Silent Ischemia in Unstable Angina Is Related to an Altered Cardiac Norepinephrine Handling

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Background. Inferential evidence suggests that silent ischemia might be related to sympathetic activity. Study of [3H]norepinephrine kinetics is a suitable tool to assess the regional sympathetic activity. This method was applied to investigate whether silent myocardial ischemia in unstable angina is related to and depends on cardiac sympathetic overactivity.

Methods and Results. Patients with active unstable angina were compared with patients with inactive unstable angina, stable effort angina, and controls. Silent myocardial ischemia was evaluated by three 24-hour Holter monitoring periods on alternate days, and [3H]norepinephrine kinetics was assessed under rest conditions and following the cold pressor test. Simultaneously, catecholamine concentrations were measured in the aortic, coronary sinus, and peripheral venous blood. Different than the other groups (p = 0.0013), in patients with active unstable angina, the majority of silent ischemic episodes occurred without increase in heart rate. These patients had a positive coronary sinus-aorta norepinephrine gradient, both at rest and following the cold pressor test. [3H]Norepinephrine kinetics demonstrated an increased selective cardiac spillover, both at rest and, even more, after the cold pressor test. Reduced cardiac [3H]norepinephrine extraction also was found. A significant relation was found between the number of ischemic episodes or the overall duration of silent ischemia and norepinephrine spillover, both at rest and following cold application.

Conclusions. During the acute phase of unstable angina (but not in the quiescent phase or in stable effort angina), a disorder in cardiac norepinephrine handling occurs. This results in a reflex cardiac sympathetic overactivity that plays a major role in the occurrence of silent myocardial ischemia. (Circulation 1993;87:1928-1937)

Key Words • angina • ischemia • norepinephrine

Silent myocardial ischemia frequently occurs in symptomatic patients with coronary heart disease, especially in those with unstable angina.1-5 Several hypotheses6-10 have been proposed to explain the occurrence of silent ischemia, but so far the responsible mechanisms are not clear. Silent myocardial ischemia frequently is associated with daily activities,11,12 and it can be induced by stimuli associated with sympathetic activation, such as mental or emotional stress or cigarette smoking.13-18 These observations suggest a possible role for the sympathetic nervous system in the occurrence of silent ischemia. Despite this evidence, investigations of the cardiac adrenergic system in relation to the occurrence of silent myocardial ischemia in anginal patients are still lacking. In one study, coronary sinus norepinephrine concentration was measured in patients with coronary artery disease after the cold pressor test.19 However, plasma norepinephrine is an inadequate tool for the evaluation of either the total or cardiac sympathetic activity because plasma levels depend on both the rate of release of norepinephrine and its rate of clearance from the plasma pool.20 In a recent study, cardiac norepinephrine spillover and clearance were assessed in anginal patients under resting conditions by infusing tritiated norepinephrine,21 but silent ischemia was not considered.

To investigate whether silent myocardial ischemia in anginal patients may be related to an increased level of cardiac sympathetic activity, we assessed the kinetics of tritiated norepinephrine in the heart and in the body as a whole, both under rest conditions and following sympathetic stimulation.

Methods

Patients Investigated

Forty-five patients (age range, 43-71 years; 38 men and seven women) suffering from angina pectoris were investigated. Fifteen had active unstable angina (12 men and three women; age range, 43-70 years; mean age, 60±9 years), 16 with inactive unstable angina (all men; age range, 44-71 years; mean age, 58±10 years), 14 with
stable effort angina (10 men and four women; age range, 46–70 years; mean age, 61±8 years), and nine controls (six men and three women; age range, 44–69 years; mean age, 56±5 years) were investigated. Unstable angina was defined as typical chest pain occurring at rest or on minimal effort that was associated with reversible ST segment elevation or depression of at least 0.1 mV 80 msec after the J point. All patients fulfilling the diagnostic criteria of unstable angina were considered for the study. To avoid interference due to calcium antagonists and β-blockers with the assessment of norepinephrine kinetics, we enrolled only patients with active unstable angina whose symptoms could be stabilized with nitrates alone (Braunwald22; class II B 1/2). The group of patients with inactive unstable angina was formed by patients who had a history of proved unstable angina that had occurred 8–12 weeks previously and had been free of angina for at least 4 weeks. The stable effort angina group was formed by patients with typical anginal pain during effort and no history of anginal attacks at rest, ECG-positive exercise stress test, and 20'Tl scintigraphy positive exercise stress test. Both patients with inactive unstable angina and those with stable effort angina were treated with nitrates (10 mg isosorbide dinitrate every 6 hours). None of the patients investigated, including those with active unstable angina, had taken a nitrate tablet for at least 4 hours before the norepinephrine kinetics was measured. Patients with recent (within 3 months) myocardial infarction, conduction system disease, or heart failure were not considered in the study.

