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RESEARCH ARTICLE

Uncoordinated regulation of atrial natriuretic factor and brain natriuretic peptide in lipopolysaccharide-treated Rats

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Abstract

We investigated the expression and secretion of the natriuretic peptides (NPs) ANF and BNP in lipopolysaccharide (LPS)-induced sepsis and its association with cytokines and other biologically active substances. LPS treatment increased plasma levels of ANF and BNP. The latter increase was larger than the increase in plasma ANF. LPS also increased cardiac content and gene expression of BNP but not of ANF. LPS treatment significantly increased gene expression cytokines, chemokines and proteases, which significantly correlated with BNP gene expression. SB203580, a p38 MAP kinase inhibitor, inhibited the elevation of BNP in plasma. The present work suggests that during inflammation, BNP gene expression and secretion is uniquely related to changes in gene expression in the absence of hemodynamic changes and hence differentiates ANF and BNP as biomarkers of cardiac disease.

Keywords: Natriuretic peptides, ANF, BNP, lipopolysaccharide, inflammation

Introduction

Cardiac pathologies such as acute coronary syndrome, congestive heart failure or hypertensive heart disease are accompanied by an increase in plasma concentration of the natriuretic peptides (NPs) ANF and BNP (Ramos & de Bold 2006). However, while plasma ANF elevation correlates with left ventricular (LV) dysfunction, plasma BNP elevation is not always associated with volume overload or LV dysfunction (Maeder et al. 2005; Shor et al. 2006; Burjonroppa et al. 2007). Intriguingly, in acute cardiac allograft rejection episodes, plasma BNP levels increase without a corresponding increase in plasma ANF levels. Successful treatment of the rejection episode using the anti-T cell monoclonal antibody OKT3 decreases the elevated plasma BNP levels to prerejection values suggesting that T-lymphocytes and inflammation in general, may participate in the specific elevation of BNP in the rejection episode (Masters et al. 1999). We have confirmed this view in past investigations both *in vivo* and *in vitro* showing that inflammatory challenges selectively upregulate the synthesis of BNP (Ma et al. 2004; Meirovich et al. 2008; Ogawa et al. 2008). These investigations also demonstrated that the BNP-promoting effect of cytokines and related substances is completely abolished by the MAPK p38 inhibitor SB203580.

Related to the above observations and to their possible impact in the specificity of plasma NPs as biomarkers of cardiac disease, there are reports of both ANF and BNP blood levels increases in severe sepsis or septic shock patients. In fact, measurement of blood levels of NPs has been proposed as useful for the diagnosis and prognosis of sepsis or septic shock (Charpentier et al. 2004; Chua & Kang-Hoe 2004; Wang et al. 2004; Brueckmann et al. 2005; Maeder et al. 2005; Morgenthaler et al. 2005; Hoffmann et al. 2006; Shor et al. 2006; Ueda et al. 2006; Burjonroppa et al. 2007; Nikolaou et al. 2007; Roch 2007; Yucel et al. 2008).

The above evidence suggests that plasma BNP is elevated by inflammation in addition to a hemodynamic challenge while plasma ANF is elevated mainly through hemodynamic changes. It is not known if changes in the expression of the NP clearance receptor "C" may also contribute to the observed changes in NP plasma levels.

In the present work, we investigated the evolution of the expression of NPs and natriuretic peptide receptor-C (NPR-C) in lipopolysaccharide (LPS)-induced sepsis in

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the rat and its association with expression and secretion of biologically active substances including cytokines, chemokines and proteases. SB203580 a p38 MAPK inhibitor was administered to LPS-treated animals to evaluate the involvement of the p38 MAPK pathway in the observed changes in NP gene expression and secretion.

Material and methods

Animals and experimental protocol

The experimental protocol was carried out in accordance with the Canadian Council on Animal Care Guide to the Care and Use of Experimental Animals. The experiments were conducted using 7-week old Sprague Dawley rats obtained from Charles River (Wilmington, MA). Three experimental protocols were followed: Group 1. Dosing experiments ($n=8$ in each group). One mL of saline or water containing the p38 MAPK inhibitor SB203580-HCl (Tocris, Ellisville, Missouri; final concentration = 2 mg/kg body weight) was administered intraperitoneally (i.p.). One hour later, one mL of saline alone or containing LPS (Escherichia coli 0111: B4, Sigma; final concentration: 0.5, 1, 2, 5 or 15 mg/kg body weight) were administered i.p. Six hours later, the rats were killed by decapitation. Group 2. Time course experiments ($n=8$ in each group). One mL of LPS solution (final concentration = 5 mg/kg) was i.p. administered. Three, 6, 12 or 18 h, or 1, 2, 4 or 8 days later, the rats were killed by decapitation. A group of equivalent animals but without treatment was used as control. Group 3. Chronic LPS dosing ($n=4$ in each group). Time release pellets (Innovation Research of America, Sarasota, FL) containing 0.3 or 3 mg of LPS or pellets containing matrix only were implanted subcutaneously (Smith et al. 2006). The pellets were designed to deliver the LPS dose over 90 days. Twelve weeks later, the rats were sacrificed by decapitation. In all experiments, the rats had free access to rat chow and water.

Systolic blood pressure was determined by the tail cuff method just before decapitation. Trunk blood was collected in chilled tubes containing EDTA and immediately centrifuged at 4°C. After centrifugation, the plasma was stored at –80°C. After blood collection, the heart, lungs and kidneys were excised and hearts from Group 1 animals were dissected in cold saline. The heart was dissected into right and left atrium and right and left ventricle with their respective septa as part of the left chambers. After weighing, the tissues were wrapped in aluminum foil and snap frozen in liquid nitrogen for determination of peptide content and mRNA levels of natriuretic peptides and cytokines.

