Effects of B-type natriuretic peptide on cardiovascular biomarkers in healthy volunteers

M. Resl,1,5 G. Vila,1 G. Grimm,2 B. Heinisch,3 M. Riedl,1 B. Dieplinger,4 T. Mueller,4 A. Luger,1 and M. Clodi5

1Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; 2Department of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria; 3Department of Internal Medicine III, Division of Gastroenterology, Medical University of Vienna, Vienna, Austria; 4Department of Laboratory Medicine, Konventhospital Barmherzige Brueder, Linz, Austria; and 5Department of Internal Medicine, Konventhospital Barmherzige Brueder, Linz, Austria

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Cardiovascular disease is by far the leading cause of death in developed countries (4). On the basis of pathophysiology of atherosclerosis, many biomarkers have been evaluated for cardiovascular risk prediction (8, 17, 32, 41). Among them, midregional-proadrenomedullin (MR-proADM), C-terminal proendothelin-1 (CT-proET-1), growth differentiation factor-15 (GDF-15), midregional-proatrial natriuretic peptide (MR-proANP), copeptin, and procalcitonin in healthy volunteers. Ten healthy male subjects (mean age 24 yr) participating in a randomized, placebo-controlled, single-blinded crossover study received placebo or 3.0 pmol·kg⁻¹·min⁻¹ human BNP 32 during a continuous infusion lasting for 4 h. Effects of BNP on other cardiovascular biomarkers were assessed. BNP did not change concentrations of MR-proADM, copeptin, CT-proET1, GDF-15, or procalcitonin. In contrast, MR-proANP was significantly decreased during BNP infusion. BNP as an established cardiovascular biomarker did not affect plasma concentrations of other cardiovascular biomarkers in a model of healthy volunteers.

cardiovascular biomarkers; BNP; multimarker approaches; healthy volunteers

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Cardiovascular disease is by far the leading cause of death in developed countries (4). On the basis of pathophysiology of atherosclerosis, many biomarkers have been evaluated for cardiovascular risk prediction (8, 17, 32, 41). Among them, midregional-proadrenomedullin (MR-proADM), C-terminal proendothelin-1 (CT-proET-1), growth differentiation factor-15 (GDF-15), midregional-proatrial natriuretic peptide (MR-proANP), copeptin, and procalcitonin (PCT) have shown excellent performance in cardiovascular risk prediction reflecting different pathophysiologic pathways of atherosclerosis.

Adrenomedullin, originally isolated from human pheochromocytoma, mediates vasodilation and natriuretic properties through cyclic adenosine monophosphate, nitric oxide, and the renal prostaglandin system (12). Because adrenomedullin is rapidly cleared from the circulation, the more stable precursor, MR-proADM, has been identified as a reliable method for determining adrenomedullin levels (3). In a recently conducted study, MR-proADM was an excellent predictor of mortality in patients with heart failure (20). CT-proET1 is a stable precursor of endothelin-1, representing one of the most potent vasoconstrictors. Endothelin-1 directly targets endothelial and vascular smooth muscle cells by inhibiting nitric oxide formation (1, 34, 35). In patients with stable coronary artery disease CT-proET1 is an independent predictor of cardiovascular death and heart failure (28). Furthermore, endothelin-1 interacts with insulin resistance and plays an important role in the pathogenesis of hypertension in insulin-resistant states (29). GDF-15, belonging to the transforming growth factor-beta (TGF-β) superfamily, is produced by macrophages, cardiomyocytes, and endothelial cells. It exerts antiapoptotic effects and is secreted in response to tissue injury and proinflammatory cytokines (13, 37). Furthermore, GDF-15 reliably predicts mortality in patients with non-ST-elevation acute coronary syndrome, providing prognostic information beyond established markers (42). According to recently published data, GDF-15 is an even better predictor of long-term cardiovascular events than NT-proBNP in patients free from cardiovascular disease at baseline (6). MR-proANP is the stable precursor of atrial natriuretic peptide, being secreted by the atria in response to volume overload. The prognostic value of MR-proANP in heart failure has been reported to be comparable to other natriuretic peptides in heart failure (20, 36). C-terminal provasopressin (copeptin) is co-secreted with arginine vasopressin by the posterior pituitary in response to changes in plasma osmolality. It is involved in osmoregulation and in cardiovascular homeostasis. Copeptin has been discovered to be a noncardiac biomarker in patients with acute coronary syndrome and in heart failure (25).