The control group was composed of nine patients who were free of ischemic heart disease. They underwent cardiac catheterization, coronary angiography, and ventriculography for diagnostic purposes. Two patients were found to be affected by mild aortic stenosis (transvalvular gradient, <40 mm Hg), two had mitral prolapse, and five had atypical chest pain. All these patients had negative exercise stress tests and angiographically normal coronary arteries. Control subjects stopped or discontinued all therapy at least 1 week before the study. All patients gave written informed consent before cardiac catheterization. The four groups investigated did not differ in any clinical or demographic characteristic (Table 1).

### TABLE 1. Clinical Characteristics of the Four Study Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>Active unstable angina</th>
<th>Inactive unstable angina</th>
<th>Effort stable angina</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>6</td>
<td>12</td>
<td>16</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>9</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>1.203</td>
<td>NS</td>
</tr>
<tr>
<td>Women</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56±5</td>
<td>59±9</td>
<td>58±10</td>
<td>61±8</td>
<td>1.300</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72±2</td>
<td>66±3</td>
<td>72±3</td>
<td>74±7</td>
<td>1.846</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterolemia (mmol/L)*</td>
<td>4.4±0.2</td>
<td>5.6±0.2</td>
<td>5.5±0.3</td>
<td>5.5±0.4</td>
<td>2.04</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>73.5±2.2</td>
<td>62.1±2.4</td>
<td>65.6±3.2</td>
<td>67.2±3.6</td>
<td>1.846</td>
<td>NS</td>
</tr>
<tr>
<td>No. of stenosed vessels</td>
<td>-</td>
<td>1.8±0.6</td>
<td>1.8±0.7</td>
<td>1.7±0.7</td>
<td>0.059</td>
<td>NS</td>
</tr>
<tr>
<td>Proximal stenosis score</td>
<td>-</td>
<td>3.2±1.6</td>
<td>3.0±1.4</td>
<td>2.9±1.6</td>
<td>0.125</td>
<td>NS</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction. Values are mean±SD. *To convert cholesterol values to milligrams per deciliter, multiply by 38.67.

**Evaluation of Silent Myocardial Ischemia and Enrollment**

Silent ischemia was assessed when patients on nitrates had been free from anginal attacks for 5 days. They underwent three 24-hour three-channel Holter monitoring periods (Del Mar Avionics model 459, Irvine, Calif.) on alternate days. The number of ischemic episodes and the overall duration of ischemia were evaluated. If patients experienced anginal attacks again, they were considered ineligible for the study; thus, only patients free from anginal attacks during the monitoring period were enrolled. Only (progressive) ST segment displacements of at least 0.1 mV lasting more than 60 seconds were considered evidence of myocardial ischemia. At the end of the monitoring period, coronary angiography was performed, and [3H]norepinephrine kinetics was studied. The Holter recordings were assessed by observers who were unaware of the other patients' data and of the norepinephrine kinetics. To standardize the environmental conditions of the three groups of anginal patients—in particular, exposure to stressful stimuli, which can influence the occurrence of silent ischemic episodes—both the patients with inactive unstable angina and those with stable effort angina were hospitalized. During this period, they underwent coronary angiography and three 24-hour Holter monitoring periods, following the same procedure of the patients with active unstable angina.

**Experimental Procedure**

Patients were always investigated in the morning following an overnight fast. Tea, coffee, cigarettes, and alcohol were withheld for a minimum of 24 hours before the study. Patients were premedicated with oral diazepam (10 mg) 1 hour before the study. [3H]Norepinephrine kinetics was assessed during a period of supine rest 10 minutes after the insertion of venous and arterial catheters but before coronary angiography. Catheter positioning and the sampling of aortic, coronary sinus, and peripheral vein samples were performed according to the procedure previously described in detail. Cold pressor test was performed by immersion of a foot in ice water (0–2°C) and by simultaneous application of ice on the lateral cervical region for 120 seconds. A 7F coronary-sinus thermodilution catheter (Goodale-Lu-
bin 19 woven Dacron) was introduced into the coronary sinus through an antecubital venous sheath, and coronary sinus flow was measured by the thermodilution technique.26

During the infusion of tritiated norepinephrine, blood samples for catecholamine assays were taken before (10 minutes after the insertion of catheters), during (beginning at the 100th second), and at 4 and 10 minutes after the beginning of cold application. Routine left heart catheterization, including coronary angiography, then was performed by conventional techniques, as previously described.24 The patients were continuously monitored with ECG.

