Paradoxical Clearance of Natriuretic Peptide between Pulmonary and Systemic Circulation: A Pulmonary Mechanism of Maintaining Natriuretic Peptide Plasma Concentration in Obese Individuals

Taro Date, Teiichi Yamane, Seigo Yamashita, Seiichiro Matsuo, Masato Matsushima, Keiichi Inada, Ikuo Taniguchi, and Michihiro Yoshimura
Division of Cardiology, Department of Internal Medicine (T.D., T.Y., S.Y., S.M., K.I., I.T., M.Y.), and Division of Clinical Epidemiology (M.M.), The Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan

Context: Although it has been reported that obese patients have low levels of natriuretic peptide, the metabolism of natriuretic peptide in this population remains unclear.

Objectives: This study aimed to examine the effects of body mass index on the natriuretic peptide clearance rate from the pulmonary and systemic circulation.

Design: We conducted a prospective observational cohort study.

Setting/Patients: Thirty patients with atrial fibrillation undergoing pulmonary vein isolation in single center participated in the study.

Main Outcomes and Measures: We measured pulmonary and systemic atrial/brain natriuretic peptide clearance and clinical parameters including body mass index and pulmonary oxygen levels.

Results: Significantly lower atrial natriuretic peptide levels were found in all pulmonary veins when compared with the pulmonary artery. The pulmonary atrial natriuretic peptide clearance rate was negatively correlated with body mass index. In contrast, the systemic atrial natriuretic peptide clearance rate was positively correlated with the body mass index. A reciprocal relationship therefore exists between pulmonary and systemic atrial natriuretic peptide clearance. Regional pulmonary atrial natriuretic peptide clearances in the inferior lung were significantly negatively correlated to oxygen pressure in the inferior pulmonary veins. There was a similar tendency for brain natriuretic peptide, but the differences between the pulmonary artery and each pulmonary vein were not significant.

Conclusions: Overweight patients have higher systemic atrial natriuretic peptide clearance, whereas they show a lower pulmonary atrial natriuretic peptide clearance, which might be related to pulmonary tissue hypoxia. (J Clin Endocrinol Metab 97: E14–E21, 2012)

A-type or atrial natriuretic peptide (ANP) and B-type or brain natriuretic peptide (BNP) have a wide range of biological effects, including vasodilation, natriuretic action, inhibition of the renin-angiotension-aldosterone system, and inhibition of the sympathetic nervous system (1–4). It has recently been reported that obese subjects have low circulating natriuretic peptide (NP) levels (5–8). Obesity is an underlying risk factor for hypertension and other cardiovascular diseases (9), and therefore elucidating the NP metabolism is especially important in obese subjects. There are two possible explanations for the low NP levels in obese individuals: increased NP clearance,
and diminished myocardial NP release. The reason for low NP levels in obese subjects, however, has not been elucidated. In this study, we determined the NP clearance from the systemic and pulmonary circulation in humans and examined the effects of body mass index (BMI) on the NP clearance from each.

Patients and Methods

Patients

This study included 30 patients with atrial fibrillation (AF; 27 males and three females; mean age, 54 yr; AF type, 15 paroxysmal, 15 persistent) who underwent catheter ablation. All patients were informed of the nature of catheter ablation and its possible complications and gave their informed consent to participate in the study protocol, which was approved by the Human Research Committee at this institution. Two-dimensional echocardiography was performed in all patients in the left lateral decubitus position, using an ultrasonic device equipped with a 2.5-MHz transducer. The left ventricular diastolic and systolic dimensions were measured in the parasternal long-axis view according to the standard recommendations (10). Clinical parameters including BMI (kilograms per square meter) were also examined.