ANF and BNP determination in plasma and tissue

Plasma samples were acidified by adding 100 μL/mL of 1 N HCl and passed through Sep-Pak C18 cartridges (Millipore) that were pre-wetted with 5 mL of 80% ACN in 0.1% TFA and 10 mL of 0.1% TFA. The cartridges with the absorbed peptides were washed with 20 mL of 0.1% TFA and eluted with 3 mL of 60% ACN in 0.1% TFA. Tissue samples were homogenized in 10 vol of an extracting mixture consisting of 0.1N HCl, 1.0 mol/L acetic acid, and 1% NaCl and centrifuged at 10,000 g for 30 min at 4°C. The supernatants were then extracted with Sep-Pak C18 cartridges as described above for plasma except that elution was with 80% ACN in 0.1% TFA. The eluates from tissue or plasma were freeze dried and processed for radioimmunoassay (RIA). ANF and BNP were determined by RIA using αANP (1–28; Rat, Mouse) antibody (Phoenix Pharmaceutical Inc.) and BNP-32 (Rat) antibody (Phoenix Pharmaceutical Inc.), respectively. The ANF and BNP antisera showed <0.01% cross-reactivity with BNP and ANF peptides, respectively.

Plasma cytokine array

Plasma cytokine concentration was evaluated using RayBio Rat Cytokine Antibody Array (RayBiotech). One mL of the plasma was applied to the array and was processed according to the manufacturer's protocol. Membrane signals were quantitated by lucigenin-enhanced chemiluminescence using a Kodak 1D chemiluminescence imaging system. Analysis of image files was done using the RayBio® Analysis Tool program. The results were expressed as relative densities compared with positive controls included in each membrane.

RNA extraction and RT-PCR

RNA from tissue samples ($n=4$, each chamber, each group) was extracted using Trizol (GIBCO BRL). Reverse transcription was performed with Transcriptor First Strand cDNA Synthesis Kit using an Oligo (dt)18 primer (Roche) according to the manufacturer's instruction. Real time PCR was performed using a LightCycler 480 SYBR Green I Master (Roche). The primers sequences are shown in Table 1. Results were normalized to glucose-6-phosphate dehydrogenase mRNA as an internal control, and the results are thus shown as a relative mRNA levels. Results were normalized to a calibrator and corrected for efficiency.

Statistical analysis

All the results are expressed as mean ± SEM. ANOVA was performed to determine statistical differences among multiple groups using Systat®. When significance was obtained by ANOVA, Fisher's least squares difference post hoc analysis was used to determine pairwise differences. Correlations between each gene of NPs and biologically active substances were determined by Pearson's simple correlation. A level of $p<0.05$ was considered significant.

Results

Anatomical and hemodynamic parameters

Heart chamber weight to body weight ratios as well as lung and kidney weight to body weight ratios did not change in any of the groups belonging to the different protocols. Systolic blood pressure of the rats treated with the highest dose (15 mg/kg LPS) was significantly lower compared to the control rats in the dosing experiment

Table 1. Primer pairs and product size utilized for PCR.

Primer sequences		product (bp)
ACTA-1 (sense)	5′-CTCTCTCTCCTCAGGACGACAATC-3′	207
ACTA-1 (antisense)	5′-CAGAATGGCTGGCTTTAATGCTTC-3′	
ANF (sense)	5′-GCCGGTAGAAGATGAGGTCA-3′	269
ANF (antisense)	5′-GGGCTCCAATCCTGTCAATC-3′	
BNP (sense)	5′-TCTGCTCCTGCTTTTCCTTA-3′	258
BNP (antisense)	5′-GAACTATGTGCCATCTTGGA-5′	
CINC-1 (sense)	5′-TGAGCTGCGCAGTCAGTGCCTGCA-3′	195
CINC-1 (antisense)	5′-ACACCCTTTAGCATCTTTTGGACT-3′	
Fractalkine (sense)	5′-CCACAAGATGACCTCGCCAATC-3′	297
Fractalkine (antisense)	5′-TCCACTGTGGCTGACTCAGGCT-3′	
G6PD (sense)	5′-CCAGCCTCCTACAAGCACCTCA-3′	406
G6PD (antisense)	5′-AATAGCCCCACGACCCTCAGTA-3′	
ICAM-1 (sense)	5′-ACCCTGGAGAGCACAAACAGCAGAG-3′	231
ICAM-1 (antisense)	5′-ACCGTGGGCTTCACACTTCACAGTT-3′	
IL-1β (sense)	5′-CTTCAAATCTCACAGCAGCATCTCG-3′	102
IL-1β (antisense)	5′-CCACGGGCAAGACATAGGTAGC-3′	
LIX (sense)	5′-GTTCACACTGCCACAGCATC-3′	300
LIX (antisense)	5′-GCTGATCTGACCAGTGCAAG-3′	
MCP-1 (sense)	5-CTGTCTCAGCCAGATGCAGTTAAT-3′	300
MCP-1 (antisense)	5-TATGGGTCAAGTTCACATTCAAAG-3′	
MIP-3-α (sense)	5′-CACAACACAGATGGCCGACGAAG-3′	185
MIP-3-α (antisense)	5′-TTGGGCTGTGTCCAATTCCATCC-3′	
MMP-8 (sense)	5′-CAAGGAGTGTCCAAGCCATT-3′	179
MMP-8 (antisense)	5′-CTGCTGGAAAACTGCATCAA-3′	
NPR-C (sense)	5′-GACAAACTCGAGAGAAACTGCTACT-3′	248
NPR-C (antisense)	5′-ATGTTGAAGAAAGCATAGTCTCCAC-3′	
RANTES (sense)	5′-ACTCCCTGCTGCTTTGCCTACC-3′	123
RANTES (antisense)	5′-TTGGCGGTTCCTTCGAGTGAC-3′	
TIMP-1 (sense)	5′-CGAGACCACCTTATACCAGCG-3′	216
TIMP-1 (antisense)	5′-CAGGAAGCTGCAGGCAGTGAT-3′	
TNFα (sense)	5′-ACTCCCAGAAAAGCAAGCAA-3′	211
TNFα (antisense)	5′-CGAGCAGGAATGAGAAGAGG-3′	

Note: ACTA-1: α1 skeletal muscle actin; ANF: atrial natriuretic factor, BNP: brain natriuretic peptide, CINC-1: cytokine-induced neutrophil chemoattractant-1; G6PD: glucose-6-phosphate dehydrogenase; ICAM-1: intercellular adhesion molecule-1; IL: interleukin; LIX: lipopolysaccharide-induced CXC chemokine; MCP-1: monocyte chemoattractant protein-1; MIP-3-alpha: macrophage inflammatory protein 3 alpha; MMP-8: matrix metaroproteinase-8; NPR-C: natriuretic peptide receptor C; RANTES: regulated on activation, normal T-cell expressed and secreted; TIMP-1: tissue inhibitor of metalloproteinase; TNFα: tumor necrosis factor α.