The coronary angiograms were interpreted by physicians of the hemodynamic laboratory staff who were unaware of the results of the Holter monitoring or catecholamine assays. Coronary artery lesions were classified using two indexes based on the number of diseased vessels and on the site (proximal or distal) of the lesions.27

Total and Cardiac Norepinephrine Kinetics

The norepinephrine kinetics was measured according to the technique of Esler et al.28 Tritiated norepinephrine (1.2 μCi levo-[7-3H]norepinephrine per minute; specific activity, 11–16 Ci/mmol; New England Nuclear, Boston) was infused intravenously at a constant rate. Total and cardiac norepinephrine spillover into the plasma and total and cardiac plasma norepinephrine clearance were calculated with the following equations29:

\[
\text{Whole body norepinephrine spillover} = \frac{\text{Infusion rate (dpm/min)}}{\text{Specific radioactivity of plasma (dpm/pg)}}
\]

\[
\text{ Whole body norepinephrine clearance} = \frac{\text{Infusion rate (dpm/min)}}{\text{Plasma } [^{3}\text{H}] \text{norepinephrine (dpm/mL)}}
\]

Cardiac spillover-CSPF×[(NEcs−NEa)+(NEa×NEe)]

Cardiac norepinephrine clearance=CSPF×NEe

where CSPF is coronary sinus plasma flow, NEe is fractional extraction of [3H]norepinephrine by the coronary circulation, NEcs is coronary sinus norepinephrine concentration, NEa is arterial norepinephrine concentration, and dpm is disintegrations per minute of [3H]norepinephrine.

Plasma Catecholamine Assay

Norepinephrine and epinephrine were assayed by electrochemical detection after separation by high-pressure liquid chromatography.30 Blood samples (5 mL) were drawn into cold heparinized tubes; after centrifugation, the plasma was frozen and stored at −70°C until the analysis was performed. In our laboratory, the normal ranges of plasma norepinephrine and epinephrine concentrations are 130–400 pg/mL and 25–100 pg/mL, respectively; the limit of detection was 10 pg/mL for norepinephrine and epinephrine. The intra-assay coefficient of variation was 4.3% for norepinephrine and 3.1% for epinephrine, and the interassay coefficient of variation was 7.9% for norepinephrine and 6.5% for epinephrine. The concentration of tritiated norepinephrine was determined by liquid scintillation counting after extraction with alumina.

Lactate Assay

Lactate assays were performed by enzymatic analysis using a commercial kit (Boehringer Mannheim, Germany).

Statistical Analysis

The differences among the data of the four groups were analyzed by the one-way ANOVA; multiple comparisons between the groups were performed with Scheffe's test. The different behaviors of norepinephrine coronary sinus-aorta gradient, cardiac norepinephrine spillover, and clearance in the four groups were analyzed by two-way ANOVA. Total and cardiac kinetics were further analyzed by applying the Scheffe's test to the differences between baseline values and those found during cold application. The analysis of variables recorded before and after cold application was performed by the Student's t test, except for the coronary sinus-aortic gradient; the Wilcoxon's rank sum test was used for this analysis because in patients with active unstable angina, the distribution of values was not normal. The differences among the groups of patients regarding the proportion of ischemic episodes preceded (or not) by an increase in heart rate were analyzed by applying the Kruskal-Wallis test to the ratio between the number of episodes preceded by increased heart rate and total number of episodes for each individual patient. The relation between the number and the duration of silent ischemic episodes and the baseline and the post–cold pressor test spillover was tested by linear regression analysis. Results are expressed as mean±SD.

Results

Coronary Pathoanatomy and Occurrence of Silent Myocardial Ischemia

Angiographic coronary lesions were not different among the three groups of anginal patients. In patients with active unstable angina, the number of narrowed coronary vessels was similar (index 1.8±0.6) to that of patients with stable effort angina (1.7±0.7) or inactive unstable angina (1.8±0.71) (Table 1). Also, the proximal stenosis score was not significantly different among the three groups (Table 1).