Blood sampling before the electrophysiological study

An electrophysiological study was performed as described previously (11). The left atrium and pulmonary vein (PV) were explored through either a patent foramen ovale or transseptal catheterization with two catheters: one for circumferential PV mapping, the other a quadriporal mapping/ablation catheter. Before blood sampling from each patient, direct visualization of all four PV was performed using selective venography, which was performed during midexpiration by hand injection of contrast media in biplane views. The findings were displayed during the procedure to show the venous anatomy and the location of the left atrium-PV junction. After the venography was performed, a 5F NIH catheter was placed in the main trunk of the pulmonary artery, the femoral vein, the left superior PV, the left inferior PV, the right superior PV, the right inferior PV, the middle of left atrium, or the coronary sinus via either a subclavian or femoral vein. Blood was sampled within 2 min at each location before delivering any radiofrequency application in either sinus rhythm (n = 13) or AF (n = 17). Arterial blood was also obtained from a femoral artery. The blood samples of PV were obtained from deep inside each PV to avoid contamination with the atrial blood, as described previously (12). The oxygen pressure (PO₂) and carbon dioxide pressure (PCO₂) values were measured within approximately 10 min of their sampling by a blood gas analyzer (ABL715; Radiometer, Copenhagen, Denmark). Two-point and one-point calibrations were set up every 8 and 4 h, respectively. Neither sedative-hypnotic agents nor oxygen was given to the patients before taking all of the blood samples. None of the studied subjects received any oxygen before or during the blood sampling. Catheter intervention for AF was performed after the blood sampling without cardioversion for AF.

Natriuretic hormone analysis

Blood samples were taken into plastic syringes and transferred to chilled siliconized disposable tubes with EDTA (1 mg/ml), then immediately placed on ice and centrifuged at 4 °C. Plasma was extracted, aliquoted, and stored at −80 °C until analysis. The plasma ANP and BNP concentrations were measured using a chemiluminescent enzyme immunoassay specific for each human NP using a commercial kit (MI02 Shionogi ANP and BNP; Shionogi Pharmaceutical Co., Tokyo, Japan), as we previously described (13). The mean interassay variance for determination of ANP and BNP was 4.5% (range, 4.1–5.3%) for plasma samples with a concentration of 30.8 to 434.8 pg/ml) and 8.2% (range, 5.6–11.8%) for plasma samples with a concentration of 23.3 to 759.4 pg/ml), respectively. The pulmonary and systemic NP clearance rates were defined as (NPFA − NPFA)/NPFA and (NPFA − NPFA)/NPFA, (where PA, LA, FA, and FV represent pulmonary artery, left atrium, femoral artery, and femoral vein) respectively, as previously described, with some modifications (14). Each regional pulmonary ANP clearance was also defined as (ANPFA − ANPFA)/ANPFA. The total body ANP clearance was defined as (ANPFA − ANPFA)/ANPFA.

Ventilation function test

Ventilation function tests (spirometry, CHESTAC-9800; Chest Co., Tokyo, Japan) were performed in 12 of 30 patients within a few days after the ablation procedure. The value of forced expiratory volume in 1 sec (FEV1.0%) represents the ratio of the measured FEV1.0 value/vital capacity. The reference value (predicted value) of the vital capacity, corrected for height, sex, and age, was calculated using Baldwin’s equation.

Statistical analysis

Quantitative data are expressed as means ± SEM. The difference between 1) the BNP levels in the pulmonary artery and each PV, and 2) the BNP level in the femoral vein and those in the femoral artery, the coronary sinus, the pulmonary artery, and the left atrium were tested with Wilcoxon’s signed-ranks test. A P value < 0.0125 was considered significant after Bonferroni correction for multiple tests. The differences between pulmonary regional ANP clearances were tested by paired Student’s t test, and a P value < 0.05 was considered to be significant for it. Pearson (linear) correlation tests were used for correlation analysis. A P value < 0.05 was considered to be significant for correlation analysis. A multiple linear regression analysis was used to determine factors independently associated with the dependent variables (ANP clearance of inferior PV). Statistical analyses were performed with the use of a statistical software program (SPSS for Windows version 11.5; SPSS, Inc., Chicago, IL).

Results

Patient characteristics

The studied subjects included nine patients with hypertension and one patient with coronary spastic angina. The mean left ventricular ejection fraction was 63.6%, and none of the subjects had a less than 55% left ventricular ejection fraction. The mean left atrial dimension was 38.2 mm. The mean BMI was 24.5 (19.2–29.8) kg/m². Twelve
patients were treated with a β-blocker for rate control or hypertension and two patients with an angiotensin II receptor blocker for hypertension. All drugs were withdrawn for 24 h before the ablation procedure.

