Clinical Studies

Effect of Exercise on Circulating Atrial Natriuretic Peptide and Left Ventricular Ejection Fraction in Healthy Persons and Patients with Coronary Artery Disease

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SUMMARY

Radionuclide angiographic measurements of left ventricular ejection fraction were performed at rest and during exercise in 10 normal persons and 11 patients with coronary artery disease. Exercise was continued on a supine bicycle exercise table up to a symptom-limited maximum. Plasma levels of atrial natriuretic peptide (ANP) were also determined at rest and during exercise.

Ejection fraction in the normal volunteers was $59 \pm 3\%$ (mean $\pm$ SEM) at rest and increased significantly ($p < 0.01$) to $69 \pm 3\%$ during exercise. Ejection fraction in the patients was $47 \pm 5\%$ at rest and did not change significantly during exercise ($51 \pm 7\%$). Plasma ANP in the normals rose significantly ($p < 0.01$) from 62 ± 16 pg/ml at rest to 454 ± 94 pg/ml during exercise. Plasma ANP in the patients also rose significantly ($p < 0.01$) from 231 ± 102 pg/ml to 794 ± 170 pg/ml. The response of plasma ANP to exercise was enhanced significantly ($p < 0.05$) in the patients as compared with the normals in relation to ejection fraction by analysis of covariance. In both the normals and the patients, plasma ANP was inversely and significantly correlated with ejection fraction during exercise ($r = -0.46$, $p < 0.05$, $n = 21$), however, not at rest. Because it has been reported that plasma ANP is correlated positively with pulmonary artery wedge pressure, the estimation of plasma ANP during an exercise stress test might be used for the evaluation of cardiac reserve in coronary artery disease.

Additional Indexing Words:

Atrial natriuretic peptide   Coronary artery disease   Radionuclide

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angiography   Ejection fraction   Renin   Aldosterone

ATRIAL natriuretic peptide (ANP), a new peptide with potent natriuretic activity, was isolated from human atrial tissue, sequenced and synthesized.¹⁾ Synthetic ANP causes natriuresis and diuresis and decreases blood pressure when infused into normal persons.¹⁾ It has been demonstrated²⁾ that circulating ANP is elevated in proportion to the increase in right atrial pressure or pulmonary artery wedge pressure (PAWP) in patients with congestive heart failure, supporting the hypothesis^{3),4)} that atrial distension stimulates the release of the peptide. On the other hand, several investigators^{5),6)} have observed that physical exercise raises the plasma level of ANP in healthy persons and cardiac patients.

It has been recognized⁷⁾ that many patients with coronary artery disease have normal left ventricular systolic function at rest and develop abnormalities only during exercise. Radionuclide ventriculography performed with the patient at rest and during exercise is an established method of evaluating suspected coronary artery disease.^{7)–10)} In the present study, we determined plasma levels of ANP at rest and during exercise in normal volunteers and patients with coronary artery disease. Ejection fraction was also measured by gated radionuclide ventriculography in an attempt to clarify the relationship between circulating ANP and cardiac function.

SUBJECTS AND METHODS

We studied 11 patients (9 males and 2 females) aged 41–67 years (56 ± 3 years, mean $\pm$ SEM), with coronary artery disease. Four patients were referred to our hospital because of a recent history of angina pectoris and 7 with myocardial infarction. No patient had any evidence of other cardiac abnormalities, and all patients underwent left heart catheterization with contrast left ventriculography and coronary arteriography before the study. The study was performed at least 24 hours after cessation of propranolol and at least 4 hours after administration of nitroglycerin.

As a control, we studied 10 normal healthy male volunteers aged 28–42 years (34 ± 2 years); physical examination, chest X-ray, electrocardiography (ECG) and biochemical findings were normal, although cardiac catheterization was not performed.