During the three 24-hour Holter-monitoring periods, the total number of ischemic episodes and the overall duration of silent ischemia were significantly higher in the group of patients with active unstable angina than in the other groups (Table 2). In these patients, 15 of 109 (14%) silent ischemic episodes were preceded by an increase in heart rate ≥10 beats per minute in the minute before the onset of ST displacement; thus, in the patients of this group, the ischemic episodes that were preceded by an increase in heart rate occurred in a significantly lower proportion compared with the patients of the other groups (F=13.340, p<0.0013 with two degrees of freedom) (Table 2). No relation was found between the frequency of silent ischemic episodes and
the severity or extent of angiographic coronary lesions, either in patients with active unstable angina ($r=0.217$, $r=0.146$) or in patients with inactive unstable ($r=0.009$, $r=-0.194$) and stable effort angina ($r=0.302$, $r=0.276$).

None of the control patients had ST segment displacement during the monitoring period.

**Norepinephrine Kinetics and the Effect of Cold Application**

The concentrations of norepinephrine in the aorta, coronary sinus, and peripheral venous blood did not differ ($F=2.627$, $F=2.160$, $F=2.014$) among the five subjects with atypical chest pain without cardiac disease, the two patients with mild aortic stenosis, or the two patients with mitral valve prolapse. Likewise, the whole body norepinephrine spillover and clearance were similar ($F=2.011$ and $F=2.124$, respectively). Therefore, these patients were considered reference control group.

Coronary blood flow and coronary vascular resistance under rest conditions were not different between the control group and the three groups of anginal patients (Table 3). Similarly, among controls, patients with effort angina, and those with inactive unstable angina, there were no differences in the baseline norepinephrine concentrations in the aorta, coronary sinus, or periph-

### Table 2. Occurrence and Severity of Silent Ischemia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Active unstable angina (n=15)</th>
<th>Inactive unstable angina (n=16)</th>
<th>Effort stable angina (n=14)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with ischemia (%)</td>
<td>15/15 (100)</td>
<td>6/16 (38)</td>
<td>5/14 (36)</td>
<td>F=3.815, $p&lt;0.04$</td>
<td></td>
</tr>
<tr>
<td>Total ischemic episodes (range)</td>
<td>109 (1–21)</td>
<td>17 (1–7)</td>
<td>12 (1–6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall duration ischemia (range)</td>
<td>856 (5–184)</td>
<td>66 (2–30)</td>
<td>47 (4–20)</td>
<td>F=3.859, $p&lt;0.04$</td>
<td></td>
</tr>
<tr>
<td>No. of ischemic episodes preceded by increased heart rate (≥10) (%)</td>
<td>15/109 (14)</td>
<td>9/17 (53)</td>
<td>8/12 (66)</td>
<td>$F=13.340^*, p&lt;0.0013$</td>
<td></td>
</tr>
</tbody>
</table>

*The different proportion of ischemic episodes not preceded by increase in heart rate was analyzed by the Kruskal-Wallis test applied to the ratio between the number of episodes preceded by increased heart rate and total number of episodes for each individual patient.

### Table 3. Effect of Cold Pressor Test on Hemodynamic Parameters (Values Obtained at the 100th Second of Cold Pressor Test)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>Active unstable angina</th>
<th>Inactive unstable angina</th>
<th>Stable effort angina</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>8.3±2.3</td>
<td>8.3±3.3</td>
<td>9.1±3.3</td>
<td>9.0±3.2</td>
<td>0.313</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>8.5±2.6</td>
<td>8.4±3.2</td>
<td>9.5±3.1</td>
<td>9.1±3.1</td>
<td>0.433</td>
<td>NS</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>80.4±17.9</td>
<td>82.5±7.8</td>
<td>85.3±11.9</td>
<td>78.7±13.6</td>
<td>0.920</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>86.7±13.3</td>
<td>94.0±11.7†</td>
<td>93.8±13.9*</td>
<td>88.8±18.6†</td>
<td>1.389</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>95.7±11.3</td>
<td>101.4±6.8</td>
<td>102.5±6.7</td>
<td>99.2±4.7</td>
<td>0.548</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>115.5±3.6</td>
<td>127.5±9.5*</td>
<td>117.2±7.2*</td>
<td>123.6±6.7*</td>
<td>0.496</td>
<td>NS</td>
</tr>
<tr>
<td>DP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>8,035±714</td>
<td>8,455±575</td>
<td>7,795±349</td>
<td>8,828±667</td>
<td>0.948</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>10,063±764</td>
<td>11,707±822*</td>
<td>10,265±669</td>
<td>11,586±748*</td>
<td>2.381</td>
<td>NS</td>
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<tr>
<td>CBF (mL/min)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>75.6±7.1</td>
<td>72.2±10.8</td>
<td>71.7±5.3</td>
<td>70.3±3.2</td>
<td>1.0298</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>109.8±13.6</td>
<td>79.6±8.1†</td>
<td>81.7±8.2</td>
<td>82.7±4.1†</td>
<td>29.13</td>
<td>0.001</td>
</tr>
<tr>
<td>CVR (mm Hg/mL/min)</td>
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<tr>
<td>Basal</td>
<td>1.26±0.25</td>
<td>1.58±0.30</td>
<td>1.53±0.36</td>
<td>1.63±0.40</td>
<td>2.39</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>1.07±0.16†</td>
<td>1.87±0.22†</td>
<td>1.82±0.29†</td>
<td>1.98±0.26†</td>
<td>28.02</td>
<td>0.001</td>
</tr>
<tr>
<td>A-CS O₂ diff (mL/100 mL)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>9.9±1.02</td>
<td>10.1±1.2</td>
<td>9.9±1.1</td>
<td>9.9±1.08</td>
<td>0.173</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>11.2±1.1 *</td>
<td>14.1±0.9†</td>
<td>12.0±0.9*</td>
<td>12.0±0.8*</td>
<td>1.625</td>
<td>NS</td>
</tr>
<tr>
<td>MVo₂ (mL/min)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>8.9±1.7</td>
<td>9.1±1.1</td>
<td>9.2±1.8</td>
<td>8.2±1.9</td>
<td>0.732</td>
<td>NS</td>
</tr>
<tr>
<td>CPT</td>
<td>12.3±1.9*</td>
<td>11.5±1.3†</td>
<td>10.9±2.1*</td>
<td>10.5±2.8†</td>
<td>1.377</td>
<td>NS</td>
</tr>
</tbody>
</table>

