Plasma Norepinephrine Responses to Head-Up Tilt Are Misleading in Autonomic Failure

Ian T. Meredith, Graeme Eisenhofer, Gavin W. Lambert, Garry L. Jennings, Jane Thompson, and Murray D. Esler

The failure of plasma norepinephrine to rise during upright posture is accepted as a diagnostic sign of autonomic nervous failure in patients with postural hypotension. Our clinical experience has been that this test is misleading, with an increase in plasma norepinephrine commonly occurring. To test whether this might result from absent reflex postural venous constriction lowering cardiac output and plasma norepinephrine clearance, we measured norepinephrine plasma kinetics during recumbency and 30° head-up tilting in six patients with pure autonomic failure and eight healthy subjects. Mean arterial pressure fell by 54±8 mm Hg with head-up tilt in the patients with pure autonomic failure. The plasma norepinephrine concentration (arterial sampling) increased 73±29 pg/ml (mean difference±SED, p<0.02), solely because of a 36% reduction in the clearance of norepinephrine from plasma (0.78±0.09 l/min, p<0.0001). In normal subjects, plasma norepinephrine concentration rose by 112±20 pg/ml (p<0.001), largely because of a 24% increase in norepinephrine spillover to plasma (190±20 ng/min, p<0.005). When the postural fall in blood pressure and cardiac output in the pure autonomic failure patients was prevented by the selective vasoconstrictor dihydroergotamine (10 μg/kg i.v.), no fall in plasma clearance or rise in plasma concentration of norepinephrine occurred. Measurement of the change in plasma norepinephrine with postural stimulation in patients with orthostatic hypotension is not a reliable diagnostic test for autonomic failure because elevations can occur in the plasma concentration that are entirely attributable to reduced plasma norepinephrine clearance. (Hypertension 1992;19:628–633)

KEY WORDS • catecholamines • norepinephrine • sympathetic nervous system • hypotension, orthostatic

The plasma concentration of norepinephrine, the principal neurotransmitter of postganglionic sympathetic nerves, is often used as a measure of sympathetic nervous activity in humans and with some justification.1-3 In pure autonomic failure (PAF) (Bradbury-Eggleston syndrome), the plasma concentration of norepinephrine at rest has been found to be either within4-5 or below6-9 the normal range. It has been proposed that the plasma concentration of norepinephrine and that of its precursors and metabolites9 may serve to differentiate central from peripheral nervous disorders of autonomic failure. A common application of plasma norepinephrine measurements in the clinical assessment of PAF is as an indicator of sympathetic nervous activation during autonomic reflex testing. The failure of the plasma concentration of norepinephrine to rise during upright posture is widely accepted as a diagnostic sign of autonomic failure in patients with postural hypotension.1,6,10,11

The usefulness of measurements of the plasma concentration of norepinephrine as an indicator of overall sympathetic nervous activity is limited, however, by its dependence on both the rate at which norepinephrine diffuses away from the synaptic cleft and "spills over" into the plasma pool and the rate at which it is subsequently cleared from plasma.12-14 The plasma norepinephrine clearance rate is determined by the combined processes of neuronal and extraneuronal uptake and metabolism12-17 and is dependent on hemodynamic influences, such as regional organ blood flows and cardiac output.13,14,18 Factors such as a change in cardiac output, which alters the plasma clearance of norepinephrine, undermine the validity of the plasma norepinephrine concentration as a clinical measure of sympathetic activity.

Our clinical experience has been that the plasma norepinephrine response to head-up tilting is misleading as a diagnostic test in PAF and commonly is in conflict with compelling evidence of autonomic failure demonstrated by other tests. We wondered whether this might result from a lack of vasoconstriction in the venous capacitance vessels during upright posture in patients with PAF leading to a significant reduction in cardiac output19 and plasma norepinephrine clearance.13,14,18 We therefore sought to examine the relative contribution of whole body plasma norepinephrine clearance and spillover to the plasma concentration of norepinephrine during head-up tilting in PAF patients. Our hypothesis was that in PAF patients a significant rise in plasma norepinephrine concentration might occur in response to head-up tilting purely as a result of a fall in plasma norepinephrine clearance, in the absence

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of sympathetic nervous activation, such as to invalidate the test.