Pulmonary and systemic ANP clearance

Table 1 shows that the ANP in the pulmonary artery was significantly increased compared with the femoral vein because of cardiac secretion of ANP, as indicated by the higher levels of ANP in the coronary sinus (901.1 ± 197.1 pg/ml). In contrast, the BNP levels in pulmonary artery were not significantly different from the femoral vein, although higher levels of BNP were noted in the coronary sinus (237.1 ± 36.5 pg/ml). An analysis of the overall pulmonary ANP clearance rate, which was obtained by the difference in ANP levels between the pulmonary artery and left atrium, demonstrated a significant negative correlation between pulmonary clearance and the BMI (Fig. 1A). We also examined the effect of BMI on the systemic ANP clearance, which was represented by that in the lower extremity calculated by the difference in ANP between the femoral artery and the femoral vein. There was a positive correlation between the systemic ANP clearance and the BMI (Fig. 1B; \( P < 0.05 \)). No significant relationship between total body ANP clearance and the BMI was observed (\( P = 0.427; R = -0.151 \)). Importantly, a reciprocal relationship was noted between the pulmonary and systemic ANP clearance (Fig. 1C). Taken together, these data indicate that the reduced ANP levels due to accelerated systemic clearance were accompanied by decreased pulmonary ANP clearance in overweight or obese subjects.

Regional pulmonary ANP clearance, obesity, and pulmonary hypoxia

We next compared the NP in each PV and pulmonary artery to examine the relative contribution of pulmonary natriuretic clearance. There were no significant differences in the BNP levels between the pulmonary artery and each PV (mean value, 70.9 ± 10.9, 80.1 ± 10.3, 75.0 ± 11.4, and 82.5 ± 10.7 pg/ml in the left superior, left inferior, right superior, and right inferior PV, respectively; 

\[ P = \text{not significant vs. BNP level in pulmonary artery}, \]

although approximately average 13 and 7% pulmonary BNP clearance was observed in left superior and right inferior PV, respectively. In contrast, significantly lower ANP levels were noted in all four PV when compared with that in the pulmonary artery (\( P < 0.001; \) Fig. 2, A and B). The ANP level in the left atrium was significantly higher than that in each PV (\( P < 0.05 \)). The regional pulmonary ANP clearance rate was significantly higher in the left and right superior PV compared with that in the left or right inferior PV, respectively (Fig. 2C; \( P < 0.05 \)).

**TABLE 1.** Plasma ANP and BNP levels in the femoral vein, coronary sinus, pulmonary artery, left atrium, and femoral artery in the study subjects (n = 30)

<table>
<thead>
<tr>
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<th>ANP (pg/ml)</th>
<th>BNP (pg/ml)</th>
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<tbody>
<tr>
<td>Femoral vein</td>
<td>61.1 ± 6.0</td>
<td>79.6 ± 10.8</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>901.1 ± 197.1</td>
<td>237.1 ± 36.5</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>153.3 ± 18.5</td>
<td>84.0 ± 11.1</td>
</tr>
<tr>
<td>Left atrium</td>
<td>133.6 ± 17.3</td>
<td>92.2 ± 12.4</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>101.8 ± 12.8</td>
<td>90.8 ± 12.1</td>
</tr>
</tbody>
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\( a P = 0.005 \) vs. femoral vein.

\( b P = 0.03 \) vs. femoral vein.
We next examined the relationship between the regional pulmonary clearance of ANP and the clinical parameters. There was no significant relationship between age, sex, the presence of hypertension, echocardiographic parameters, the parameters of ventilation function tests (tidal volume, FEV$_{1.0}$%, %vital capacity), renal function, and pulmonary clearance of ANP. Interestingly, the regional pulmonary ANP clearance rate was also negatively correlated with the BMI value in the inferior lung regions, but not in superior lung regions (Fig. 3). In the blood gas analysis, the PO$_2$ was significantly higher in the superior PV than in the inferior PV (mean value, 88.7 ± 2.7, 66.4 ± 3.3, 91.8 ± 2.0, and 77.0 ± 2.8 mm Hg in the left superior, left inferior, right superior, and right inferior PV, respectively; P < 0.01). In addition, PO$_2$ values of less than 60 mm Hg were observed in 12 PV in 11 cases, all of which were identified in the inferior PV. As we reported previously (12), there is an inverse relationship between the BMI and PO$_2$ in the inferior PV (P = 0.004; R = −0.420). The regional pulmonary clearance of ANP in the inferior lung regions was strongly correlated to PO$_2$ in each PV (Fig. 4; P < 0.001). In contrast, no significant relationship was noted between the regional ANP clearance and oxygenation levels in the superior PV. Finally, a multiple linear regression analysis demonstrated that the PO$_2$ in the inferior PV (coefficient, 0.008; P = 0.007; 95% confidence interval, 0.003 to 0.014) but not the BMI (coefficient, −0.003; P = 0.835; 95% confidence interval, −0.032 to 0.026) is independently correlated with the regional ANP clearance in the inferior lungs (n = 46 inferior PV).

