Postexercise Ischemia Is Associated With Increased Neuropeptide Y in Patients With Coronary Artery Disease

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Background—Neurohormones may influence vascular tone both during and after exercise. Neuropeptide Y (NPY), which is costored and released with norepinephrine (NE) during sympathetic activity, is a potent vasoconstrictor with a relatively long half-life. We therefore examined its possible association with the ischemic response to exercise in patients with coronary artery disease.

Methods and Results—Twenty-nine male patients with effort-induced angina pectoris underwent a symptom-limited exercise test. In addition to conventional ST-segment analysis, we examined ischemia on the basis of heart rate (HR)-adjusted ST-segment changes through calculation of the ST/HR slope during the final 4 minutes of exercise and of the ST/HR recovery loop after exercise. Blood samples were taken before, during, and after exercise for an analysis of several neurohormones. Mean ST-segment depression was $-223 \pm 20.2 \, \mu V$ ($P<0.0001$) just before the termination of exercise, followed by a gradual normalization, but it remained significant after 10 minutes ($-49 \pm 8.9 \, \mu V$, $P<0.0001$). At the end of exercise, the ST/HR slope, which reflects myocardial ischemia, was $-6.0 \pm 0.77 \, \mu V/HR$. In most patients, ST-segment levels at a given HR were lower during recovery than during exercise, here referred to as ST “deficit.” Exercise increased the plasma levels of NPY, NE, epinephrine, and N-terminal proatrial natriuretic peptide, but big endothelin remained unchanged. Although NE and epinephrine peaked at maximal exercise, the highest levels of NPY and N-terminal proatrial natriuretic peptide were observed 4 minutes after exercise. The maximal increase in the NPY correlated significantly with ST-segment depression at 3 minutes after exercise ($r=-0.61$, $P=0.0005$), the ST deficit at the corresponding time point ($r=-0.66$, $P=0.0001$), and the duration of ST-segment depression after exercise ($r=0.42$, $P=0.02$). In contrast, no such correlations were found for NE.

Conclusions—The present study has for the first time demonstrated a correlation between plasma NPY levels and the degree and duration of ST-segment depression after exercise in patients with coronary artery disease, which suggests that NPY may contribute to myocardial ischemia in these patients. (Circulation. 2000;102:987-993.)

Key Words: coronary disease ■ exercise ■ ischemia ■ peptides

In patients with angina pectoris, myocardial ischemia during physical exercise is caused by oxygen demand that exceeds the supply and is mainly due to activation of the sympathetic nervous system. The release of norepinephrine (NE) leads to increased myocardial oxygen demand via a $\beta$-adrenoceptor–mediated increased heart rate (HR) and contractility, as well as limitation of the increase in oxygen supply due to an $\alpha$-adrenoceptor–mediated coronary vasoconstriction. Early studies suggest a significant role of $\alpha$-adrenergic coronary vasoconstriction during exercise-induced myocardial ischemia in patients with stable angina pectoris. However, it remains unknown whether the non-adrenergic sympathetic cotransmitter neuropeptide Y (NPY) contributes.

NPY is a 36-amino-acid peptide that belongs to the NPY family. It is widely distributed but is particularly abundant in the perivascular sympathetic nerve fibers, where it is costored and released with NE. Its cardiovascular effects are multiple and not fully explored, but a long-lasting vasoconstriction, which is not mediated via $\alpha$-adrenergic receptors, is prominent. In a recent study in dogs, the myocardial release of NPY elicited by cardiac sympathetic nerve stimulation correlated significantly with the coronary vasoconstrictor response, which persisted after $\alpha$- and $\beta$-adrenoceptor blockade. It was also demonstrated that the NPY (Y1) receptor antagonist BIBP 3226 attenuated the vasoconstrictor response resistant to $\alpha$-adrenoceptor blockade. The intracoronary infusion of NPY in patients with angina pectoris induces myo-
cardial ischemia with typical chest pain and ECG changes. This may indicate that NPY also is a mediator of coronary vasoconstriction in humans.