(Table 2). In the time course (conducted using 5 mg LPS/kg) and chronic experiments, systolic blood pressure did not show any significant changes. Heart rate did not change significantly in any of the protocols.

Changes in plasma concentration of NPs

Dosing experiments

Plasma ANF levels increased significantly when more than 5 mg/kg LPS was administered while plasma BNP increased significantly when only 1 mg/kg LPS was administered (Figure 1). Administration of 2 mg/kg SB203580 prior to the administration of 5 mg/kg LPS significantly reduced plasma BNP levels. Plasma ANF and BNP levels continued to increase with increasing LPS doses. At 15 mg/kg LPS induced significant hemodynamic changes as evidenced by a significant reduction in systolic blood pressure (Table 2). For this reason, a 5 mg/kg LPS was used for the time course experiments.

Time course experiments

In these experiments, both plasma ANF and BNP levels in rats decapitated at 6 and 12 h following administration of LPS were significantly increased compared to the pretreatment time point. After one day following LPS administration, ANF plasma levels were significantly lower compared to the 12-h time point. Plasma BNP levels at 18 h postinjection were significantly lower compared to the 12-h time point. In these experiments, although both ANF and BNP were elevated, the increase in BNP was always relatively larger than the increase in ANF.

Chronic experiments

In the chronic experiments, plasma BNP levels in rats that were implanted 0.3 mg or 3 mg LPS pellets significantly increased compared to the control rats while plasma ANF remained unchanged.

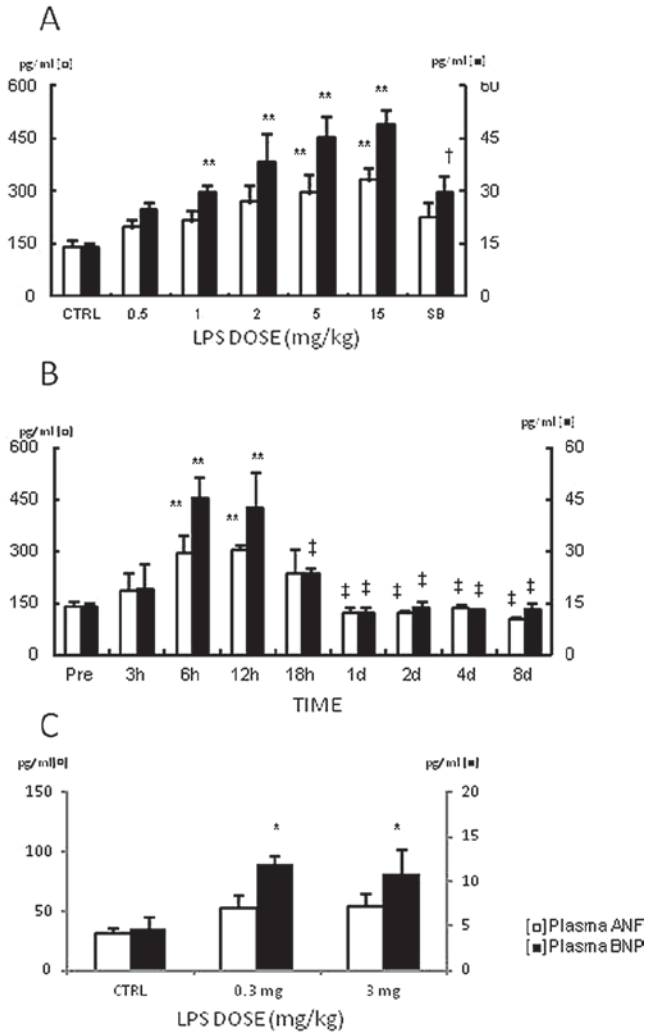

Figure 1. Effect of dose- (A), time- (B) or chronic administration (C) of lipopolysaccharide (LPS) on plasma atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) plasma levels. CTRL = control; 0.5 = LPS 0.5 mg/kg; SB = SB203580; Pre = before treatment. $n = 8$ in each group in Figure A and Figure B. $n = 4$ in each group in Figure C. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control or pretreatment. $\dagger p < 0.05$ vs. LPS 5 mg/kg rats. $\ddagger\ddagger p < 0.01$, $\ddagger p < 0.05$ vs. 12-h rats.

Table 2. Systolic blood pressure and Heart Rate in animals treated with increasing doses of lipopolysaccharide (LPS).

LPS (mg/kg)	Systolic BP (mmHg)	Heart rate (/min)
—	120 ± 2.7	415 ± 13
0.5	120 ± 3.7	436 ± 18
1	133 ± 11.6	416 ± 28
2	116 ± 3.3	433 ± 17
5	115 ± 5.5	426 ± 11
15	96 ± 4.7*	436 ± 4
5.0 + SB	118 ± 2.1	421 ± 19

Note: Values are Mean ± SE. BP = blood pressure; SB = SB203580. n = 8 in each group. $^{*}p < 0.05$ vs. control group.

Changes in plasma concentration of biologically active substances

Plasma cytokine-induced neutrophil chemoattractant-1 (CINC-1), fractalkine, intercellular adhesion molecule-1 (ICAM-1), lipopolysaccharide-induced CXC chemokine (LIX), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein 3 alpha (MIP-3-alpha), matrix metalloproteinase-8 (MMP-8) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in 5 mg/kg LPS rats were significantly increased compared to control rats (Figure 2).