LVEDP, left ventricular end-diastolic pressure; CPT, cold pressor test; bpm, beats per minute; HR, heart rate; MAP, mean aortic pressure; DP, double product; CBF, coronary blood flow; CVR, coronary vascular resistance; A-CS O₂ diff, oxygen aortic coronary sinus difference; MVo₂, myocardial oxygen consumption.

*p<0.001, †p<0.01 compared with basal values (values are mean±SD).
eral venous blood ($F=0.608$ [p=NS], $F=0.654$ [p=NS], and $F=0.839$ [p=NS], respectively). In all these patients, the concentrations of norepinephrine in the coronary sinus were lower than in the aorta. In contrast, patients with active unstable angina had higher coronary sinus norepinephrine concentrations than did controls or the other groups of anginal patients ($p<0.001$ by multiple-comparison test), even though norepinephrine concentrations in the peripheral venous and aortic blood were not different from those of the other groups. Moreover, in patients with active unstable angina, norepinephrine concentration in coronary sinus blood was significantly higher than in the aorta ($p<0.001$), thus suggesting a release of norepinephrine (Figure 1).

In the three groups of anginal patients, baseline whole body norepinephrine spillover and clearance were not significantly different from those of the control group (Table 4). On the contrary, baseline cardiac norepinephrine spillover was significantly higher in patients with active unstable angina ($23.3\pm12.6$ ng/min) ($p<0.0001$), by an average of 290%, compared with patients with effort ($7.3\pm2.9$ ng/min) or inactive unstable angina ($8.5\pm15.5$ ng/min) and with controls ($6.4\pm3.7$ ng/min) (Figure 2). In patients with active unstable angina, cardiac norepinephrine spillover was elevated disproportionately compared with the total norepinephrine spillover ($p<0.0001$) (Table 4).

Cardiac norepinephrine extraction at rest was similar in the control group, in patients with stable effort angina, and in those with inactive unstable angina ($F=3.106$), but it was significantly reduced in patients with active unstable angina ($p<0.001$) (Figure 3). In this group of patients, the cardiac norepinephrine clearance was also lower than in the other groups of patients (at least $p<0.001$).

Cold application caused an increase in heart rate and aortic blood pressure in all patients without significant differences in the double product among the four groups (Table 3). Likewise, no significant differences were found among the groups following the cold pressor test regarding calculated myocardial oxygen consumption, cardiac oxygen extraction, or left ventricular end-diastolic pressure (Table 3). Changes in coronary blood flow, norepinephrine concentration, and norepinephrine kinetics induced by cold stimulation peaked during cold application; therefore, values reported refer to that time.
TABLE 4. Cardiac and Whole Body \(^3\)H Norepinephrine Kinetics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Active unstable angina</th>
<th>Inactive unstable angina</th>
<th>Stable effort angina</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac norepinephrine clearance (mL/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>48±10</td>
<td>32±6</td>
<td>43±8</td>
<td>42±5</td>
<td>12.090</td>
<td>0.001†</td>
</tr>
<tr>
<td>CPT*</td>
<td>53±7</td>
<td>35±3</td>
<td>43±9</td>
<td>44±4</td>
<td>12.660</td>
<td>0.001†</td>
</tr>
<tr>
<td>CPT-basal</td>
<td>5.2±10.9</td>
<td>3.2±4.2</td>
<td>-0.03±2.01</td>
<td>2.3±3.9</td>
<td>2.022</td>
<td>NS‡</td>
</tr>
</tbody>
</table>

