The Changes in Circulating Levels of Vasoactive Intestinal Polypeptide During Exercise and Its Reproducibility for Detection of Myocardial Ischemia

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SUMMARY

Vasoactive intestinal polypeptide (VIP) contributes to the regulation of coronary vaso-motor tone and circulating levels of VIP have been reported to increase during acute myocardial infarction. However, the changes in VIP concentration during exercise-induced ischemia have not been studied yet. Therefore, we sought to determine whether circulating levels of VIP change during treadmill exercise testing and whether they could be used as a marker of exercise-induced myocardial ischemia. Twenty-nine subjects with definitive positive (group-I) and 20 subjects (group-II) with negative results on treadmill exercise testing were included in this study. In order to assess circulating levels of VIP, blood samples were collected in both groups before exercise, at 5 minutes of exercise, at peak exercise, and at 10 minutes in the recovery period. There were no differences between the two groups with respect to the baseline demographics of age, sex, heart rate, or blood pressure. The metabolic equivalents (METs) values, peak heart rate achieved, peak systolic-diastolic blood pressure, and exercise duration did not differ between the two groups. No significant differences were found in the circulating levels of VIP at any stage of the exercise between the two groups (10.5 ± 2.5 versus 11.0 ± 3.5 pmol/L, \( P = 0.5 \), 10.6 ± 2.3 versus 10.6 ± 3.3 pmol/L, \( P = 0.9 \), 10.9 ± 3.1 versus 11.5 ± 3.4 pmol/L, \( P = 0.5 \), and 10.7 ± 1.8 versus 11.7 ± 4.1 pmol/L, \( P = 0.3 \), respectively).

There was no relationship between the circulating level of VIP and exercise-induced myocardial ischemia, and therefore it could not be used as a marker of exercise-induced myocardial ischemia. (Int Heart J 2005; 46: 363-371)

Key words: Vasoactive intestinal polypeptide, Ischemia, Exercise

Vasoactive intestinal polypeptide (VIP), a peptide first isolated from porcine duodenum, has been reported to be a potent nonadrenergic and noncholinergic vasodilator in a number of vascular beds, including coronary arteries.1-3)
Studies with immunohistochemistry and radioimmunoassay techniques showed that VIP-like immunoreactive substances are present specifically in nerves of the coronary arteries of humans and other mammalian species.4,5) VIP has also been implicated in pathophysiologic aspects of hypertension, hemorrhagic shock, and heart failure.6-8) In addition, its plasma concentration has been shown to increase in patients with acute coronary occlusion and the plasma VIP concentration has been reported to increase by 33-62% within 6 hours of the onset of symptoms but then abruptly decrease below the normal concentration after 24 hours in patients with acute myocardial infarction (AMI). It is involved in neuro-endocrine activation occurring in AMI and could be an additional marker of the course of AMI.9-11) On the other hand, after short-term exercise the change in the level of circulating VIP concentration has been studied both in healthy subjects and in those with cardiac failure.12,13) Accordingly, during strenuous exercise, plasma VIP levels have been found to increase by as much as 100% and persist for more than 20 minutes after the termination of exercise.14)

Treadmill exercise testing is one of the most common noninvasive methods used to detect myocardial ischemia. However, changes in the circulating level of VIP during treadmill exercise testing and its clinical utility in the detection of exercise-induced myocardial ischemia have not been studied to date. In the present study, therefore, we attempted to test whether the circulating level of VIP changes in response to treadmill exercise testing and whether it could be used as an additional marker of exercise-induced myocardial ischemia.

**METHODS**

Forty-nine subjects fulfilling the inclusion criteria were included in this study. All participants eligible for inclusion were selected from among those who underwent treadmill exercise testing due to typical or atypical chest pain. They were divided into two groups according to their test results. Twenty-nine subjects with definitive positive ischemic exercise testing in whom coronary artery disease (CAD) was later confirmed by diagnostic coronary angiography constituted the patient group (group I) and 20 subjects without any ischemic changes during exercise constituted the control group (group II).

The subjects were excluded from the study if they had a history of congestive heart failure, valvular heart disease, previous myocardial infarction, coronary artery bypass graft (CABG) surgery, or any other systemic disorder and drug usage that could affect VIP concentration such as angiotensin converting enzyme inhibitors. CAD was defined by the coronary arteriographic evaluation performed in multiple angulated views according to the standard Judkins technique.
and the presence of 70% or more severe stenosis in at least one major coronary artery was accepted as significant.

**Treadmill exercise testing:** All study subjects underwent treadmill exercise testing using the modified Bruce protocol. Prior to testing, all subjects were instructed not to eat, drink any beverages, or smoke for 3 hours before the testing. Heart rate, ECG, and blood pressure were recorded at the onset and immediately after exercise. During exercise the level of the ST segment depression was determined in a blinded manner by 2 independent observers, and disagreements were resolved by consensus. Angina, fatigue, diagnostic ST-segment depression, or persistent arrhythmias were considered reasons for discontinuing the exercise test. ST-segment level was measured 60 ms after the J point in all 12 leads. Exercise-induced significant ST-segment depression was defined as: horizontal or downsloping ST segment depression $\geq 1$ ms, or upsloping ST segment depression, $\geq 2$ mm in any lead, present within the first 2 minutes of the recovery period.