All subjects fasted for 12 hours prior to the study. On the day of data collection, each subject had a 20-gauge teflon i.v. catheter placed percutaneously into an external jugular or antecubital vein for radionuclide injection or obtaining venous blood. ECG leads were placed on the chest and a

sphygmomanometer cuff was placed on the arm that was not used for injection. After the patient was in the recumbent position for at least 1 hour, exercise was performed on a supine bicycle exercise table. It began at a work load of 25 watts and exercise loads were increased by 25 watt increments at 3 min intervals, culminating in a load that produced symptoms of angina or dyspnea, or fatigue of sufficient severity to limit further exercise. Heart rate and blood pressure were recorded with the patients at rest and at maximal work load (immediately after ceasing the stress test). Heart rate and rhythm were recorded by ECG and blood pressure was measured by the standard cuff technique.

Samples of venous blood were obtained at rest and at maximal work load for measurements of plasma ANP, plasma renin activity (PRA), and plasma concentrations of aldosterone and cortisol. PRA and plasma concentrations of aldosterone and cortisol were measured by radioimmunoassay, as reported previously.¹¹⁾ For measurement of immunoreactive ANP, 1 ml plasma aliquots were extracted on Sep-PAK C18 cartridges (Waters Associates, Milford, Massachusetts, USA) before radioimmunoassay, as described previously.¹²⁾ Synthetic α -human atrial natriuretic peptide (α -hANP) was purchased from Peninsula Laboratories (Belmont, CA, USA). ¹²⁵I- α -hANP and antibody against atriopeptin I were kindly provided by the Mitsubishi Yuka Laboratory of Medical Science. The lowest level of α -hANP that significantly inhibited ¹²⁵I- α -hANP-binding to the antibody was 7.0 pg/tube. The efficacy of the extraction procedure was estimated by recovery of synthetic α -hANP added to plasma. When 50–500 pg/ml synthetic α -hANP were added, plasma recovery was $81 \pm 2\%$ (mean $\pm$ SEM, $n=12$) after the extraction and radioimmunoassay procedure. The intra- and interassay coefficients of variation were 7.0 and 9.1% ($n=10$), respectively.

Radionuclide angiography was performed by using erythrocytes labeled with technetium-99 m and gated cardiac blood-pool imaging. Red blood cells were tagged with stannous pyrophosphate and labeled with 30 mCi of technetium 99 m using an in vivo technique after a 15 min delay. Cardiac images in the left anterior oblique projection that best separated both ventricles were obtained with a standard gamma camera with suitable caudal angulation, at rest and at maximal work load. Collection of images was made with a multitriggered system, triggered by the R wave of the ECG. Counts were recorded for 300 to 500 cardiac cycles. Left ventricular ejection fractions at rest and at maximal exercise were determined, by using a commercially available operator-interactive program, from count rates in the left ventricular region of interest corrected for background activity by means of a dedicated computer (Scintipack 1200, Shimadzu, Tokyo, Japan).

Values at rest and during exercise were evaluated by Student's t-test for paired observations. Analyses of covariance were employed for comparison of the response of plasma ANP with changes in heart rate, systolic blood pressure or ejection fraction in the 2 groups (Figs. 1-3). Linear regression analysis was performed according to the standard procedure (Fig. 4). Values are presented as mean $\pm$ SEM. A p value of less than 5% was considered significant.

RESULTS

All patients had angiographic evidence of significant coronary artery disease by left heart catheterization (reduction of luminal diameter of more than 50% in at least one major coronary artery). Maximal work load in normal volunteers and the patients with coronary artery disease were 145 ± 5 watts and 59 ± 11 watts, respectively. Six patients experienced angina pectoris during exercise. Table I summarizes the data of PRA and plasma levels of aldosterone and cortisol. PRA and plasma levels of aldosterone and cortisol did not differ significantly during exercise as compared with the values at rest.

Plasma ANP in normal persons was 62 ± 16 pg/ml at rest and increased significantly ($p<0.01$) to 454 ± 94 pg/ml during exercise. Plasma ANP in the patients was also increased significantly ($p<0.01$) from 231 ± 102 pg/ml to 794 ± 170 pg/ml. Figures 1 and 2 show a comparison between the 2 groups in the response of plasma ANP in relation to heart rate and systolic blood pressure, respectively. Heart rate in normal persons rose significantly ($p<$

Table I. Plasma Renin Activity and Plasma Levels of Aldosterone and Cortisol at Rest and during Exercise