| Whole body norepinephrine clearance (mL/min) |                   |                        |                          |                     |     |       |
| Basal                | 1,727±78          | 1,750±80               | 1,701±80                 | 1,743±91            | 0.494 | NS†   |
| CPT*                | 1,682±71          | 1,732±86               | 1,683±85                 | 1,722±75            | 0.611 | NS†   |
| CPT-basal           | -44.8±20.8        | -17.9±24.9             | -17.7±25.7               | -20.7±27.5          | 2.707 | NS‡   |

| Cardiac norepinephrine spillover (ng/min) |                   |                        |                          |                     |     |       |
| Basal                | 6±4               | 23±12                  | 8±3                      | 7±3                 | 17.010 | 0.001† |
| CPT*                | 7±5               | 53±20                  | 15±7                     | 11±4                | 42.800 | 0.001† |
| CPT-basal           | 1.3±2.6           | 27.9±19.6              | 7.01±6.6                 | 3.6±3.6             | 16.217 | 0.001† |

| Whole body norepinephrine spillover (ng/min) |                   |                        |                          |                     |     |       |
| Basal                | 635±63            | 672±73                 | 653±83                   | 665±53              | 0.777 | NS†   |
| CPT*                | 684±6             | 723±69                 | 713±90                   | 703±55              | 0.631 | NS†   |
| CPT-basal           | 48.5±25.68        | 48.7±17.5              | 40.8±23.6                | 38.3±17.8           | 2.779 | NS‡   |

| Ratio of cardiac to whole body spillover (%) |                   |                        |                          |                     |     |       |
| Basal                | 0.024±0.013       | 0.053±0.021            | 0.021±0.009              | 0.019±0.010         | 12.403 | 0.001† |
| CPT                  | 0.010±0.0079      | 0.072±0.026            | 0.021±0.010              | 0.014±0.0066        | 36.177 | 0.001† |

Values are mean±SD.
*Values obtained at the 100th second of cold pressor test (CPT).
CPT-basal, difference between baseline values and those found during cold application.
†Data were analyzed by ANOVA.
‡Data were analyzed by Scheffe’s test.

In the control group, coronary blood flow increased greatly from 75.6±7.1 mL/min to 109.8±13.6 mL/min (p<0.0001); although all the groups of anginal patients showed an increase in coronary blood flow with cold application (at least p<0.01), the increase was lesser than that in the control group (+34.2±7.9% versus +10.0±5%, +7.3±4.6%, and +12.4±5.9%, respectively; F=43.658 [p<0.0001] [Table 3]). Coronary vascular resistance decreased in all control patients (p<0.01), whereas it increased in anginal patients. There were no significant differences among the three groups of patients (Table 3). The positive coronary sinus-aortic norepinephrine gradient found under rest conditions in patients with active unstable angina further increased following the cold pressor test: it rose from 144±158 pg/mL at rest to 432±228 pg/mL (p<0.0001) (Figure 1); thus, the behavior of patients with unstable angina was completely different from that of the other groups (F=21.560, p<0.0001) (Figure 1).

Whole body norepinephrine spillover and clearance increased after the cold pressor test in comparison to basal conditions. There were no significant differences between controls and anginal patients (Table 4). Cardiac norepinephrine spillover markedly increased only in patients with active unstable angina (from 23.3±12.6 to 53.4±20.6 ng/min); multiple-comparison testing showed that after sympathetic stimulation, only the cardiac norepinephrine spillover of this group of patients differed significantly from the values of the other groups (p<0.001) (Figure 2). The cardiac norepinephrine clearance was not significantly affected by cold application, and the behavior of patients with active unstable angina was significantly different from that of the other groups (p=0.004) (Table 4). Likewise, the extraction of tritiated norepinephrine (Figure 3) was not modified by the cold pressor test, with the exception of the controls, in which it significantly decreased in comparison to the rest values (p<0.001), probably because of the increase in coronary blood flow.