**Methods**

**Patient and Healthy Subject Selection**

Six patients with PAF and eight healthy age-matched normal subjects participated in the study, which was performed with written informed consent and the approval of the Alfred Hospital Human Research Ethics Committee. Most of the patients with PAF had a long history of symptomatic postural hypotension (7±3 years, mean±SD) and disturbance of other autonomic functions (see Table 1). None of the patients suffered from Parkinson’s disease or had clinical features of a central nervous system defect to suggest a “central” origin for their autonomic failure. No patient suffered from diabetes mellitus, amyloidosis, autoimmune disease, or carcinoma. There were no cases of dopamine-β-hydroxylase deficiency. No patient had clinical evidence of a peripheral neuropathy.

The diagnosis was confirmed in all six cases using standard noninvasive and invasive tests of autonomic reflex function10,11,21,22 (Table 1). All six patients had abnormal phase II and phase IV arterial blood pressure responses to a Valsalva maneuver, according to previously established criteria.10,11,21 The ratio of the longest R-R interval to the shortest R-R interval on electrocardiographic recording during the Valsalva maneuver, the Valsalva ratio,22,23 was abnormal in all PAF patients. In all patients, there was biochemical evidence of almost total postganglionic sympathetic denervation of the heart, based on 78% to 98% reduction in the spillover from the heart of norepinephrine, the norepinephrine precursor dihydroxyphenylalanine, and the intraneuronal metabolite dihydroxyphenylglycol.24 Normal subjects were recruited from the community by advertisement and were screened by history, examination, and routine laboratory tests.

**Study Protocol**

All participants were studied at rest in the supine position 2 hours after they had eaten a standardized light breakfast (juice and toast) containing insufficient glucose and calories to precipitate postprandial hypotension. In two patients, postprandial hypotension had been noted previously, but this did not occur during the test. Tea, coffee, and alcohol were withheld for a minimum of 12 hours before the study. Patients with PAF had ceased their medications for a minimum of 48 hours.

On the morning of the study, a 21-gauge cannula was inserted percutaneously into a brachial artery with participants under local anesthesia, and a 23-gauge peripheral intravenous line was placed for infusion of tritiated norepinephrine (0.70 μCi/min Levo-[7-3H]norepinephrine, New England Nuclear, Boston; specific activity, 14–20 Ci/mmol). During the infusion, arterial plasma was sampled at 40, 60, and 80 minutes to ensure that steady-state plasma concentrations had been established.12 Calculations of total norepinephrine spillover to plasma and total plasma clearance at rest were based on the average of the assay values from the 40-, 60-, and 80-minute arterial plasma samples.
Patients with PAF were then tilted 30° head-up on a tilt table with foot support, and arterial blood was drawn at 3, 6, 10, 15, and 20 minutes to establish the time to a new steady state for total norepinephrine spillover and clearance. A single-lead electrocardiograph (lead II), heart rate, and intra-arterial blood pressure were recorded at the same time intervals. Patients were then returned to the supine position, and repeat supine measurements were taken 15 minutes later. Healthy subjects were tilted at 30° head-up for 15 minutes.

**Dihydroergotamine administration.** In an effort to prevent the fall in cardiac output and possibly plasma clearance of norepinephrine during 30° head-up tilt, PAF patients were given 10 µg/kg dihydroergotamine mesylate (Sandoz, Australia), infused intravenously at a rate of 50 µg/min (total dose given, 450–700 µg). All measurements were then repeated after a further 15 minutes supine and again at 30° head-up tilt.