	Rest	Exercise
Normal Persons (n=10)		
Plasma renin activity (ng/ml/hr)	3.1 ± 1.0	2.3 ± 0.4
Plasma		
Aldosterone (pg/ml)	103 ± 6	116 ± 6
Cortisol (μ g/dl)	9.4 ± 1.4	10.5 ± 1.3
Patients with Coronary Artery (n=11)		
Plasma renin activity (ng/ml/hr)	2.6 ± 0.7	2.7 ± 0.6
Plasma		
Aldosterone (pg/ml)	119 ± 11	125 ± 10
Cortisol (μ g/dl)	12.6 ± 2.0	12.3 ± 1.7

Values are mean $\pm$ SEM. There were no significant differences between the values at rest and during exercise by Student's t-test for paired observations.

Fig. 1. Relation between plasma levels of ANP and heart rate at rest and during exercise. The lines joining the points in the left and middle columns represent individual values in normal persons and patients with coronary artery disease, respectively. In all subjects, both normals and patients, plasma level of ANP was increased during exercise as compared with the value at rest. The right column represents the group mean values for plasma levels of ANP and heart rate at rest and during exercise. Open and closed circles indicate normal persons and patients, respectively. Bars in the right column indicate mean $\pm$ SEM. CAD=coronary artery disease; HR=heart rate.

Fig. 2. Relation between plasma levels of ANP and systolic blood pressure at rest and during exercise. The lines joining the points in the left and middle columns represent individual values in normal persons and the patients with coronary artery disease, respectively. In each subject, both normals and patients, plasma level of ANP was increased during exercise as compared with the value at rest. The right column represents the group mean values for plasma levels of ANP and systolic blood pressure at rest and during exercise. Open and closed circles indicate normals and patients, respectively. Bars in the right column indicate mean $\pm$ SEM. CAD=coronary artery disease; SYST BP=systolic blood pressure.

0.01) from 67 ± 3 beats/min at rest to 141 ± 4 beats/min during exercise. Heart rate in the patients also rose significantly ($p < 0.01$) from 64 ± 3 beats/min to 106 ± 6 beats/min. Systolic blood pressure was elevated significantly ($p < 0.01$) from 131 ± 3 mmHg to 209 ± 7 mmHg in normal persons, and from 139 ± 4 mmHg to 178 ± 8 mmHg in the patients. The response of plasma ANP was enhanced significantly ($p < 0.05$) in the patients with coronary artery disease as compared with normal persons in relation to heart rate or systolic blood pressure.

Figure 3 represents the response of plasma ANP related to ejection fraction. Ejection fraction was increased in all normal volunteers, whereas it decreased in 5 patients during exercise. Ejection fraction in normal persons was $59 \pm 3\%$ at rest and increased significantly ($p < 0.01$) to $69 \pm 3\%$ during exercise. Ejection fraction in the patients was $47 \pm 5\%$ at rest and did not change significantly during exercise ($51 \pm 7\%$). There was a significant difference between the 2 groups in the response of plasma ANP to exercise in relation to ejection fraction. Figure 4 shows a regression analysis between plasma ANP and ejection fraction. In all subjects of the 2 groups together, plasma ANP was inversely and significantly correlated with ejection fraction

Fig. 3. Relation between plasma levels of ANP and ejection fraction at rest and during exercise. The lines joining the points in the left and middle columns represent individual values in normal persons and the patients with coronary artery disease, respectively. In each subject, both normals and patients, plasma level of ANP was increased during exercise as compared with the value at rest. The right column represents the group mean values for plasma levels of ANP and ejection fraction at rest and during exercise. Open and closed circles indicate normals and patients, respectively. Bars in the right column indicate mean $\pm$ SEM. CAD = coronary artery disease; EF = ejection fraction.

Fig. 4. Regression analysis between plasma ANP and ejection fraction. Left and right columns represent the data at rest and during exercise, respectively. Open and closed circles indicate normals and patients, respectively. The lines in the right column are regression lines. n.s.=not significant; EF=ejection fraction.

during exercise ($r = -0.46$, $p < 0.05$, $n = 21$), but not at rest.