Epinephrine concentrations in the peripheral venous, aortic, and coronary sinus blood were not significantly different among the four groups of patients (Table 5).

Lactate release in the coronary sinus following cold application occurred in six of 15 patients (40%) with active unstable angina, in two of 16 (13%) with inactive unstable angina, and in none with stable effort angina. The relative proportion of occurrence of myocardial ischemia occurring following cold application, as evaluated by the increase of lactate in coronary sinus, was significantly higher in patients with active unstable angina than in the other groups of patients (Fisher’s exact test p=0.0069). However, in only two patients with unstable angina was lactate release associated with ST segment displacement. One patient experienced anginal pain.

Silent Myocardial Ischemia and Norepinephrine Kinetics

In the 15 patients with active unstable angina, a significant correlation was found between the number of
silent ischemic episodes ($r=0.901$, $p<0.0001$) or the overall duration of ischemia ($r=0.682$, $p<0.001$) and the coronary sinus-aorta norepinephrine gradient following the cold pressor test, but not under resting conditions ($r=0.461$, $p=NS$). Most important, norepinephrine spillover was significantly related to both the number ($r=0.823$, $p<0.001$) and the overall duration of silent ischemic episodes ($r=0.834$, $p<0.001$) both in resting conditions and following sympathetic stimulation ($r=0.849$, $p<0.001$, and $r=0.857$, $p<0.001$, respectively) (Figure 4). In patients with inactive unstable or effort stable angina, no relation was found between the overall duration of silent myocardial ischemia and norepinephrine spillover or the coronary sinus-aorta norepinephrine gradient both in rest conditions ($r$ always <0.1) and following cold pressor test ($r$ always <0.1).

To evaluate whether the altered cardiac norepinephrine handling was a consequence of acute myocardial ischemia, we compared the resting cardiac [3H]norepinephrine spillover of patients from different anginal groups who had had the same number of ischemic episodes (one through seven) during the entire period of Holter monitoring (72 hours). By multiple-comparison testing, only spillover values of patients with active unstable angina significantly exceeded those of the other groups ($p<0.01$), thus indicating that the altered norepinephrine spillover was independent of an acute or immediate episode of myocardial ischemia but rather the effect of a long-term subacute condition.

**Discussion**

These results provide evidence that silent myocardial ischemia in patients with active unstable angina is closely associated, and probably due, to cardiac adrenergic dysfunction. In these patients, the study of [3H]norepinephrine kinetics demonstrates selective increase in cardiac norepinephrine spillover, both under rest conditions and, even more, in response to sympathetic stimulation induced by the cold pressor test. Moreover, at variance with a previous study,21 in these patients we also found reduced cardiac extraction of tritiated norepinephrine. This discrepancy probably is due to a difference in the selection of anginal patients because our patients seemed to be in a more acute phase of angina than those of the other study.21 Indeed, patients with unstable angina studied long after the acute phase (patients with inactive unstable angina) showed a cardiac extraction of tritiated norepinephrine that did not differ from that of controls or patients with stable effort angina.

Because in the patients with active unstable angina there was no evidence of a flow-dependent increase in norepinephrine washout, which could influence the norepinephrine cardiac extraction,31,32 its reduction probably indicates defective neuronal reuptake.20,33 However, a lower norepinephrine extraction unrelated to neuronal reuptake cannot be excluded because of perfusion by collaterals of areas of myocardium previously denervated by ischemia.

The comparison between cardiac and whole body [3H]norepinephrine kinetics in patients with active unstable angina showed a selective increase in cardiac norepinephrine spillover, in addition to reduced norepinephrine uptake. This enhanced baseline spillover may indicate an increased firing rate of the cardiac sympathetic nerves,34 although this cannot be stated with certainty because the reduced norepinephrine extraction might affect norepinephrine spillover values.35 In comparison to the other two groups of anginal patients, the enhanced cardiac norepinephrine spillover at baseline further increased disproportionately following sympathetic stimulation, even in the absence either of changes in norepinephrine extraction or of significant differences in coronary blood flow. The increased car-
diac norepinephrine spillover following sympathetic stimulation was found in all patients with active unstable angina, including those in whom cold application did not induce ischemia evaluated by lactate release. This finding makes it very unlikely that the increased cardiac norepinephrine spillover results from an ischemia-related neurotransmitter release with consequent mismatching of the nerve firing rate. Therefore, the increased norepinephrine spillover following cold application found in patients with active unstable angina appears to be evidence of reflex cardiac sympathetic overactivity.