Procalcitonin (PCT), the prohormone of calcitonin, is a reliable marker of inflammation (5,6). Recently, studies have shown that PCT correlates with the metabolic syndrome, thereby predicting cardiovascular events and death (2, 5, 30).

Taken together, MR-proADM, copeptin, CT-proET-1, and MR-proANP can be used to predict outcome in patients with heart failure and in those with myocardial infarction (14–16, 26). In the BACH trial, MR-proANP was as good as B-type natriuretic peptide (BNP) for diagnosing acute heart failure in patients with dyspnea, whereas MR-proADM was the best predictor of mortality (20). In patients with type 2 diabetes mellitus, MR-proADM, CT-proET-1, copeptin, and MR-proANP were independently related to cardiovascular events and impaired renal function, thereby linking diabetic nephropathy and cardiovascular disease (19). Although many of these
biomarkers are influenced by inflammation, it is currently not known whether BNP concentrations influence the levels of other markers of cardiovascular disease. We therefore investigated the effects of a BNP infusion on clinically used cardiovascular biomarkers in 10 healthy volunteers.

MATERIALS AND METHODS

Study subjects. In this study, 10 healthy volunteers free from any disease and medication were examined in a crossover design. Volunteers were recruited using flyers that were displayed in Vienna’s general hospital. Before inclusion, a careful medical examination; biochemical tests for glucose, HbA1c, NT-proBNP, electrolytes, C-reactive protein, lipid values, and renal, liver, and thyroid function; and an electrocardiogram were performed.

All participants gave written informed consent according to the Declaration of Helsinki and its amendments. The trial was approved by the institutional review board.

Study protocol, blood sampling, and assays. The two study days started at 8:00 A.M. After an overnight fast, BNP was administered in a randomized, crossover, single-blinded design. All volunteers received placebo (NaCl 0.9%) and once an intravenous infusion of 3 pmol·kg⁻¹·min⁻¹ (10.881 ± 7.8 pg·kg⁻¹·min⁻¹) BNP-32 (amino acids 77–108 of the preproBNP, American Peptide, Sunnyvale, CA) during a period of 4 h. This dose, which has been adapted by previously published data, has been shown to increase natriuresis and diuresis while blood pressure drops only in sitting and standing positions but not in the supine position (18). Blood samples were collected at time points −5 min, and at 0, 1, 2, 3, and 4 h. All patients remained supine during the entire study period, and blood pressure was measured at time points −5 min, and at 0, 1, 2, 3, and 4 h.

The main aim of the study was to assess the effect of an intravenous BNP infusion on MR-proADM, CT-proET-1, GDF-15, MR-proANP, copeptin, and procalcitonin.

MR-proANP, CT-proET-1, MR-proADM, copeptin, and procalcitonin were determined from EDTA plasma and analyzed via sandwich immunoluminometric assays by BRAHMS (Hennigsdorf, Berlin, Germany) as described before (21–24, 27).

BNP was measured using the ARCHITECT BNP chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott Park, IL) as previously described (3). The assay range was from 1.0 to 5,000 pg/ml. The intra- and interassay CVs ranged from 0.9% to 5.6%, and 1.7% to 6.7%, respectively.

GDF-15 was measured using a sandwich ELISA kit (DGD150; R&D Systems) with intra- and interassay CVs of <2.8% and <6%, respectively.