In humans, plasma NPY levels are enhanced during reflex sympathetic activation, which suggests release from sympathetic nerves. Compared with NE, NPY is eliminated more slowly from the vicinity of its action site and from the circulating blood. As a consequence of this, the exercise-induced increase in the systemic plasma level of NPY is characterized by a more delayed time course than that of NE. There also is evidence of a myocardial release of NPY in humans during exercise. Interestingly, the cardiac overflow of NPY, relative to that of NE, is increased during exercise under hypoxic conditions. However, the role of NPY in sympathetic regulation of myocardial blood flow under physiological conditions or in patients with coronary artery disease is unknown. In patients with coronary artery disease, an evaluation of the ischemic ECG response to exercise remains the most widely used method for the diagnosis and evaluation of antianginal therapy. The objective of the present study was to examine whether the ischemic response to a bicycle exercise test was related to the plasma levels of NPY in patients with coronary artery disease. The ischemia was estimated through a determination of both levels of NPY in patients with coronary artery disease. The ischemia was characterized by a more delayed time course than that of NE. The introduction of this analysis has greatly improved the estimation of the functional severity of coronary obstruction and revealed the possible presence of a delayed coronary vasocostriction in the recovery phase. Of importance, a recent study by Rywik et al demonstrated ST-segment changes in the recovery period to be of independent adverse prognostic value. We therefore found it of particular interest to assess whether the release of the long-lasting coronary vasoconstrictor NPY is associated with myocardial ischemia in the recovery phase after exercise. The NPY plasma levels were measured before, during, and after exercise. Corresponding measurements were also made of the plasma levels of NE and epinephrine (E) and of 2 other endogenous substances, big endothelin (big ET) and N-terminal proatrial natriuretic peptide (Nt-pro-ANP); all may modulate coronary vascular resistance.

Methods

Patients

Initially, 36 patients who were referred for coronary angiography for suspected coronary artery disease were recruited for the study. Seven were later excluded (1 had normal coronary arteries, 3 did not show ST-segment depression during exercise, and the blood samples were inappropriate in 3). The study population therefore consisted of 29 male patients with chronic effort-induced angina pectoris for >3 months and without concomitant disease of importance (Table 1). Significant coronary artery disease was confirmed in all except 1 patient, who eventually refused angiography. The patients’ clinical and hemodynamic conditions were stable; they experienced no myocardial infarction during the preceding 3 months; and medication (Table 1) had not changed during the preceding month. Informed consent was obtained from all participants, and the ethics committee of the hospital approved the protocol.

Exercise Testing

The subjects were instructed not to take part in physical training on the day before the exercise test and to eat a normal mixed diet. They were not allowed to eat, drink coffee, or smoke during the 4 hours before the study, and the morning medication was postponed. The tests were performed at the same time of the day and under similar conditions.

The bicycle exercise test was carried out on an electrically braked cycle ergometer (Siemens Elema) with a constant pedal rate of 60 rpm. Workload was initiated at 40 W with subsequent increments of 20 W every other minute. The exercise was stopped because of anginal pain (19 patients), exhaustion (9 patients), or dyspnea (1 patient). A 12-lead ECG and blood pressure were recorded before exercise, during exercise, and at 1, 3, 5, 10, and 30 minutes in the postexercise period. All postexercise ECGs in the fourth minute of recovery and later were taken with the patient in the supine position. According to standard criterion, a positive ischemic response was defined as ≥0.1-mV (1-mm) J-point depression with ST segment flat or downsloping at peak exercise or in the recovery period in any lead. ST levels were measured at 60 ms after the end of QRS with a section of the PQ level as reference. The values were measured in V5 except for 1 patient in whom V5 was used. ST-segment depression was calculated with values at rest before exercise. An ST/HR slope was calculated during the final 4 minutes of exercise and expressed as microvolts per HR.

The ST-segment depression (in µV) measured during exercise and at 1, 3, 5, and 10 minutes of recovery was plotted against the corresponding HRs. A rate-recovery loop could therefore be constructed (Figure 1). In most patients, ST-segment depression was related to HR in the recovery period compared with the exercise phase, producing a clockwise loop of ST-segment depression. With this loop, we measured the difference between the ST-segment depression during and after exercise at corresponding HRs. Because the measured value was negative in most cases, it is referred to here as ST “deficit.” A time curve for the ST deficit in each of the first 10 minutes of recovery was constructed in which missing values were determined through interpolation. From this curve, calculations were made of the mean ST deficit in the first 10 minutes after exercise. The duration of ST-segment depression after exercise was assessed through determination of the “ST-segment depression time” (ie, the time that elapsed in the recovery until the ST-segment depression had returned to 100 µV below the preexercise resting level).