**Measurement of total body norepinephrine spillover and clearance.** At steady state during peripheral intravenous infusion of a tracer dose of tritiated norepinephrine, the total norepinephrine spillover to plasma and total plasma norepinephrine clearance can be calculated as follows:

\[
\text{Total NE spillover} = \frac{[3H] \text{NE infusion rate (dpm/min)}}{\text{plasma NE specific activity (dpm/pg)}}
\]

and

\[
\text{Total NE clearance} = \frac{[3H] \text{NE infusion rate (dpm/min)}}{\text{plasma [3H]NE concentration (dpm/ml)}}
\]

where NE is norepinephrine, dpm is disintegrations per minute of tritiated NE, and [3H]NE is tritiated norepinephrine.

**Catecholamine Assays.**

Arterial blood samples (5 ml) were transferred immediately to ice-chilled tubes containing an anticoagulant and antioxidant (EGTA plus glutathione). Plasma was separated by centrifugation at 4°C, and samples were subsequently stored at −70°C until assayed.

Endogenous norepinephrine and epinephrine plasma concentrations were measured by high-performance liquid chromatography with electrochemical detection according to our previously published method.28 Timed collection of the eluant leaving the electrochemical cell allowed fractionation of tritiated norepinephrine into scintillation vials for counting by liquid scintillation spectroscopy. The intra-assay coefficients of variation were 2.8% for endogenous norepinephrine and 3.2% for tritiated norepinephrine. The interassay coefficients of variation were 5.4% and 7.4%, respectively.

**Statistics.**

Between-group comparisons (PAF versus normal subjects) were made using one-way analysis of variance (ANOVA). Data are expressed as mean±SED calculated from the error mean squared of the respective ANOVA table.28 Supine posture, head-up tilt, and dihydroergotamine therapy were regarded as “treatments,” and within-patient comparisons were made using a two-way ANOVA.26 The incremental change in measured variables between treatments was compared between groups using one-way ANOVA. The null hypothesis was rejected at p<0.05.

**Results.**

Patients with PAF did not differ from their normal counterparts in age (63 versus 60 years, SED=2 years) or weight (73 versus 74 kg, SED=2.7 kg). Supine plasma norepinephrine concentration was 58% lower in the patients with PAF compared with the normal age-matched subjects (123 versus 298 pg/ml, respectively, SED=14 pg/ml, p<0.001; Table 2), the plasma concentration perhaps not being reduced as much as might be expected in the presence of autonomic failure because of the accompanying reduction in norepinephrine plasma clearance. Total plasma clearance of norepinephrine was 24% lower than in the healthy subjects (2.04 versus 2.70 l/min, SED=0.08 l/min, p<0.03). The total body norepinephrine spillover rate to plasma was 68% lower in the PAF patients (251 versus 797 ng/min, SED=43 ng/min, p<0.001). The supine plasma epinephrine concentration was 66% lower (p<0.007).

Head-up tilt of 30° resulted in a blood pressure fall of 80/40 mm Hg in the patients with PAF (SED=13 and 6 mm Hg for systolic and diastolic blood pressure, respectively, p<0.001), whereas in the normal subjects, the blood pressure remained substantially unchanged. Heart rate was increased by 14 beats per minute in the normal subjects, whereas no mean rise in heart rate was apparent in PAF patients. Table 3 shows the between-group comparisons in blood pressure and heart rate responses during head-up tilt.

Analysis of the time course of change of the plasma concentration of endogenous and tritiated norepinephrine was completed in four patients. This revealed that the plasma concentrations had achieved a new steady state by 15 minutes after head-up tilt (p<0.009, Figure 1). On the basis of this, all kinetic calculations were made using arterial blood samples drawn 15 minutes into each phase of testing: 30° head-up tilt, repeat baseline, dihydroergotamine treatment, and repeat head-up tilt after dihydroergotamine therapy.

The plasma norepinephrine concentration increased 73±29 pg/ml in the patients with PAF (mean difference±SED, p<0.02) and 112±20 pg/ml (p<0.001) in the normal subjects during 30° head-up tilt. Whereas the increment in plasma norepinephrine was greater in the normal subjects, four of the six patients with PAF had responses similar to the normal subjects (Figure 2). The plasma concentration of epinephrine also increased with tilting in PAF patients, by 45%, from the initial low value of 20±2 to 29±4 pg/ml (p<0.05, paired t test).