DISCUSSION

Physiologic assessment of patients with stable coronary artery disease is inadequate when performed only with the patients at rest.⁷⁾ In this state, myocardial oxygen supply and demand are in balance. However, during exercise, supply-demand imbalance and myocardial ischemia may occur which result in transient pathophysiologic abnormalities. Noninvasive evaluation of left ventricular performance during exercise has utilized the gated cardiac blood pool imaging technique. Many investigators⁷⁾⁻¹⁰⁾ have used radio-nuclide angiography to measure left ventricular function during rest and exercise and have demonstrated the presence of impaired global and regional left ventricular reserve during exercise in patients with coronary artery disease.

The present study has shown that exercise increases the plasma level of ANP in both normal persons and patients with coronary artery disease. The patients had a greater response of plasma ANP than did the normal persons in relation to changes in heart rate, systolic blood pressure or ejection fraction, indicating an enhanced exercise-induced rise in plasma ANP in these patients. The physiologic basis for the release of ANP during exercise is important. A direct action of catecholamines is unlikely, because Katsube

et al¹³⁾ have reported that the response of ANP to vasoconstrictors like catecholamines is indirect and is brought about by elevation of right atrial pressure induced by these substances. On the other hand, the increase in heart rate might contribute to the rise in plasma ANP, since supraventricular tachycardia is accompanied by the increase in plasma ANP; however, Nicklas et al¹⁴⁾ have shown that patients with sinus tachycardia have no significant increase in plasma ANP, and that there is no relationship between the peak ANP level and the heart rate in supraventricular tachycardia. They have concluded that the primary stimulus for release of ANP during supraventricular tachycardia is not an increase in heart rate per se, but a rise in right atrial pressure due to simultaneous contraction of the atrium and the ventricle with the tricuspid valve closed.

Increasing atrial pressure in rats through intravenous infusion of saline solution³⁾ or intraatrial balloon dilatation⁴⁾ increases the circulating level of ANP. We¹⁵⁾ and others²⁾ have reported that plasma ANP levels are highly correlated with mean PAWP in patients with cardiac impairment. Since PAWP reflects left atrial pressure, it appears that plasma ANP alters in proportion to left atrial pressure in cardiac patients.

It has been demonstrated that the mean PAWP and left ventricular filling pressure increase during exercise in both normal persons and patients with coronary artery disease.^{16),17)} The elevation of PAWP is reported to be increased in patients with coronary artery disease as compared with normal persons.¹⁸⁾ The mechanism of the increase in left ventricular filling pressure during exercise-induced angina is still controversial. Angiographic studies during exercise-induced angina have revealed increased end-diastolic volume,¹⁹⁾ whereas reduced myocardial compliance may play a role when ischemia is produced by cardiac pacing.^{20),21)} Regardless of the mechanism for the increase in left ventricular filling pressure during exercise-induced myocardial ischemia, the elevation in left ventricular filling pressure implies impaired left ventricular function in these patients and contributes to the increase in PAWP and circulating ANP. The present study has shown that there is a significant negative correlation between plasma ANP and ejection fraction only during exercise.

Ejection fraction was decreased after supine ergometer exercise in 5 patients with coronary artery disease, but increased in all normal volunteers. Because of the heterogeneous nature of coronary artery disease, a direct correlation between the anatomic extent of disease and the functional impairment of the ventricle during exercise could not be anticipated.⁸⁾ The patient's age, sex, level of exercise conditioning, previous cardiac medications, level of excitement, level of stenosis, caliber of patent vessels and amount of collateral

flow are some of the many factors that might influence the response of the left ventricle during exercise stress.²²⁾ Because plasma ANP may reflect atrial pressure or ventricular filling pressure, the estimation of plasma ANP during a standardized ergometer test may contribute to the evaluation of cardiac reserve in patients with coronary artery disease.

In conclusion, we have demonstrated that supine ergometer exercise testing raises the circulating levels of ANP in patients with coronary artery disease. The exercise-induced rise in plasma ANP is increased in patients with coronary artery disease as compared with normal persons. Plasma ANP is inversely and significantly correlated with ejection fraction only during exercise. It is suggested that the estimation of plasma ANP during an exercise stress test may be used for the evaluation of cardiac reserve in patients with coronary artery disease.

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