Changes in tissue levels of NPs

Regardless of LPS dose, neither atrial ANF and BNP levels nor ventricular ANF showed changes (Figure 3). Ventricular BNP significantly increased by treatment with 5 and 15 mg/kg LPS. Administration of 2 mg/kg SB203580 in rats treated with 5 mg/kg LPS significantly reduced ventricular BNP content.

Changes in gene expression of NPs, biologically active substances and NPR-C

ANF mRNA levels remained unchanged (Figure 4) but BNP mRNA levels in atria and ventricles significantly increased when more than 2 mg/kg LPS was administered. Administration of 2 mg/kg SB203580 to rats treated with 5 mg/kg LPS significantly reduced relative BNP mRNA levels compared to 5 mg/kg LPS rats.

MCP-1, MMP-8, TIMP-1 mRNA levels in atria and ventricles increased in all LPS-treated animals (Table 3). Administration of 2 mg/kg SB203580 in addition to 5 mg/kg LPS significantly reduced relative mRNA levels of these chemokine or proteases compared to 5 mg/kg LPS rats. CINC-1, tumor necrosis factor-α (TNFα), interleukin (IL)-1β, fractalkine, ICAM-1 and MIP-3-alpha and LIX mRNA levels also significantly increased in atria and ventricles in LPS-treated rats. However, administration of 2 mg/kg SB203580 addition to 5 mg/kg LPS did not change their mRNA levels compared to 5 mg/kg LPS rats. Finally, relative mRNA levels of regulated on activation, normal T cell expressed and secreted (RANTES) and α1 skeletal muscle actin (ACTA-1) did not change following LPS treatment (Table 3).

Relative NPR-C mRNA levels in the heart, lungs and kidneys from rats treated with 5 mg/kg LPS were significantly reduced compared to control rats (Figure 5).

Correlation between NPs and cytokines and other factors

Correlation significance for mRNA data from 16 rats (ANF, BNP and other biologically active substances) were calculated using Pearson's simple correlation. There was no correlation between gene expression of ANF and of BNP (Table 4). Gene expressions of MCP-1, MMP-8 and TIMP-1 correlated significantly with gene expression of BNP in all four chambers of the heart. Gene expressions of CINC-1, TNFα, ICAM-1 and MIP-3-alpha correlate with BNP in one or two chambers of the heart. ANF gene expression did not show a significant correlation with any of these genes.

Discussion

The measurement of plasma BNP as a marker of cardiovascular disease is widely utilized. We have previously

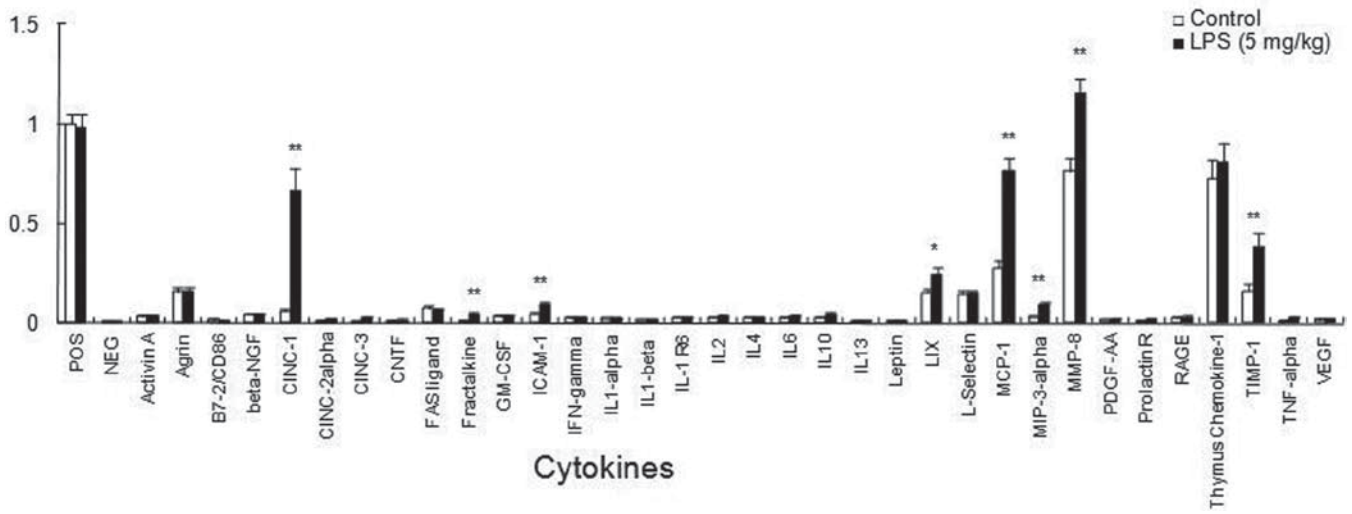

Figure 2. Cytokine plasma levels in control and rats treated with 5 mg/kg LPS. $n = 8$ in each group. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control.