**Blood sampling and biochemical analysis:** All subjects were studied in the morning and after insertion of a cannula into an antecubital vein and rested quietly in a supine position for at least 10 minutes before removal of the first blood sample. Blood samples were drawn at the following time points: just before the exercise test, at 5 minutes of exercise, at peak exercise, and at 10 minutes in the recovery period. The blood for VIP determination was collected in ice chilled 10 mL vacuum tubes containing ethylenediamine tetra acetic acid (EDTA) and aprotinin (5000 I.U./mL). They were kept on ice until centrifugation. Blood for serum collection was taken in vacuum tubes, allowed to clot at room temperature, and then centrifuged after about 30 minutes. The plasma and serum were then frozen on dry ice immediately after centrifugation and kept frozen at -70°C until analyzed. Plasma VIP values were determined using a commercially available kit (Euro-Diagnostica AB, Malmo, Sweden) according to a competitive radioimmunoassay method with a detection limit of 3 pmol/L.

**Statistical analysis:** Statistical analysis was performed with SPSS for Windows version 10.0 (SPSS Inc. Chicago, Illinois). Data are expressed as the mean value $\pm$ SD. The paired or unpaired Student's $t$ test was used for continuous variables and the chi-square test was used for categorical variables. A $P$ value $< 0.05$ was considered to indicate statistical significance.

**RESULTS**

The baseline characteristics of all participants are given in Table I. There were no significant differences between the two groups with respect to the baseline demographics of age, sex, heart rate, or blood pressure. All subjects completed the test successfully. No significant rhythm disturbance or hemodynamic
abnormality was detected during exercise testing in any subject. Metabolic equivalent (METs) values, peak heart rate achieved, exercise duration, and peak systolic-diastolic blood pressure were not different in either group (Table II). In group I, typical angina or angina-like symptoms such as palpitations, shortness of breath, or fatigue occurred in 15 cases during exercise and in 10 cases in group II. In group I, maximum (2.1 ± 1.1 mm) ST-segment depression (in 4.2 ± 0.3 leads at 60 ms after the J point) occurred during peak exercise. Group II showed no significant ST segment depression at peak exercise (max. 0.3 ± 0.2 mm at 60 ms after the J point in 0.8 ± 0.4 leads). Baseline VIP levels were similar in both groups (10.5 ± 2.5 versus 11.0 ± 3.5 pmol/L, \( P = 0.5 \)). Also, there was no statistically significant difference in the level of VIP at any stage of exercise between the two groups (10.6 ± 2.3 versus 10.6 ± 3.3 pmol/L, \( P = 0.9 \), 10.9 ± 3.1 versus 11.5 ± 3.4 pmol/L, \( P = 0.5 \), 10.7 ± 1.8 versus 11.7 ± 4.1 pmol/L, \( P = 0.3 \), respec-

### Table I. Baseline Demographic Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I</th>
<th>Group II</th>
<th>( P ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Men/women</td>
<td>17/12</td>
<td>12/8</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years</td>
<td>62 ± 9</td>
<td>60 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 3</td>
<td>28 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>81 ± 17</td>
<td>81 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>135 ± 15</td>
<td>134 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>80 ± 5</td>
<td>82 ± 5</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = statistically not significant; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure.

### Table II. Exercise Test Results in the Two Study Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I</th>
<th>Group II</th>
<th>( P ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>METs, mL/kg/dk</td>
<td>7.8 ± 1.4</td>
<td>8.4 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Peak heart rate, beat/min</td>
<td>146 ± 16</td>
<td>154 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Peak SBP, mmHg</td>
<td>183 ± 31</td>
<td>191 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td>Peak DBP, mmHg</td>
<td>92 ± 15</td>
<td>96 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>Exercise duration, seconds</td>
<td>704 ± 25</td>
<td>770 ± 110</td>
<td>NS</td>
</tr>
<tr>
<td>Max ST segment depression, mm</td>
<td>2.1 ± 1.1</td>
<td>0.3 ± 0.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Number of leads</td>
<td>4.2 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

NS = statistically not significant; METs = metabolic equivalent values; SBP = systolic blood pressure; DBP = diastolic blood pressure.
Figure 1. Comparison of plasma VIP levels in the two study groups. The bars on the left represent VIP levels in subjects with positive exercise testing (patient group) and the right bars represent VIP levels in subjects with negative exercise testing (control group). The rectangles represent the changes in plasma VIP levels: basal, at 5th minute of exercise, at peak exercise, and at 10th minute of recovery period, respectively.