The rise in plasma norepinephrine concentration in the patients with PAF during head-up tilting was due to a 36% reduction in the clearance of norepinephrine from plasma (0.78±0.09 l/min, p<0.0001), without any increase in the rate of norepinephrine spillover, whereas in the normal subjects, the rise in plasma norepinephrine concentration was primarily due to a 24% increase in total norepinephrine spillover to plasma (190±20 ng/min, p<0.005). When the ortho-
static fall in blood pressure in the PAF patients was prevented by prior intravenous infusion of 10 μg/kg dihydroergotamine (mean fall in blood pressure, 0.2 mm Hg), the plasma norepinephrine concentration, norepinephrine spillover, and plasma clearance were unchanged by the 30° head-up tilting (Figure 3). Dihydroergotamine failed to produce the increase in plasma norepinephrine clearance with the supine posture expected of a drug that increases cardiac output.13-20 It is possible that this was an artifact attributable to the failure of the plasma titrated norepinephrine concentration to fully return to baseline after the first tilt (Figure 1).

### Discussion

The results of this study indicate that the concentration of norepinephrine in plasma provides an unreliable index of the degree of sympathetic nervous activation during postural stimulation in patients with PAF. We observed that head-up tilting in PAF patients increased the plasma concentration of the sympathetic neurotransmitter norepinephrine by 59%, without any apparent sympathetic nerve activation and increase in norepinephrine release. In four of the six subjects, the magnitude of this increase in plasma norepinephrine concentration was similar to that observed in normal subjects. In all six patients, including patient 6 from Table 1 in whom the relatively short duration of illness (2 years) would otherwise make the diagnosis of PAF uncertain, the diagnosis of PAF seems secure, despite the rise in plasma norepinephrine with tilting. All of these patients with postural hypotension exhibited biochemical evidence of almost total postganglionic sympathetic denervation of the heart, based on 78% to 98% reduction in the spillover from the heart of norepinephrine, the norepinephrine precursor dihydroxyphenylalanine, and the intraneuronal metabolite dihydroxyphenylglycol.24

The net rise in the plasma concentration of norepinephrine with tilting in PAF patients resulted solely from a reduction in the clearance rate of norepinephrine from plasma. In the healthy subjects, however, the rise in the plasma concentration was largely the result of sympathetic nervous activation and increased total norepinephrine spillover to plasma, with a much smaller reduction in the plasma clearance of norepinephrine than in PAF patients. In patients with PAF, the concentration of epinephrine in plasma also rose with tilting, by 45% from the low baseline of 20±2 pg/ml, again possibly because of a reduction in the plasma clearance with tilting.

Clearance of norepinephrine from plasma involves the combined processes of neuronal uptake into the sympathetic nerves and extraneuronal uptake and metabolism by a variety of tissues capable of catecholamine metabolism.12-18 The overall rate of norepinephrine removal from plasma is, however, in part determined by the cardiac output and regional organ blood flows.13,14,18 Factors that alter cardiac output, such as β-adrenergic blockade,13,27 congestive heart failure,26,28 and head-up tilting,14,30 typically reduce the rate of norepinephrine clearance from plasma. In the present study, we observed a 36% reduction in the plasma norepinephrine clearance rate during postural stimulation in PAF patients. This most likely occurred as a result of a substantial fall in the cardiac output consequent upon a defective or absent reflex postural vasoconstrictor response in the venous capacitance circulation.11,19 Previous work in this laboratory has demonstrated a reduction of 25% in central blood volume and cardiac output during 30° head-up tilting in PAF.20

In PAF patients, intravenous dihydroergotamine increases central blood volume and cardiac output at rest, largely because of an α-adrenergic-mediated constriction of venous capacitance vessels,20,21 and reduces the falls in central blood volume and cardiac output during 30° head-up tilting.20 When tilting was repeated after dihydroergotamine administration in our PAF patients, the fall in blood pressure was substantially reduced, reflecting the maintenance of the cardiac output, and the plasma norepinephrine clearance rate remained unchanged from recumbent levels. Under these conditions, in which the fall in plasma norepinephrine clearance was prevented, no rise in the plasma concentration of norepinephrine was found.