Statistical evaluation. Data were analyzed using SPSS statistical software (release 16.0; SPSS, Chicago, IL). Baseline characteristics are shown as means ± SE. Effects of BNP infusion of the biomarkers of interest were assessed by repeated measures ANOVA (RM-ANOVA) with the interaction between time and treatment (time * treatment) being the term of interest. Kolmogorov-Smirnov tests were used to test for normal distribution. Paired t-tests were performed to compare intervention-induced changes at each time point when RM-ANOVA showed significantly different results. Based on Chauvenet’s criterion, the copeptin value of patient 8 at time point 3 h during BNP infusion was considered an outlier and was therefore discarded.

RESULTS

Data derived from 10 healthy male volunteers were analyzed. Mean age was 24 ± 2 yr, and mean body mass index was 24 ± 1.3 kg/m². All participants were healthy and free from any previously known disease.

The intravenous infusion of 3 pmol·kg⁻¹·min⁻¹ BNP significantly increased plasma BNP concentrations, which remained constant between 400 and 500 ng/liter during the last 2 h of the study (38). Blood pressure (mean baseline blood pressure 121/72 vs. 116/70 after 4 h of BNP infusion) and serum sodium levels remained constant throughout BNP infusion. At baseline, the mean sodium level was 137.7 mmol/liter; after 4 h of BNP infusion the mean sodium level was 137.2 mmol/liter (P = 0.53).

Vascular biomarkers MR-proADM (RM-ANOVA P = 0.192, Figure 1A), CT-proET-1 (RM-ANOVA P = 0.132, Fig. 1B) and copeptin (RM-ANOVA P = 0.590, Fig. 1C) remained unaffected by infusion of BNP. On the basis of Chauvenet’s criterion the copeptin value of patient 8 at time point 3 h during BNP infusion was considered an outlier and was therefore discarded.

Fig. 1. A: effects of B-type natriuretic peptide (BNP) and placebo on midregional-proadrenomedullin (MR-proADM) at time points −5 min, and 0, 1, 2, 3 and 4 h. B: effects of BNP and placebo on C-terminal proendothelin-1 (CT-proET-1) at time points −5 min, and 0, 1, 2, 3 and 4 h. C: effects of BNP and placebo on copeptin at time points −5 min, and 0, 1, 2, 3 and 4 h.
LPS), and cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (10, 33). The most important effects of adrenomedullin are an increase in natriuresis, an inhibition of the renin-angiotensin-aldosterone system, and a reduction in salt and water intake. In patients with acute heart failure MR-proADM is a better predictor of 90-day mortality than NT-proBNP (20). Furthermore, MR-proADM is strongly related to cardiovascular outcome in patients with stable angina. The predictive value of a single determination of MR-proADM is comparable to that of NT-proBNP, one of the best predictors of cardiovascular events and outcome (32). Our data demonstrate that administration of BNP does not increase the production of adrenomedullin measured by its stable precursor, MR-proADM.

MR-proANP, as a member of the same peptide family as BNP, decreased significantly during BNP infusion given that exogenous administration of BNP resulted in a significant reduction in cardiac preload. This finding is well in line with data by Hillock and colleagues demonstrating that BNP infusion in acute myocardial infarction significantly reduced NT-proANP production and delayed the increase in atrial natriuretic peptide (9).

Copeptin, which is the stable precursor of vasopressin, has also been established as a cardiovascular biomarker in patients with chronic heart failure and in patients with coronary artery disease (26, 40).

Vasopressin is a major regulator of osmolality, whereas BNP reduces preload by increasing natriuresis and diuresis. Taken together, both peptides regulate fluid homeostasis via different pathways, which is also demonstrated by our study because copeptin concentrations remained unaffected by BNP.

Inflammatory pathways of atherosclerosis are well represented by GDF-15 as a member of the TGF-β superfamily. In a recently conducted study, GDF-15 was an excellent predictor of mortality, adding incremental prognostic information to NT-proBNP (6). However, infusion of BNP did not increase GDF-15 or procalctonin levels. Interestingly, mediators of inflammation such as LPS and TNF-α stimulate the production of BNP, thus supporting the concept of a unidirectional link between BNP and inflammation (11, 39). In fact, this might in part explain the excellent prognostic and diagnostic performance of BNP because it sums up subclinical cardiovascular dysfunction and inflammation.