Blood Sampling

Blood samples were drawn from an arm vein (contralateral to the used for blood pressure measurements) at rest, at submaximal (100 W) and peak exercise, and after 4, 10, and 30 minutes of recovery. Plasma was separated immediately and frozen at −70°C until analyzed. NE and E were determined with cation exchange HPLC.

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**TABLE 1. Clinical Characteristics of Study Population**

| Age, y | 62 ± 2* |
| Hypertension, n (%) | 7 (24) |
| NYHA class, I/II/III | 2/14/13 |
| Medication for angina, n |  |
| β-Blocker | 26 |
| Calcium channel-blocker | 10 |
| Long-acting nitrate | 13 |
| Aspirin | 22 |
| Lipid-lowering medication | 18 |
| No. of vessels affected, 1/2/3 | 6/12/10 |
| Left ventricular ejection fraction | 0.68 ± 0.3* |
| Left ventricular end-diastolic pressure, mm Hg | 14 ± 1* |

*Values are mean ± SEM or numbers (n = 29).
with electrochemical detection, and NPY, big ET, and Nt-pro-ANP [ANP(1-98)] were assessed with radioimmunoassay. The coefficients of intra-assay variation were 3.8%, 3.5%, 7.0%, 5.6%, and 6.9% for NE, E, NPY, big ET, and Nt-pro-ANP, respectively.

**Statistical Analysis**

ANOVA for repeated measurements was used to analyze the effect of exercise on the different variables. If statistically significant differences were found, post hoc Student’s *t* tests with Bonferroni’s correction were performed. Pearson’s correlation analysis was performed to test the correlation between hemodynamic and neurohumoral responses. In the statistical evaluation, the exercise-induced effects on the plasma levels of measured neurohormones were calculated as the change between the levels obtained at rest before exercise and the maximal plasma level measured at peak exercise or 4 minutes after exercise (maximal change). Values are given as mean±SEM unless otherwise stated. Correlation coefficients and differences were considered significant at *P*<0.05 (2-tailed test).

**Results**

**Exercise Data**

The exercise data are given in Table 2. The duration of the bicycle exercise test varied between 6 and 14 minutes, with maximal load between 100 and 220 W. The HR increased from 67±2 bpm at rest to 110±4 bpm (*P*<0.0001) at 100 W and to 135±4 bpm (*P*<0.0001) at the maximal load (Figure 2). At 10 minutes after exercise, the HR remained increased above the preexercise level (81±3 bpm, *P*<0.0001). Systolic blood pressure increased during exercise (Table 2), whereas diastolic blood pressure remained virtually unchanged.

Progressive ST-segment depression developed in all patients during exercise (Figure 2). The mean ST-segment depression compared with the preexercise level was −223±20.2 μV (*P*<0.0001) just before the termination of exercise. The degree of ST-segment depression declined during recovery but remained significant after 10 minutes (−49±8.9 μV, *P*<0.0001). The relation between ST-segment depression and HR during and after exercise was determined (an example for 1 patient is shown in Figure 1). The ST/HR slope constructed during the final 4 minutes of exercise, which provides an estimate of myocardial ischemia at the end of exercise, was −6.0±0.77 μV/HR. The ST/HR slope tended to increase with severity of coronary artery disease from 1- to 2- and 3-vessel disease [−4.7±0.84 (n=6), −5.9±1.1 (n=12), and −7.0±1.6 (n=10) μV/HR, respectively], but the differences were not statistically significant.

In most patients, the ST-segment depression was greater in the recovery phase than during exercise at the same HR (Figure 2). This ST deficit in the recovery phase was determined at 1, 3, 5, and 10 minutes after exercise and was significant at the latter 3 time points (Figure 3). Peak deficit was recorded in the third minute (−120±16.1 μV, *P*<0.0001). Mean ST deficit during the first 10 minutes of recovery did not correlate with the ST/HR slope determined during the final 4 minutes of exercise (r=0.32, NS).