This is not the first time a rise in plasma norepinephrine concentration on the assumption of upright posture has been observed in PAF patients,4 but this is generally qualified as "a blunted response"72 without critical examination of its cause. The reason previous studies have not observed this rise in plasma norepinephrine concentration may be in part explained by the time at which blood samples were taken after patients assumed the upright posture, generally after 2–10 minutes.4,6,7,10 Our experience in the present study, in which we drew multiple arterial blood samples to determine the time course of the effect of tilting on plasma norepinephrine concentration and clearance, was that only after 15 minutes or so of head-up tilt was a new steady state

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**Table 2. Plasma Catecholamines and Norepinephrine Kinetics in Primary Autonomic Failure Patients and Normal Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primary autonomic failure patients (n=6)</th>
<th>Normal subjects (n=8)</th>
<th>SED</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma epinephrine concentration (pg/ml)</td>
<td>21</td>
<td>53</td>
<td>4</td>
<td>0.007</td>
</tr>
<tr>
<td>Plasma norepinephrine concentration (pg/ml)</td>
<td>123</td>
<td>298</td>
<td>14</td>
<td>0.001</td>
</tr>
<tr>
<td>Total norepinephrine clearance rate (l/min)</td>
<td>2.04</td>
<td>2.70</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>Total norepinephrine spillover rate (ng/min)</td>
<td>251</td>
<td>797</td>
<td>43</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 3. Incremental Responses to 15 Minutes of 30° Head-Up Tilting**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primary autonomic failure patients</th>
<th>Normal subjects</th>
<th>SED</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSystolic BP (mm Hg)</td>
<td>−80</td>
<td>−6</td>
<td>5</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔDiastolic BP (mm Hg)</td>
<td>−40</td>
<td>3</td>
<td>3</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔMAP (mm Hg)</td>
<td>−54</td>
<td>0</td>
<td>3</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔHeart rate (bpm)</td>
<td>2</td>
<td>14</td>
<td>1</td>
<td>0.001</td>
</tr>
</tbody>
</table>
reached for the plasma norepinephrine concentration. Blood samples taken earlier may have been misleading.

We find that the plasma norepinephrine concentration does increase in response to postural stimulation in patients with an established diagnosis of PAF, solely because of a reduction in the plasma clearance of norepinephrine, although on average less than in healthy subjects. In some patients, in the face of large reductions in blood pressure and cardiac output, the plasma norepinephrine response lies within the normal range. We conclude that an elevation of the plasma norepinephrine concentration during postural stimulation in patients with postural hypotension does not reliably exclude a diagnosis of autonomic failure.

**References**


**Figure 1.** Graphs showing time course of changes in arterial plasma tritiated norepinephrine (top panel) and endogenous norepinephrine (bottom panel) during 30° head-up tilting before and after intravenous dihydroergotamine administration (DHE).

**Figure 2.** Bar graph showing increment in arterial plasma norepinephrine concentration (Δ Plasma NE Conc.) with 30° head-up tilting in pure autonomic failure (PAF) patients and normal subjects. Individual observations are shown with a filled circle.

**Figure 3.** Bar graphs showing changes in arterial plasma norepinephrine concentration (top panel), plasma clearance of norepinephrine (middle panel), and total norepinephrine spillover rate (bottom panel) in patients with pure autonomic failure during 30° head-up tilting before and after intravenous dihydroergotamine administration (DHE). Data are shown for patients in the supine position (open bars) and after 30° head-up tilting (shaded bars). The rise in plasma norepinephrine with tilting before dihydroergotamine and the underlying fall in plasma norepinephrine clearance were abolished by the drug.


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