SP during 6 weeks of treatment, with similar values at week 12. However, the degree of cardiac hypertrophy increased progressively and was time-dependent until week 12. These results suggest that cardiac hypertrophy developed in this experimental model is not completely related to increased SP. These findings are consistent with those published by Brown et al., (29) who studied rats with 4 and 8 weeks of DS treatment .

Cardiac hypertrophy has been associated with increased synthesis and secretion of ANP in the

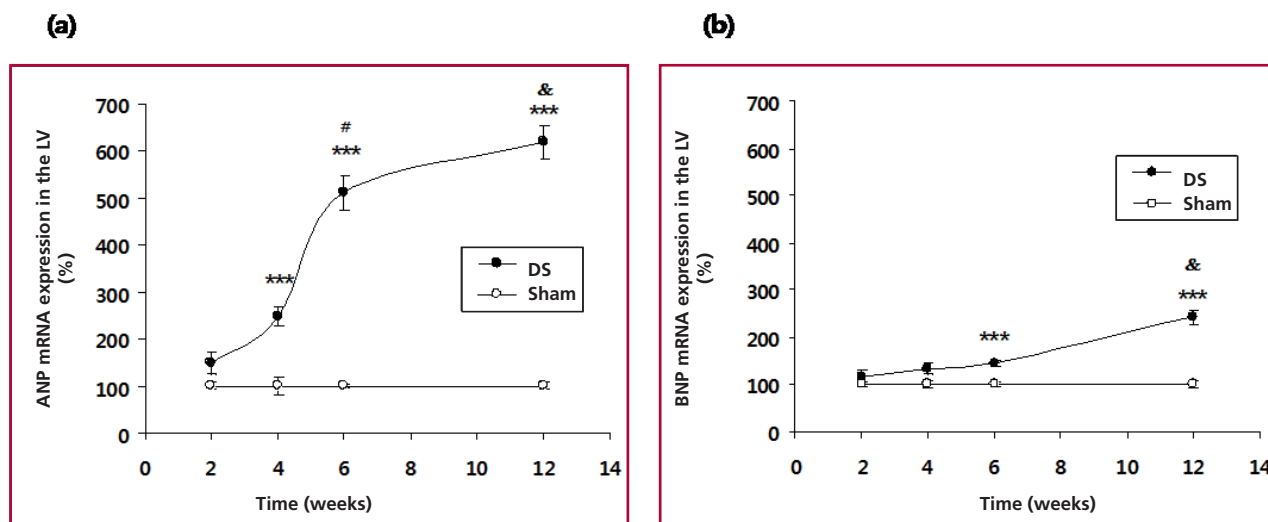
**Table 1.** Hypertrophy of the different cardiac chambers

Cardiac chambers hypertrophy (%)	LVW/BW	RVW/BW	LAW/BW	RAW/BW
Sh2	100 ± 3,5	100 ± 7,8	100 ± 6,4	100 ± 7,8
DS2	112 ± 1,6*	109 ± 9,6	115 ± 4,1	110 ± 4,8
Sh4	100 ± 3,5	100 ± 4,8	100 ± 3,7	100 ± 5,9
DS4	128 ± 4,3**	102 ± 4,2	125 ± 5,9**	107 ± 4,6
Sh6	100 ± 1,9	100 ± 3,2	100 ± 3,7	100 ± 5,6
DS6	130 ± 6,9**	136 ± 9,1*	144 ± 6,7*	126 ± 4,6*
Sh12	100 ± 2,6	100 ± 2,8	100 ± 2,2	100 ± 3,3
DS12	164 ± 11,1***	141 ± 9,9**	184 ± 10,7***&	173 ± 11,3***