Combining endothelial biomarkers with inflammatory markers has been reported to increase the accuracy of cardiovascular prognosis. Schnabel and colleagues have shown that among 12

![Image](http://jap.physiology.org/)

Fig. 2. A: effects of BNP and placebo on procalctonin at time points −5 min, and 0, 1, 2, 3 and 4 h. B: effects of BNP and placebo on growth differentiation factor-15 (GDF-15) at time points −5 min, and 0, 1, 2, 3 and 4 h.

excluded. Also, after excluding this value the effect of BNP infusion on copeptin levels remained not significant (RM-ANOVA P = 0.617).

Correspondingly inflammatory biomarkers procalctonin (RM-ANOVA P = 0.416, Fig. 2A) and GDF-15 (RM-ANOVA P = 0.680, Fig. 2B) were also not affected by administration of BNP.

In contrast, MR-proANP was significantly decreased during BNP infusion (RM-ANOVA P = 0.006, Fig. 3) with significant differences observed at time points 2, 3, and 4 h (P = 0.05, P = 0.0009, P = 0.0005, respectively post hoc t-tests).

**DISCUSSION**

We evaluated the influence of BNP on established cardiovascular biomarkers. Our data show that intravenous administration of BNP in healthy volunteers did not affect plasma levels of MR-proADM, CT-proET-1, GDF-15, copeptin (a stable precursor of vasopressin), or procalctonin.

MR-proADM, copeptin, MR-proANP, and CT-proET-1, representing vascular and endothelial function, can be used to predict cardiovascular risk (20, 32). Adrenomedullin, being mainly synthesized and secreted by vascular endothelial cells, is considered to be an endothelial-derived relaxing factor (12). The production of adrenomedullin is increased by endothelin-1, thyroid hormones, angiotensin II, lipopolysaccharide (LPS), and cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (10, 33). The most important effects of adrenomedullin are an increase in natriuresis, an inhibition of the renin-angiotensin-aldosterone system, and a reduction in salt and water intake. In patients with acute heart failure MR-proADM is a better predictor of 90-day mortality than NT-proBNP (20). Furthermore, MR-proADM is strongly related to cardiovascular outcome in patients with stable angina. The predictive value of a single determination of MR-proADM is comparable to that of NT-proBNP, one of the best predictors of cardiovascular events and outcome (32). Our data demonstrate that administration of BNP does not increase the production of adrenomedullin measured by its stable precursor, MR-proADM.

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different biomarkers, NT-proBNP, GDF-15, cystatin C, and MR-proADM were the strongest predictors of cardiovascular outcome in patients with stable angina (31).

In this study, a multimarker approach was strongly related to outcome, adding incremental risk information (32). Our results might provide a basis for further research in possible interactions of different cardiovascular biomarkers because BNP administration has no effect on endothelial and inflammatory biomarkers.

A possible limitation of our study is that it has been performed in healthy volunteers. Adaptations of vascular and inflammatory biomarkers to longstanding cardiovascular disease and heart failure and different reaction to increased BNP concentrations cannot be analyzed in our model. Although the duration of BNP administration was relatively short, we have already previously demonstrated effects of BNP on glucose metabolism and satiety, thereby underscoring the validity of the model (7, 38).

In summary, our data demonstrate that acute increases in BNP in a model of healthy men did not affect plasma concentrations of the most important regulatory peptides of the cardiovascular system, thus emphasizing the independency of these biomarkers and the complexity of regulators and mediators of the vascular system.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M. Resl, G.V., and M.C. conception and design of research; M. Resl, G.V., G.G., B.B.H., M.C. performed experiments; M. Resl, G.V., G.G., B.D., T.M., A.L., and M.C. analyzed data; M. Resl, G.V., G.G., B.B.H., M. Riedl, Riedl, A.L., and M.C. interpreted results of experiments; M. Resl, G.V., and M.C. drafted manuscript; M. Resl, G.V., and M.C. revised manuscript; M. Resl, G.V., and M.C. approved final version of manuscript.

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