In the patients with unstable angina, the number of silent ischemic episodes was related linearly to the cardiac norepinephrine spillover both under resting conditions and, especially, following cold application. This finding apparently is in contrast with the absence of baseline differences in heart rate, coronary resistance, and left ventricular end-diastolic pressure among patients with unstable angina, controls, and the other groups. An increased baseline norepinephrine spillover is not necessarily associated with a reduced coronary blood flow in rest conditions. The disproportionately high increase in norepinephrine spillover and the increase in coronary resistance and in frequency of myocardial ischemic episodes following cold stimulation suggest an enhanced sensitivity of the cardiac sympathetic system to different stimuli in patients with active unstable angina. The silent ischemic episodes in these patients are likely to result from the interaction between the hypersensitivity of cardiac sympathetic system and environmental stimuli rather than from the absolute increase in coronary vascular tone. The nonlinear relation of the norepinephrine concentration to the heart rate explains the high frequency (86%) of spontaneous silent ischemic episodes not preceded by an increase in heart rate.

In patients with stable effort angina who did not show enhanced cardiac norepinephrine spillover, silent myocardial

| TABLE 5. Plasma Concentrations of Epinephrine (pg/mL) in Control Subjects and in Anginal Patients |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Controls                                      | Active unstable angina                        | Inactive unstable angina                       | Stable effort angina                          |
|                                              | Aorta                                         | Aorta                                         | Aorta                                         | Aorta                                         |
|                                              | Coronary sinus                               | Coronary sinus                               | Coronary sinus                               | Coronary sinus                               |
| Basal                                         | 69±34                                        | 50±26                                        | 63±19                                        | 64±15                                        |
|                                               |                                               |                                               |                                               |                                               |
| CPT†                                          | 115±55                                       | 86±43                                        | 113±32                                       | 117±35                                       |
| 4 Minutes‡                                    | 107±50                                       | 71±28                                        | 102±30                                       | 104±36                                       |
| 10 Minutes‡                                   | 84±33                                        | 60±25                                        | 70±26                                        | 77±45                                        |

Values are mean±SD.
†At 100 seconds from the beginning of cold pressor test (CPT).
‡From the beginning of cold application.
For statistical analysis, see the text.

FIGURE 4. Plots of relation between cardiac norepinephrine (NE) spillover and the number of silent ischemic episodes (A at rest, B after cold pressor test [CPT]) or the overall duration of silent ischemia (C at rest, D after CPT) in patients with active unstable angina.
dial ischemia usually occurred in relation to a rise in heart rate and then to an increase in oxygen demand. In fact, the proportion of ischemic episodes preceded by an increase in heart rate (≥10 beats per minute) was 62% in these patients and 14% in those with active unstable angina.

In the present study, the proportion of patients with effort stable angina who experienced silent ischemic episodes during the monitoring period (36%) was smaller than usually reported in ambulatory patients. This difference probably is due to the hospitalization of our patients, which resulted in more effective protection against environmental stimuli (e.g., exposure to cold or emotional challenges) than for outpatients. The mechanisms responsible for the altered kinetics of the cardiac adrenergic system in active unstable angina remain to be clarified. The altered cardiac norepinephrine handling is not ascribable to the consequence of the severity or extent of the coronary angiographic lesions as no significant differences were found among patients with active unstable angina, stable effort angina, or inactive unstable angina with respect to their coronary pathoanatomy. The higher cardiac norepinephrine spillover does not appear to be a direct consequence of myocardial ischemia because it consistently was absent in the patients with stable effort angina and silent ischemia. Moreover, even with the same number of silent ischemic episodes, the reflex increased cardiac sympathetic overflow was detectable only in patients with active unstable angina and not in patients with inactive unstable angina or effort angina. It has to be stressed that although cardiac sympathetic activation due to local ischemia has been observed in cats, no increase in cardiac norepinephrine spillover into plasma has been found in humans during pacing-induced angina. Thus, there is no evidence that transient myocardial ischemia per se causes cardiac sympathetic activation.

In present study, only a small proportion of patients with unstable angina were investigated. For ethical reasons, we investigated only patients with angina of medium severity as anginal attacks could be controlled by nitrates alone. Thus, these patients represent only a subgroup of patients with unstable angina. Our patients had only silent ischemia without recent chest pain, although this clinical characteristic is not unusual and has been reported in the literature. Therefore, one should be cautious in generalizing the findings of present study to all patients with unstable angina.

In conclusion, during the acute phase of unstable angina, a disorder in cardiac norepinephrine handling occurs. This results in a reflex cardiac sympathetic overactivity that appears to play a major role in the occurrence of silent myocardial ischemia.

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