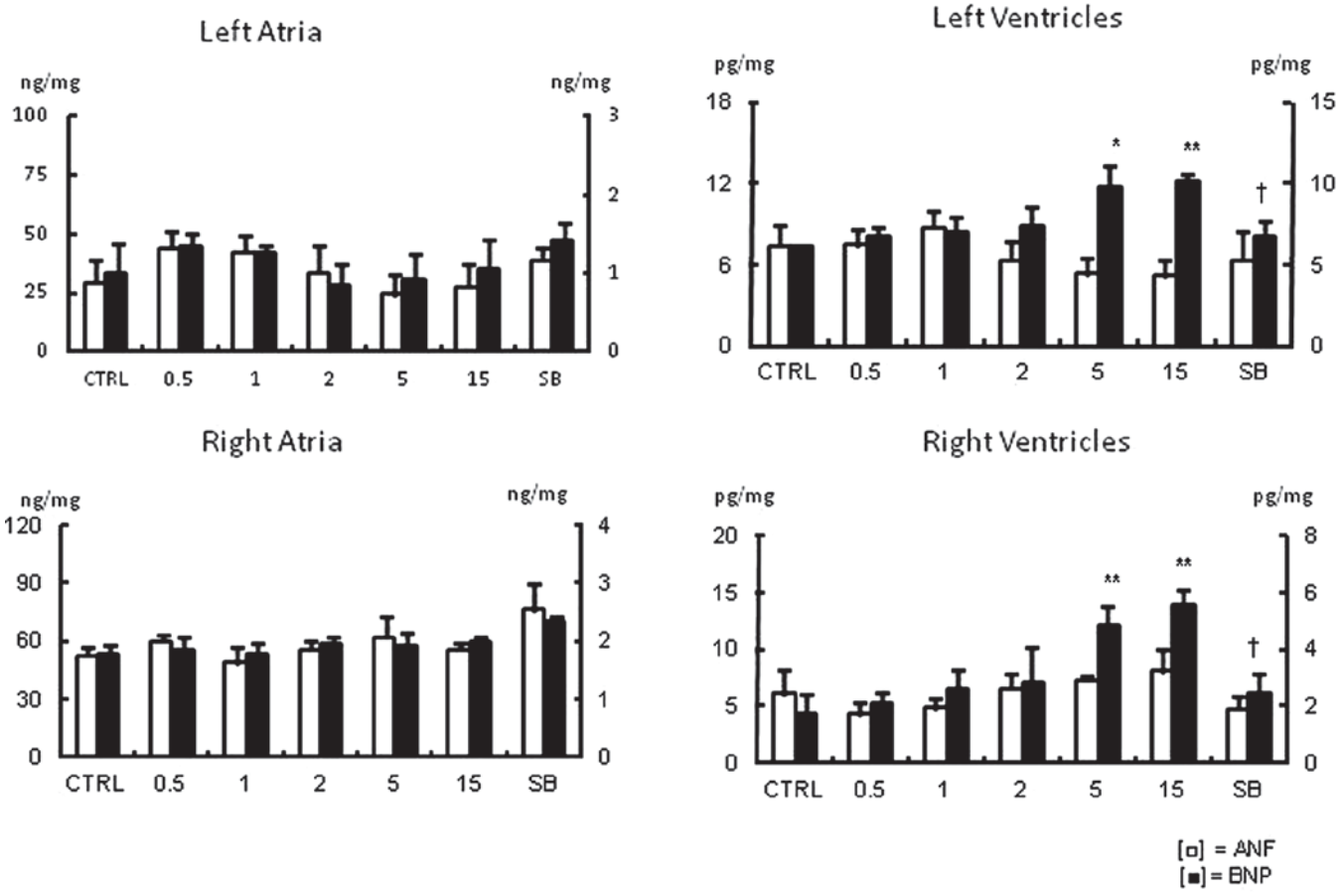

Figure 3. Cardiac atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) content. C: control; 0.5: lipopolysaccharide 0.5 mg/kg; SB: SB203580. $n = 4$ in each group. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control. $\dagger p < 0.05$ vs. LPS 5 mg/kg rats.

demonstrated that in inflammatory states of the myocardium such as in acute cardiac allograft rejection (Masters et al. 1999) and in experimentally induced autoimmune myocarditis (Ogawa et al. 2008), BNP is selectively upregulated when compared to ANF. This observation demonstrates a specific role of BNP in vivo inflammation and so it differentiates this hormone from ANF with which it is normally coregulated during hemodynamic overload and it shares general biological properties and receptor. Apart from this physiological insight, the above-mentioned observations are of relevance to the use of plasma levels of ANF or BNP as biomarkers of myocardial function because it suggests that changes in biomarker plasma concentration can occur in the absence