**Hormonal Response to Exercise**

Exercise was associated with a 5-fold increase in plasma NE at maximal exercise (*P*<0.0001), followed by normalization after 30 minutes (Figure 4). The change in plasma E (Figure 4) paralleled that of NE. Plasma NPY also increased during exercise. The degree of ST-segment depression declined during recovery but remained significant after 10 minutes (−49±8.9 μV, *P*<0.0001). The relation between ST-segment depression and HR during and after exercise was determined (an example for 1 patient is shown in Figure 1).
exercise, but in comparison with NE, the increase in NPY levels was delayed (Figure 4). From a resting level of 30 ± 2.4 pmol/L, plasma NPY was not significantly increased at a load of 100 W, but it had increased by 16 ± 4.6 pmol/l (P < 0.01) at peak exercise. In contrast to NE, NPY continued to increase after exercise, with a peak 4 minutes after exercise (27 ± 5.5 pmol/l above resting level). Even after 30 minutes of recovery, NPY remained elevated. As shown in Figure 4, Nt-pro-ANP increased slightly during exercise, with peak values at 4 and 10 minutes after exercise, whereas big ET remained unchanged.

The maximal increase in NPY was correlated with that of NE (r = 0.47, P = 0.01). A similar relationship was found between NPY and E (r = 0.46, P = 0.01) but not between NPY and Nt-pro-ANP or big ET. The maximal exercise-induced increases in plasma NPY and NE were significantly correlated with the maximal increase in HR during exercise (r = 0.42, P = 0.02; r = 0.52, P = 0.004, respectively). There was no relationship between the increase in plasma NPY levels and the degree of coronary artery disease.

**Relationship Between ST/HR Slope During Exercise or ST-Segment Changes During Recovery and Neurohormones**

The ST/HR slope obtained during the final 4 minutes of exercise did not correlate with any of the neurohormones except for a relationship between this variable and peak increase in pro-ANF (r = 0.41, P = 0.03).

A close relationship was found between exercise-induced increase in NPY and the time-related ST-segment depression and the HR-related ST deficit during recovery. The maximal exercise-induced increase in plasma NPY was significantly correlated with the duration of the ST-segment depression and with the ST deficit in the third and fifth minutes of recovery (Figure 5, Tables 3 and 4). A significant correlation was also found between the exercise-induced increase in NPY plasma level and the ST-segment depression, as well as the mean ST deficit during the 10-minute recovery period (Figure 5, Tables 3 and 4). In contrast, no significant correlations were found between changes in plasma NE, big ET, or Nt-pro-ANP and the ST-segment depression or the ST deficit during recovery (Tables 3 and 4). However, a weak correlation (r = -0.45) was found between the increase in plasma E and the ST deficit at 3 minutes of the recovery period (Table 4).

**Discussion**

Our main finding was that an exercise-induced increase in NPY was closely correlated with time-related ST-segment depression and HR-adjusted ST-segment depression during the recovery phase. This finding suggests that NPY may contribute to postexercise myocardial ischemia in patients with coronary artery disease.

**Analysis of Ischemia**

Two methods were used to analyze the ST segment during and after exercise in addition to time-related ST-segment changes: the exercise ST/HR slope and ST-segment/HR adjustments in the recovery period, or the “rate-recovery loop.” These methods not only may improve the diagnostic value of the exercise ECG but also may provide information about the functional severity of a coronary obstruction and the possible presence of a delayed coronary vasoconstriction in the recovery phase. The poor correlation found between ST-segment changes during exercise and recovery possibly reflects a difference in the pathophysiology of ischemia during and after exercise. In accordance with this, Rywik et al. recently demonstrated an independent prognostic value of exercise-induced ST-segment changes in the recovery period.

**Figure 3.** ST deficit during first 10 minutes of recovery. ST deficit represents difference between ST-segment depression during exercise and during recovery at corresponding HRs. Values are mean and SEM. ***P < 0.001 vs 0 μV.

**Figure 4.** Effect of exercise on venous plasma levels of various neurohormones at rest, during submaximal exercise (100 W), at peak exercise (mean load 163 ± 9 W), and during recovery 4, 10, and 30 minutes after exercise. Values are mean and SEM for 29 patients. *P < 0.05, **P < 0.01, ***P < 0.001 compared with resting values.
Ischemia During Exercise
In patients with ischemia due to obstructive coronary artery disease, the relation between ST-segment depression and HR is in general linear during stepwise increases in the load. The linear regression–based ST/HR slope has been shown to be a reliable index for the functional severity of coronary obstruction. The rationale for this relationship depends on the notion that myocardial oxygen demand is directly proportional to HR at higher workloads. HR changes during exercise, as a measure of changing oxygen demand, can therefore be used to adjust evolving ST-segment depression for an increasing workload and to provide a more accurate measure of the underlying coronary obstruction. Although not statistically significant, we found a tendency toward a more negative slope with advancing coronary artery disease.