Figure 2. Changes in plasma VIP levels during exercise in subjects with positive exercise testing (group I). Before exercise (VIP-basal), at 5th minute of exercise (5 minute), at peak exercise (Peak), and at 10th minute of recovery period (10 min after), respectively.
In group I, the circulating level of VIP did not show any significant alteration during any stage of the exercise (Figure 2), but the VIP level at 5 minutes of exercise decreased compared to the previous and subsequent levels in group II. (11.5 ± 3.4 versus 10.6 ± 3.3 pmol/L, \( P = 0.01 \)) (Figure 3).

**DISCUSSION**

The results of the present study indicate that the circulating level of VIP is similar in patients with CAD and control subjects both at rest and during exercise. In addition, it did not show any alteration at any stage of the treadmill exercise testing in patients with CAD. To the best of our knowledge, our study is the first attempt to examine the relationship between the circulating level of VIP and exercise-induced myocardial ischemia.

VIP is present in the coronary arterial wall and contributes to the regulation of normal coronary vasomotor tone and a decrease in VIP concentration may induce coronary spasm.2) The release of VIP in the heart and the VIP concentration in the coronary sinus blood are increased during coronary artery occlusion and during reperfusion.15,16) In isolated perfused rat hearts in which coronary perfusion is interrupted for 30 minutes, the VIP concentration in the coronary effluent increases by 250%, during the ensuing 60-min reperfusion period.15) Moreover, coronary perfusion with VIP immediately prior to the induction of ischemia significantly decreases the myocardial release of creatinine kinase and formation of hydroxyl radicals, and inhibits calcium overload in cardiac myocytes.15-17) These findings suggest that VIP promotes local blood flow in the heart.
during acute myocardial ischemia and may also have a free-radical scavenging effect. VIP plasma concentration also increases in patients with acute coronary occlusion. In patients with acute myocardial infarction, the VIP concentration in plasma increases by 33-62% within 6 hours of the onset of symptoms but then abruptly decreases below the normal concentration after 24 hours. In patients who die from acute myocardial infarction, the VIP plasma concentrations do not normalize but rather remain significantly lower than the VIP concentrations of patients who survive, and the authors concluded that VIP is involved in neuroendocrine activation occurring in AMI and could be an additional marker of the course of AMI. In this context, we decided to test whether the circulating level of VIP could be used as a novel marker of exercise-induced myocardial ischemia. However, our findings suggest that the plasma VIP concentration does not change before, during, or after exercise testing in those patients with positive exercise testing, but it decreased only at the 5th minute of exercise compared to the previous and subsequent levels in those with negative exercise testing. The mechanism of this phenomenon is unclear, however, the relatively short duration of exercise and lack of myocardial ischemia in these subjects may be responsible mechanisms.

Several previous studies have focused on the effect of exercise on the circulating level of VIP, but the effect of exercise on VIP levels is not clearly understood. In accordance with our findings, Galbo, et al. observed no significant increase in plasma levels of VIP in healthy volunteers during a short-term maximal treadmill test lasting 5 minutes. Similarly, Nicholls, et al. observed no significant difference in the circulating level of VIP at any stage of exercise between individuals with chronic heart failure and controls. Woie, et al. reported that no significant increase occurs after 7 to 15 minutes of mild exercise in patients with angina pectoris. They also reported that heavy and short-term (16-32 minutes) muscular exercise was followed by a significant increase in the concentration of VIP in the systemic circulation in healthy volunteers. However, their study included only a limited number of subjects and exercise duration was longer than our study. On the other hand, Galbo, et al. reported that plasma VIP levels increased in military cadets during a 3-hour mild bicycle exercise test but not at all during an equivalent period of rest or during short-term submaximal and maximal exercise, and they also showed that VIP levels increased during fasting. Similarly, Opstad, et al. showed that plasma levels of VIP increased during bicycle exercise testing lasting more than 20 minutes with more than 50% of VO$_2$ max and that the highest levels appeared in the early (5-10 minutes after the exercise) recovery period. Hilsted, et al. measured peripheral plasma concentrations of VIP after a 3-h period of bicycle exercise at 40% of maximal oxygen uptake in six normal men and observed a marked increase in the level of VIP. However, in
our study the lack of increase in circulating levels of VIP might have resulted from the relatively short exercise duration and intensity of exercise since we used a mild rather than heavy exercise protocol, whereas all of the studies mentioned above utilized a long-term exercise. The fact that systemic VIP levels remain normal in patients with myocardial ischemia suggests that during the treadmill exercise either VIP may be rapidly degraded by the liver or the metabolism of the peptide may be altered so that it is no longer detectable by radioimmunoassay.

In conclusion, our study results have revealed that there were no differences in VIP levels in subjects with either positive or negative myocardial ischemia whether at rest or during exercise. Thus, our findings appear to indicate there is no relationship between the circulating level of VIP and exercise-induced myocardial ischemia, and that it cannot be used as a marker of exercise-induced ischemia. Our overall findings regarding the role of VIP in myocardial ischemia require further corroboration by prospective studies and further studies to establish the role of VIP receptors at the tissue level.

REFERENCES

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