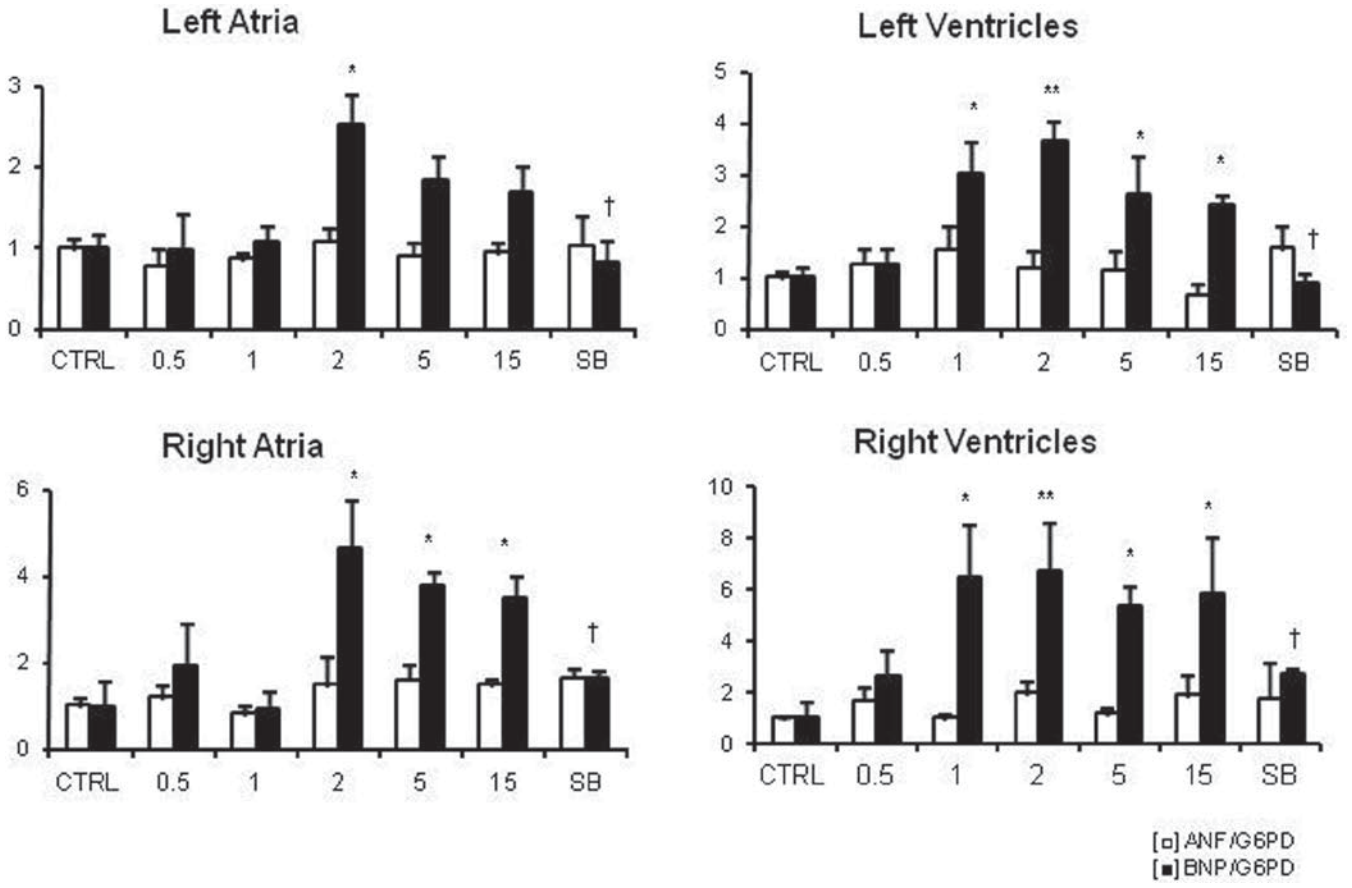

Figure 4. Relative cardiac atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) mRNA levels. CTRL = control; 0.5 = lipopolysaccharide 0.5 mg/kg; SB = SB203580. $n = 4$ in each group. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control. †$p < 0.05$ vs. LPS 5 mg/kg rats.

Table 3. Relative genetic expression of various cytokines and related substances in the heart of animals treated with various doses of lipopolysaccharide (LPS).

Gene	Control	LPS 1 mg/kg	LPS 5 mg/kg	LPS 5 mg/kg +SB
Left atria				
CINC-1	1.00 ± 0.26	342 ± 9.43**	648 ± 23**	447 ± 169
MCP-1	1.00 ± 0.33	200 ± 56**	472 ± 48**	252 ± 41†
MMP-8	1.00 ± 0.08	22.5 ± 10.6**	51.2 ± 11.4**	25.3 ± 4.71†
TIMP-1	1.00 ± 0.13	5.68 ± 1.52*	26.1 ± 2.87**	15.1 ± 1.93†
TNF-α	1.00 ± 0.23	6.51 ± 1.51*	18.5 ± 4.45*	17.9 ± 3.79
IL-1β	1.00 ± 0.30	14.3 ± 2.29*	17.4 ± 2.33*	18.2 ± 4.77
Fractalkine	1.00 ± 0.43	10.8 ± 3.96*	15.4 ± 2.74*	11.8 ± 4.15
ICAM-1	1.00 ± 0.23	5.70 ± 1.84	20.9 ± 4.69*	10.8 ± 2.76
MIP-3-alpha	1.00 ± 0.06	10.5 ± 3.95*	25.7 ± 1.93**	24.9 ± 3.29
LIX	1.00 ± 0.33	953 ± 436**	1581 ± 391**	2497 ± 534
RANTES	1.00 ± 0.21	0.76 ± 0.12	1.05 ± 0.35	1.26 ± 0.18
ACTA-1	1.00 ± 0.49	0.86 ± 0.23	1.56 ± 0.81	1.54 ± 0.83
Right atria				
CINC-1	1.00 ± 0.33	146 ± 43**	505 ± 20**	308 ± 60
MCP-1	1.00 ± 0.14	248 ± 119**	519 ± 118**	134 ± 34††
MMP-8	1.00 ± 0.38	39.6 ± 11.2**	38.3 ± 11.1**	9.35 ± 1.9†
TIMP-1	1.00 ± 0.18	9.27 ± 1.3*	29.8 ± 2.5**	17.2 ± 4.2†
TNF-α	1.00 ± 0.11	12.7 ± 2.9*	14.2 ± 2.8**	24.7 ± 6.3
IL-1β	1.00 ± 0.42	4.45 ± 1.3	10.5 ± 2.1*	7.72 ± 1.7
Fractalkine	1.00 ± 0.12	2.02 ± 1.0	2.80 ± 0.75*	4.09 ± 1.8

(Continued)

Table 3. Continued.