**Discussion**

In this study, we demonstrated a significant positive correlation to exist between the systemic ANP clearance and BMI. In contrast, BMI was negatively correlated with the pulmonary ANP clearance. We confirmed that the lower oxygenation level in the inferior PV noted in the patients with a high BMI was strongly correlated with the regional pulmonary clearance of ANP from the inferior lungs. Finally, the pulmonary ANP clearance was reciprocally correlated with the systemic ANP clearance.

It has been known that obese patients have low NP levels and also have an impaired NP response (5), thus suggesting that the NP system, counter-regulators of sodium retention and neurohormonal activation, might be impaired in obese patients. These findings indicated that the BNP-guided diagnostic strategy for cardiovascular diseases was also affected by BMI (15). The mechanism underlying this phenomenon has not been described, although there are two hypotheses for the low ANP levels in obese individuals, including increased systemic ANP clearance and diminished myocardial hormone release. The present study demonstrated, for the first time, that systemic ANP clearance is positively correlated with the BMI. Investigations of the effects of BMI on NP production or release are also of interest. A recent experimental study demonstrated that lipid accumulation in obese mice was associated with decreased NP production (16). Nevertheless, because both paroxysmal and persistent AF patients were included in this study and ongoing AF rhythm is a powerful factor for stimulating ANP release, it was not possible to analyze the relationship between myocardial NP release and BMI in this study.
there to be a similar decrease as occurred for ANP. This may be explained by the lower affinity of the NP receptor (NPR)-C and neutral endopeptidase for BNP compared with ANP (21), although a similar clearance system for ANP, BNP, and C-type NP exists in the human circulation (22). The binding affinity of BNP for the NPR-C is about 1 order of magnitude lower than those of ANP in the lung, and BNP clearance from the circulation is slower than that of ANP (23).

NP are cleared from the circulation by neutral endopeptidase and NPR-C-mediated internalization and degradation (23, 24). NPR-C is widely distributed in many tissues and cell types, including vascular endothelial cells, smooth muscle cells, and glomeruli. The main function of NPR-C has been considered to be clearance of bound ligand by internalization and degradation (25). The NPR-C expression or activity is regulated by various pathophysiological conditions (18, 26–28). In particular, hypoxia has been shown to reduce the pulmonary NPR-C expression level (29). Experimental studies have also demonstrated that neutral endopeptidase is down-regulated by hypoxia in the lung (30). In this study, our observations that the oxygenation levels in the inferior PV were lower compared with superior PV were consistent with a previous report (12). The present study demonstrated that only the BMI was significantly associated with the decreased PO$_2$ value in the inferior PV. We also showed that the oxygenation level was significantly correlated with the pulmonary clearance of ANP in inferior PV. In addition, the pulmonary ANP clearance rate was negatively correlated with the BMI. A multiple linear regression analysis clearly demonstrated that the inclusion of oxygen pressure in the PVs canceled the correlation between the regional ANP clearance in the inferior lobes and the BMI. These results suggested that the pulmonary ANP clearance was negatively correlated with obesity-associated regional hypoxic pulmonary circulation. Although the precise mechanism remains to be determined, these associations might be partly due to the down-regulation of pulmonary neutral endopeptidase by hypoxia. The down-regulation of NPR-C by hypoxia in the inferior lungs of obese subjects might be another possible cause of the low ANP clearance. It was reported that the pulmonary sympathetic nervous system activity was markedly increased by hypoxia, which was greater than the hypoxia-induced renal sympathetic nerve activation (31). Moreover, human obesity itself is associated with marked sympathetic activation (32). The NPR-C gene is transcriptionally down-regulated by stimulation of $\beta_2$-adrenergic receptors (33), which are abundantly present throughout the lung. Taken together, these lines of evidence might explain the differences in the reg-