Ischemia After Exercise
The present study was primarily focused on myocardial ischemia in the early recovery phase after exercise, as expressed by HR-adjusted ST-segment depression. In agreement with previous studies in patients with coronary artery disease, we found a greater ST-segment depression during recovery-phase HRs than at corresponding exercise HRs. As Okin et al suggested, these observations indicate that myocardial ischemia is greater relative to the HR during early recovery than during exercise. Although the size of ST-segment depression during exercise can be directly related to myocardial workload (as reflected by HR in patients with myocardial ischemia), ST-segment depression during early recovery remains greater than expected for the rapidly decreasing myocardial oxygen demand, when exercise is stopped. Thus, the relatively greater ST-segment depression during recovery, quantified as ST deficit from the exercise/recovery ST/HR plots (cf Okin et al and Herpin et al), suggests a different mechanism of ischemia. This may reflect the presence of factors that restrict oxygen supply to the ischemic region in the recovery phase.

Possible Role of NPY in Ischemia
It is well established that coronary vasoconstriction elicited by catecholamines contributes to exercise-induced myocardial ischemia in patients with angina pectoris. Thus, the intracoronary administration of α-adrenoceptor antagonists has been shown to reduce the ST/HR slope during exercise. NPY causes considerably more long-lasting coronary vasoconstriction than NE. A recent study demonstrated that NPY, released from cardiac sympathetic nerves during electrical nerve stimulation in dogs, participates in the elicitation of a long-lasting coronary vasoconstriction. This may be of interest in consideration of the maintained ischemia in the recovery phase.

The present results agree with previous reports that show plasma NPY increases during physical exercise in humans. Because NPY is co-stored with NE in perivascular sympa-
thetnic nerves and only to a minor degree in the human adrenal medulla (together with E), it is assumed that NPY during exercise is mainly released from the sympathetic nerve terminals. We found that the increases in plasma of both NE and NPY were significantly correlated with the peak increase in HR during exercise. This suggests that the increases in NPY and NE reflected the exercise-induced cardiac sympathetic nerve activation. The increase in plasma NPY, but not that of NE, correlated significantly with the magnitude and duration of both the ST-segment depression and the ST deficit during the recovery period after exercise. In consideration of the long-lasting vasoconstrictor properties of NPY, these associations may indicate that involvement of NPY in myocardial ischemia persists after exercise. This issue, however, should be further evaluated with specific NPY receptor antagonists, which are not currently available for human use.

In healthy volunteers, Morris et al. demonstrated in healthy men that the cardiac overflow of NPY, relative to that of NE, was enhanced during exercise under hypoxic conditions. It is of interest that in anesthetized pigs exposed to short-term renal ischemia, Malmström and Lundberg showed an enhanced role of neurogenically released NPY, relative to that of NE, in renal sympathetic vasoconstriction. This observation may indicate either that NPY release is preferentially enhanced compared with NE or that metabolic degradation of NPY is reduced in ischemic regions. Furthermore, there is evidence that NPY receptor-mediated vasoconstriction is augmented after ischemia. This may suggest that the role of NPY becomes more important not only in cardiac hypoxemia but also under ischemic conditions, such as in patients with coronary artery disease.

In agreement with earlier results, we found that NPY increased and decreased slower during and after exercise than NE. This difference is probably related to the fact that NPY is released mainly during high degrees of sympathetic activation but also to a slower diffusion of released NPY into the systemic circulation and to a more prolonged elimination from the blood.

NPY may affect myocardial blood flow via various mechanisms. The infusion of NPY causes a long-lasting vasoconstriction, in part as a direct effect on vascular smooth muscle cells and in part due to an amplification of the action of NE. In the coronary circulation, this effect is associated with ischemia and impaired ventricular function.

Conclusions
Exercise-induced increase in plasma NPY was significantly correlated with the duration of ST-segment depression and the ST deficit in the early recovery phase in patients with coronary artery disease. In contrast, no corresponding correlation was found between exercise-induced changes in the plasma concentrations of NE and ST-segment depression or the ST deficit in the recovery phase. The close relation between the increase in NPY and the ST deficit in recovery indicates that prolonged ischemia after exercise is associated with elevated levels of NPY in patients with angina pectoris. Thus, the present observation may indicate that NPY released from cardiac sympathetic nerves during exercise contributes to elicit coronary vasoconstriction that may maintain ischemia in the recovery phase.

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