Gene	Control	LPS 1 mg/kg	LPS 5 mg/kg	LPS 5 mg/kg +SB
ICAM-1	1.00 ± 0.51	1.71 ± 0.47	4.23 ± 1.76*	3.51 ± 0.70
MIP-3-alpha	1.00 ± 0.51	2.96 ± 1.2*	10.5 ± 2.7*	9.15 ± 2.5
LIX	1.00 ± 0.28	721 ± 351**	1931 ± 651**	2043 ± 394
RANTES	1.00 ± 0.18	0.91 ± 0.129	1.08 ± 0.18	1.09 ± 0.27
ACTA-1	1.00 ± 0.29	0.99 ± 0.26	0.83 ± 0.12	0.92 ± 015
Left ventricles				
CINC-1	1.00 ± 0.13	126 ± 34.3**	284 ± 57.4**	161 ± 41.2
MCP-1	1.00 ± 0.26	126 ± 43.4**	300 ± 58.8**	106 ± 14.5†
MMP-8	1.00 ± 0.46	7.02 ± 0.97**	12.6 ± 2.82**	3.66 ± 1.23†
TIMP-1	1.00 ± 0.11	6.51 ± 1.19**	21.0 ± 3.47**	12.8 ± 3.09†
TNF-α	1.00 ± 0.08	3.90 ± 1.54*	6.95 ± 0.99*	8.88 ± 1.03
IL-1β	1.00 ± 0.09	0.76 ± 0.08	1.61 ± 0.43	1.18 ± 0.53
Fractalkine	1.00 ± 0.24	3.79 ± 1.52	4.63 ± 1.06*	8.28 ± 2.33
ICAM-1	1.00 ± 0.27	5.46 ± 2.35	10.4 ± 1.39**	12.6 ± 2.12
MIP-3-alpha	1.00 ± 0.17	2.84 ± 1.07	7.30 ± 1.98*	12.6 ± 3.55
LIX	1.00 ± 0.50	382 ± 102**	868 ± 150**	1124 ± 481
RANTES	1.00 ± 0.36	0.68 ± 0.15	0.64 ± 0.27	0.58 ± 0.09
ACTA-1	1.00 ± 0.31	1.07 ± 0.29	0.69 ± 0.13	1.38 ± 0.36
Right ventricles				
CINC-1	1.00 ± 0.19	257 ± 103**	761 ± 171**	430 ± 211
MCP-1	1.00 ± 0.37	208 ± 86**	431 ± 62**	120 ± 21†
MMP-8	1.00 ± 0.35	11.6 ± 5.12**	41.1 ± 9.77**	15.2 ± 4.55†
TIMP-1	1.00 ± 0.04	9.88 ± 3.62**	47.3 ± 3.45**	16.9 ± 1.20†
TNF-α	1.00 ± 0.45	3.12 ± 1.22*	6.16 ± 0.48*	5.37 ± 2.27
IL-1β	1.00 ± 0.27	0.98 ± 0.33	1.07 ± 0.27	0.84 ± 0.30
Fractalkine	1.00 ± 0.18	3.96 ± 1.58	4.71 ± 1.38*	2.97 ± 1.28
ICAM-1	1.00 ± 0.59	6.36 ± 2.56	7.47 ± 1.90*	7.50 ± 1.96
MIP-3-alpha	1.00 ± 0.60	4.69 ± 2.67	3.69 ± 1.64	5.31 ± 2.41
LIX	1.00 ± 0.58	300 ± 142**	561 ± 339**	997 ± 321
RANTES	1.00 ± 0.13	0.54 ± 0.21	0.98 ± 0.16	0.78 ± 0.32
ACTA-1	1.00 ± 0.21	1.18 ± 0.22	1.03 ± 0.15	0.67 ± 0.26

Note: Values are the relative expression of target genes and the housekeeping gene glucose-6-phosphate dehydrogenase transcripts. CINC-1: cytokine-induced neutrophil chemoattractant-1; MCP-1: monocyte chemoattractant protein-1; MMP-8: matrix metaroproteinase-8; TIMP-1: tissue inhibitor of metalloproteinase-1; TNF-α: tumor necrosis factor α; IL: interleukin; ICAM-1: intercellular adhesion molecule-1; MIP-3-alpha: macrophage inflammatory protein 3 alpha; LIX: lipopolysaccharide-induced CXC chemokine; RANTES: regulated on activation, normal T-cell expressed and secreted; ACTA-1: α1 skeletal muscle actin; n = 4 in each group. *<0.05, **<0.01 vs. control group, †<0.05, ††<0.01 vs. LPS 5 mg/kg group.

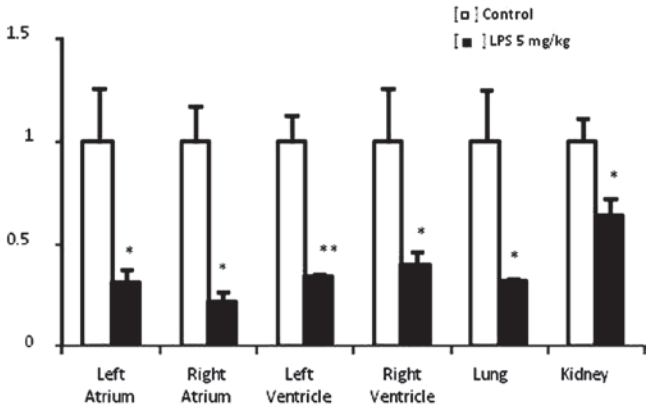

Figure 5. Natriuretic peptide receptor-C (NPR-C) mRNA levels in heart, lungs and kidneys. LPS: lipopolysaccharide; LA: left atria; RA: right atria; LV: left ventricles; RV: right ventricles. $n = 4$ in each group. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control.

of significant hemodynamic changes. Indeed, plasma BNP elevation can occur in septic patients without any clinical or echocardiographic evidences of left ventricular systolic dysfunction (Shor et al. 2006). Septic shock patients show high cardiac index and a slight increase in pulmonary capillary wedge pressure (Witthaut et al. 2003). Despite this elevation, plasma NPs levels inversely correlate with cardiac index (Hartemink et al. 2001; Witthaut et al. 2003).

In the present study, regulations of NPs were investigated using LPS-induced sepsis in the rat. To evaluate the relation between severity of sepsis and BNP levels, LPS doses were increased stepwise. In addition, because BNP gene expression increases occur earlier than those of ANF in response to pathological stimuli (Bruneau et al. 1994; Hama et al. 1995), time course and chronic investigations were also conducted. It was found that the increase

Table 4. Correlation between gene expression of NPs and cytokines or other factors.

	LA		RA		LV		RV	
Gene	ANF	BNP	ANF	BNP	ANF	BNP	ANF	BNP
ANF	—	–0.151	—	0.434	—	0.028	—	–0.06
BNP	–0.151	—	0.434	—	0.028	—	–0.06	—
CINC-1	–0.315	0.279	0.492	0.785**	0.090	0.328	–0.122	0.187
MCP-1	–0.107	0.701**	0.398	0.562*	0.008	0.656**	–0.063	0.646**
MMP-8	–0.086	0.609*	0.436	0.850**	0.059	0.755**	0.023	0.560*
TIMP-1	0.056	0.672**	0.299	0.713**	0.074	0.500*	–0.049	0.509*
TNFα	–0.219	0.525*	0.299	0.713**	0.298	0.195	–0.053	0.325
IL-1β	0.0111	0.386	0.293	0.285	0.330	0.194	–0.210	0.325
Fractalkine	0.168	0.225	0.198	0.287	0.471	0.130	0.108	0.370
ICAM-1	0.143	0.701**	0.182	0.582*	0.380	0.229	–0.058	0.481
MIP-3-alpha	0.237	0.364	0.450	0.620**	0.079	0.021	–0.073	0.585*
LIX	–0.270	0.280	0.438	0.356	0.331	0.115	0.129	0.165
RANTES	0.376	0.200	0.209	0.176	0.030	0.185	–0.054	0.058
ACTA-1	0.379	0.024	0.195	0.150	0.454	0.007	–0.035	0.085