FIG. 3. Relation between the pulmonary regional ANP clearance rate and the BMI. Correlation between the pulmonary regional ANP clearance rate and the BMI (kg/m$^2$) in right superior lung (A), left superior lung (B), right and left superior lungs (C), right inferior lung (D), left inferior lung (E), and right and left inferior lungs (F) ($n = 30$). A significant relationship between these factors was observed in the inferior, but not in the superior, lung regions.
ulation of ANP clearance under obese circumstances between the pulmonary and systemic circulation.

We demonstrated that the pulmonary ANP clearance rate was significantly higher in superior PV than that in inferior PV. We have not yet determined the reason for this. Little is known about the distribution of NPR-C or neutral endopeptidase in each region of the lungs. As we previously reported (12), the inferior PV show lower PO2 values compared with superior PV. Therefore, one of the postulated explanations for the differences in clearance of ANP is that the relative hypoxia in the inferior portion of the lungs elicits the down-regulation of NPR-C and neutral endopeptidase, which results in lower pulmonary ANP clearance by the inferior lungs in comparison with the superior lungs.

We have also shown that a significant amount of ANP was removed from the systemic circulation and that this elimination was dependent on the BMI value. NPR-C is abundant in adipose tissues, indicating that adipocytes play an important role in the removal of ANP from the systemic circulation. Although the precise mechanism underlying the accelerated ANP clearance from the lower limb circulation has not yet been determined, it is possible that the excess of adipose tissues in obese subjects participates in the increased NP clearance. Low levels of circulating ANP are associated with obesity and may contribute to perpetuating the obese state through reducing the lipolytic and thermogenic effects of NP in adipose tissues (34, 35). This effect might also be related to the development of heart failure (36) because an experimental study also demonstrated that ANP plays an important role in improvement of pulmonary gas exchange by reducing extravascular lung water and pulmonary arterial pressure (37). In this study, we found a reciprocal relationship between the pulmonary and systemic ANP clearance. Taken together, these findings indicate that decreased pulmonary clearance served to maintain ANP levels in the obese, and without that mechanism the NP levels might be reduced even further.

**Study limitations**

One of the major limitations of this study is that we investigated patients with AF. It is well known that AF patients have higher levels of NP in their venous blood compared with those without AF (13, 38). There is a possibility that the plasma NP level in the venous blood of AF patients may have a different clearance rate than for patients without AF. In addition, the increased ANP secreted from the heart might lead to relatively smaller clearance of BNP from the pulmonary circulation. Further studies in
subjects without AF would be useful, although conducting such studies in humans would be difficult for ethical reasons. Second, all of the study measurements were performed with the patients in a supine position. Posture affects the lung volumes and respiratory resistance, possibly leading to changes in the regional oxygenation status in the lungs (39). Therefore, the body position might also have influenced the data about the pulmonary ANP clearance. Third, it has been reported that obese subjects have increased circulatory congestion, with consequent alveolar capillary leak (40). The influences of these pathophysiological differences related to the obese state on pulmonary ANP clearance cannot be ruled out. Fourth, it is well recognized that there are sex differences in body fat distribution. Men tend to accumulate adipose tissue in the abdomen, whereas women tend to accumulate the gluteofemoral fat, which might affect systemic ANP clearance. Because this study included only three women, our observations may not be generalizable to women.

Conclusions

Pulmonary ANP clearance was reciprocally correlated with the systemic ANP clearance. Obese or overweight subjects demonstrated higher systemic and lower pulmonary ANP clearance. The obesity-related hypoxia of the inferior lung regions is associated with lower ANP pulmonary clearance. Taken together, these data indicate that there may be a unique mechanism for maintaining NP levels in the hypoxic lungs of overweight and obese populations.

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Address all correspondence and requests for reprints to:
Taro Date, M.D., Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan. E-mail: datet@jikei.ac.jp.

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