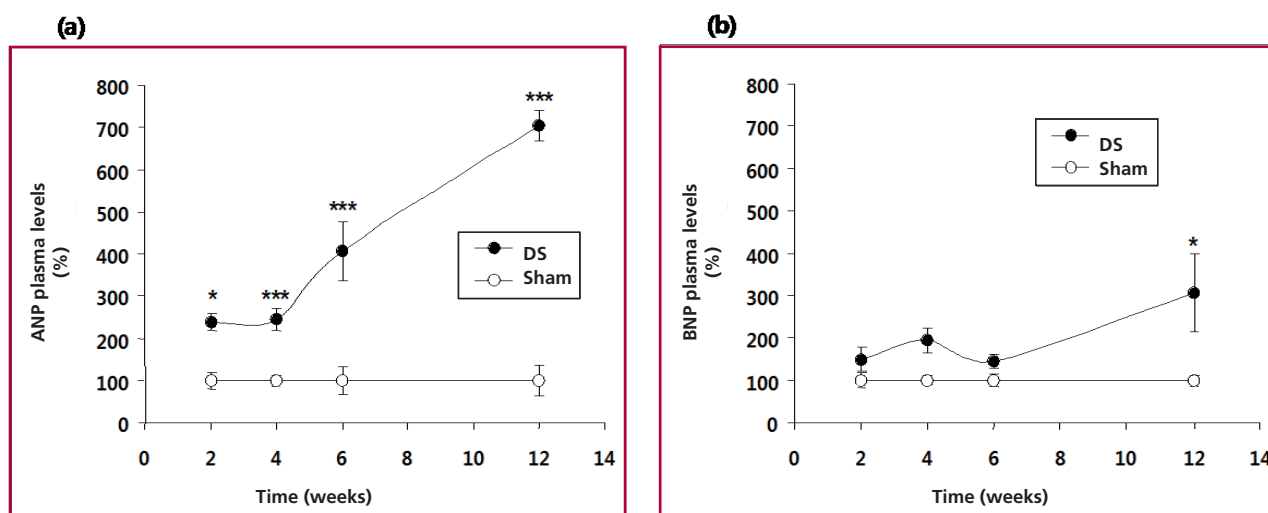
Values expressed as percentage of the corresponding value in the sham groups, mean ± SEM LVW/BW: Left ventricular weight/body weight RVW/BW: Right ventricular weight/body weight LAW/BW: Left atrium weight/body weight. RAW/BW: Right atrium weight/body weight. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 vs. sham; & p < 0.05 vs. DS6.

ventricles in one study. (30) Our results coincide with that study, as we found greater cardiac hypertrophy and left ventricular ANP mRNA expression with longer treatment time. This was demonstrated by a positive correlation between ventricular ANP mRNA expression and cardiac hypertrophy index.

The NPs expression and secretion in the heart due to hemodynamic overload has a differential response. BNP gene expression increases moderately and later, while increase in ANP expression appears earlier and has greater magnitude. ANP in the left ventricle presented a gradual increase from week 2, reached significant values at week 4 and had a time-dependent increase until week 12. Conversely, BNP increased after 6 weeks of treatment, showing the greatest increase at 12 weeks. In consequence, BNP expression did not correlate with the degree of hypertrophy and hypertension as ANP.



**Fig. 2.** Natriuretic peptide expression in the left ventricle (LV) ANP mRNA expression in the LV (panel a), and BNP mRNA expression in the LV (panel b) in relation to treatment time. All values are expressed as percentage of the corresponding value in the sham groups, mean  $\pm$  SEM (n = 4-8). \* p < 0.05 and \*\*\* p < 0.001 vs. sham; # p < 0,05 vs. DS4; & p < 0.01 vs. DS6.



**Fig. 3.** Plasma levels of ANP (panel a) y BNP (panel b) in relation to treatment time. Panels a and b: All values expressed as percentage of the corresponding value in the sham groups, mean  $\pm$  SEM (n = 4-8). \* p < 0.05 and \*\*\* p < 0.001 vs. sham.

The endocrine response of the heart related with the production or secretion of NPs to pressure or volume overload varies in relation to whether the challenge is acute, subacute or chronic. ANP expression increased earlier in the left ventricle, compared to BNP. However, as BNP expression is closely related to heart failure, it increases during the 12 weeks of treatment but with values they are lower than those of ANP. Therefore, ANP expression is related with cardiac hypertrophy and might respond selectively to volume overload. When we compared these results with those previously reported (31) studying the renovascular (RV) hypertension model one kidney, one clip (in which pressure overload predominates), we found an early increase in BNP expression after 2 weeks of treatment in the RV mode 1, while ANP expression increased