Note: Data are expressed as Pearson correlation coefficients (r values). LA: left atria; RA: right atria; LV: left ventricles; RV: right ventricles; CINC-1: cytokine-induced neutrophil chemoattractant-1; MCP-1: monocyte chemoattractant protein-1; MMP-8: matrix metaroproteinase-8; TIMP-1: tissue inhibitor of metalloproteinase-1; TNF-α: tumor necrosis factor α; IL: interleukin; ICAM-1: intercellular adhesion molecule-1; MIP-3-alpha: macrophage inflammatory protein 3 alpha; LIX: lipopolysaccharide-induced CXC chemokine; RANTES: regulated on activation, normal T-cell expressed and secreted; ACTA-1: α1 skeletal muscle actin; $n = 16$ in each gene group. *<0.05, **<0.01.

in plasma BNP was always larger than the increase in plasma ANF in both the dosing and time course experiments. In the chronic experiments it was evident that only plasma BNP but not plasma ANF increased. This plasma NP pattern is different from that elicited by aortic banding (Ogawa et al. 1996) in which both ANF and BNP increase to a similar degree and where pressure and volume overload to the heart are the main stimuli.

The cytokine array data showed that CINC-1, fractalkine, ICAM-1, LIX, MCP-1, MIP-3-alpha, MMP-8 and TIMP-1 were elevated in the LPS-treated animals. These results were confirmed by RT-PCR having been further determined that the change in BNP gene expression correlates significantly with the changes in gene expression for MCP-1, MMP-8 and TIMP-1 in all cambers, and of CINC-1, TNFα, ICAM-1 and MIP-3-alpha in one or two of the four chambers of the heart. ANF did not correlate with any of these substances. Previous reports suggest a link between BNP gene expression or secretion and MMP-8 (Weiss et al. 1985; Van et al. 2005; Laxton et al. 2009) or TIMP-1 (Li et al. 1999; Awad et al. 2010) and MIP-3-alpha (Ghadjar et al. 2009; Hirata et al. 2010), although there have been no reports which suggest a direct relation between BNP and MCP-1, CINC-1, TNFα and ICAM-1. These genes may possibly be related to BNP gene expression and secretion through their reported gene expression promoting properties of substances such as angiotensin II, IL-1β and TNF-α. Direct stimulation by LPS through toll-like receptor (Bailly et al. 1990; Genth-Zotz et al. 2002) could also contribute to the increase in BNP production and secretion during LPS challenge. It is worth pointing out that in this investigation, unlike our previous findings in human cardiac allograft rejection (Meirovich et al. 2008), gene expression for RANTES was not increased in LPS-treated rats thus demonstrating differences between the inflammatory processes observed in cardiac allograft rejection and after LPS administration to rats. It should be also pointed out that in the present investigation ACTA-1 gene expression, a marker of hypertrophic change (Berni et al. 2009), was unchanged in LPS-treated animals signifying that cardiac hypertrophy did not occur in these animals.

A common mechanism at the level of p38 MAPK signaling is suggested by the results described herein showing that administration of the specific p38 inhibitor SB203580 to LPS-treated rats prevented the elevation of cardiac and plasma BNP. This is in line with previous in vitro work from our laboratory (Ma et al. 2004; Meirovich et al. 2008; Ogawa et al. 2008).

NPs blood levels are a reflection of the rate of secretion and that of degradation. The latter occur through mechanisms involving in part the clearance NPR-C receptor. In the present investigation it was found that 5 mg/kg LPS treatment decreased NPR-C expression in the heart, lungs and kidneys, which may partly contribute the observed increase in plasma ANF and BNP but this dos not explain the difference between ANF and BNP plasma levels.

It is unlikely that the animals used in this study developed significant hemodynamic changes, because neither blood pressure and heart rate change nor did these animals show changes in body weight, relative heart weight expression of hypertrophic marker or showed signs of cardiac failure, such as dyspnea, edema or ascites.

The present study suggest that in the clinical setting, it would be important to follow the patients by using the simultaneous measurements of both plasma ANF as well as plasma BNP, especially in septic patients in which hemodynamic changes and inflammation coexist

in different proportions for the individual patient. ANF increases relatively larger than plasma BNP may signal deterioration of the hemodynamic status. Alternatively increases in plasma BNP larger than plasma ANF might indicate worsening of inflammation. Because these two pathological conditions are of different pathogenesis and so are their therapeutic strategies, measurement of both ANF and BNP simultaneously in the follow up would be beneficial to evaluate the course of disease.

It should be noted as limitations of this study that although LPS is the main bacterial component responsible for the immune response to gram negative bacterial infection, there exists the possibility that other substances of bacterial origin may elicit a different response to that observed in the present work. In addition, administration of SB202474, a structural analogue of SB203580, as a negative control for the p38 MAPK inhibitor was not carried out so that nonspecific effects of the inhibitor cannot be discarded.

In conclusion, the present findings reinforce the notion that, compared to ANF, BNP appears uniquely related to inflammation. Furthermore, LPS treatment increased gene expression of various cytokines, chemokines and proteases which correlated with the observed increase in BNP gene expression but not with that of ANF. Administration of the specific p38 MAP kinase inhibitor SB203580 inhibited the increase of BNP plasma levels in concert with the expression of MCP-1, MMP-8 and TIMP-1. Finally, the uncoordinated regulation of ANF and BNP in inflammatory states may prove useful in the follow-up of patients given that ANF plasma levels appear related to cardiac function and volume status while plasma BNP levels are additionally affected by inflammation.

Declaration of interest

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