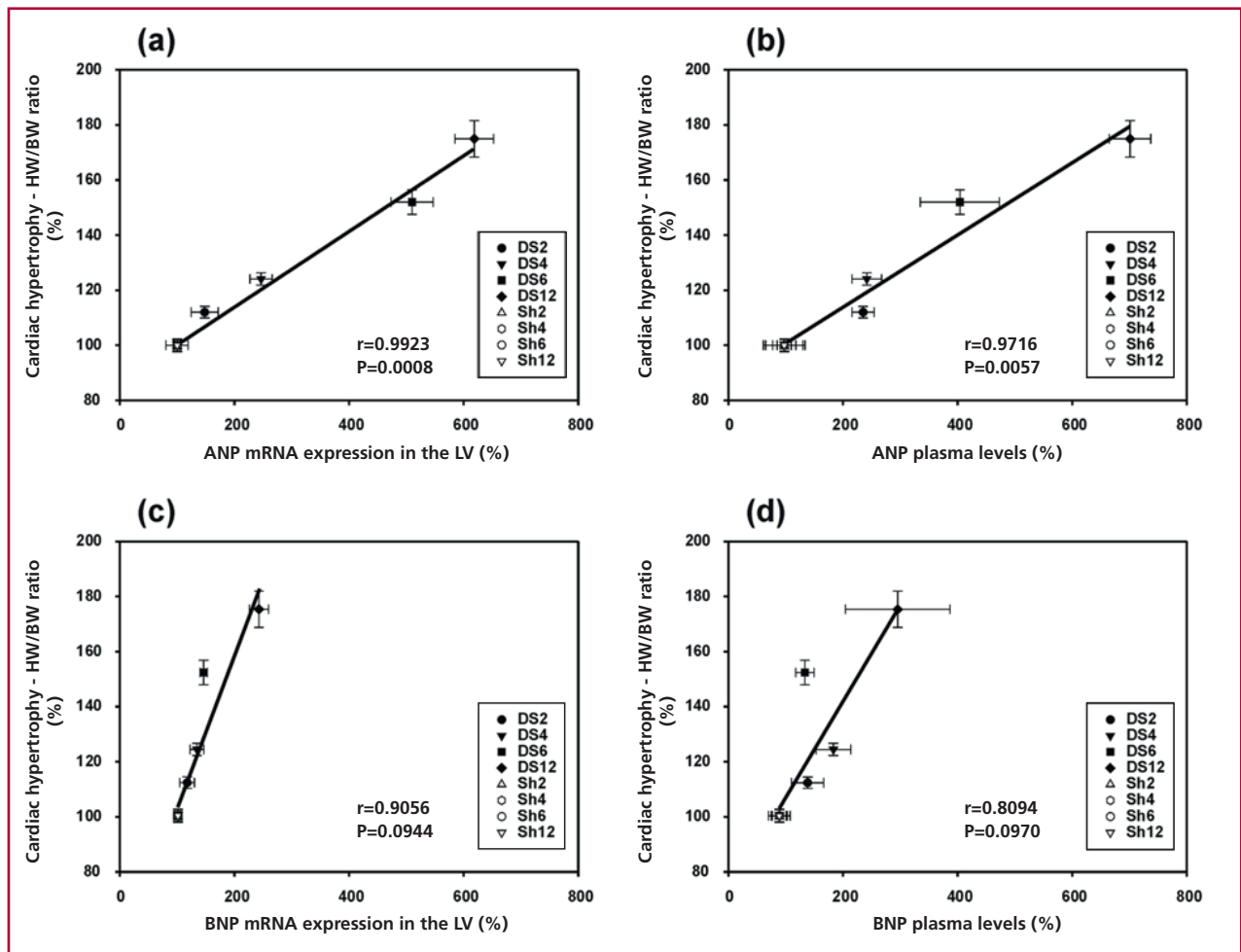
significantly at 6 weeks. In conclusion, early increase in NP expression depends on the hypertensive model used: ANP in the DS model and BNP in the RV model.

Finally, the synthesis and secretion of ANP was higher and earlier than that of BNP in absolute values and percentages across all the DS groups, suggesting that ANP might be considered a more specific marker of volume overload.

## RESUMEN

### Perfiles de síntesis y secreción de los péptidos natriuréticos durante la evolución de la hipertrofia cardíaca en ratas hipertensas DOCA-sal

Durante el desarrollo de la hipertensión arterial, las interacciones entre las sobrecargas de presión y de volumen conducen a diferentes patrones de hipertrofia cardíaca y a



**Fig. 4.** Correlation between cardiac hypertrophy and ANP expression in the LV (panel a), plasma ANP levels (panel b), BNP expression in the LV (panel c) and BNP plasma levels (panel d). Each dot in the diagram represents mean  $\pm$  SEM for each group.

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 la evolución diferencial de estos perfiles durante períodos agudos y crónicos de la hipertrofia cardíaca producida por sobrecarga de volumen. Por este motivo estudiamos ratas Sprague-Dawley con el modelo DOCA-sal a las 2, 4, 6 y 12 semanas, correlacionando la evolución de dichos perfiles con la hipertrofia cardíaca y la hipertensión arterial. El grado de hipertrofia cardíaca se correlacionó positivamente con la expresión del ANP en el ventrículo izquierdo y con los niveles de ANP en plasma. La expresión del ANP aumentó a las 4 semanas de tratamiento, mientras que la de BNP se incrementó recién a las 2 semanas. Asimismo, el BNP plasmático se incrementó sólo en el grupo con 12 semanas de tratamiento, mientras que el ANP plasmático mostró un aumento a partir de las 2 semanas de tratamiento. Durante el desarrollo de la hipertrofia cardíaca producida en el modelo DOCA-sal, la síntesis y la secreción de los PN responden en forma diferencial, con incremento precoz del ANP. Además, el aumento de éste superó al de BNP en todos los grupos DOCA-sal, lo que permitiría considerar al ANP como un marcador más específico de la sobrecarga de volumen.

**Palabras clave >** Péptidos natriuréticos auriculares - ANP  
Hipertrofia cardíaca

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#### Competing interests

None declared.

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