Tyramine-Induced Vasodilation Mediated by Dopamine Contamination
A Paradox Resolved

Giris Jacob, Alfredo Gamboa, André Diedrich, Cyndya Shibao, David Robertson, Italo Biaggioni

Abstract—We reported previously that intravenous administration of tyramine induced a paradoxical forearm vasodilation and an increase in plasma dopamine, raising the possibility that dopamine is released by or converted from tyramine in vivo. Alternatively, tyramine can be nonenzymatically oxidized into dopamine in vitro, and this contamination may be responsible for the increase in plasma dopamine and forearm vasodilation. To distinguish between these possibilities, we measured the hemodynamic and neurohumoral effects of an intravenous infusion of a specially prepared dopamine-free tyramine solution in 8 normal volunteers at a dose that increased systolic blood pressure by ∼25 mm Hg (from 107±5 to 133±5 mm Hg; P<0.001) and compared it with an equivalent dose of norepinephrine. Tyramine increased plasma norepinephrine (139±18 to 226±30 pg/mL; P<0.02), its intraneuronal metabolite dihydroxyphenylglycol (980±73 to 2245±206 pg/mL; P<0.001), and systemic vascular resistance, but not plasma dopamine or its intraneuronal metabolite dihydroxyphenylacetic acid. Tyramine and norepinephrine produced nonsignificant increases in forearm vascular resistance. We conclude that tyramine-induced forearm vasodilation reported in previous studies is explained by the presence of dopamine contamination in tyramine preparations. Intravenous administration of dopamine-free tyramine and norepinephrine produced equivalent systemic vasoconstriction. The forearm vasculature was not useful in monitoring the vasoconstrictive effects of either agent. The possibility of dopamine contamination needs to be considered when interpreting previously published studies using tyramine as a pharmacological tool to assess sympathetic function, and it must be avoided in future studies. (Hypertension. 2005;46:355-359.)

Key Words: nervous system, sympathetic ▪ blood flow ▪ catecholamines ▪ norepinephrine

Recently, we reported that systemically administered tyramine induced forearm vasodilation rather than vasoconstriction, despite eliciting an increase in norepinephrine spillover.1 This paradoxical effect of tyramine was associated with an increase in plasma dopamine, which could be responsible for the vasodilation. We hypothesized that tyramine increased plasma dopamine either by inducing its release from sympathetic nerve endings2,3 or by enzymatic (via the hepatic cytochrome P450 enzyme)4 or nonenzymatic in vivo conversion of tyramine to dopamine. Alternatively, Goldstein et al proposed that increased dopamine could be the result of contamination in the tyramine infusate.5 We did find that the tyramine solution used in our previous studies was contaminated with 0.7% to 1% of dopamine,6 but it was unclear whether this degree of contamination would explain the vasodilation and increased plasma dopamine observed during tyramine infusion.

To test these possibilities, we obtained a lyophilized tyramine preparation free of dopamine contamination and evaluated its effects on forearm and systemic hemodynamics and on plasma levels of dopamine and other catechols. We used a systemic infusion of norepinephrine as control. If tyramine induces the release of dopamine, or is converted in vivo to dopamine, then infusion of dopamine-free tyramine should still increase plasma dopamine levels and produce vasodilation.

Methods

Subjects

The study group consisted of 8 healthy subjects, 21 to 45 years of age (35±3). All investigational procedures were approved by the Vanderbilt institutional review board, and subjects gave informed consent before the study. All studies were performed at the clinical research center at Vanderbilt University. Subjects were studied while supine after fasting for ≥4 hours in a quiet, partially darkened room. Subjects restrained from food and beverages containing monoamines and caffeine for ≥24 hours.

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Hemodynamic and Biochemical Effects of Tyramine and Norepinephrine Administered Intravenously at Dose That Increased Systolic BP by 25 mm Hg

<table>
<thead>
<tr>
<th>Parameter, Units</th>
<th>Tyramine Baseline</th>
<th>Tyramine-25</th>
<th>P Value</th>
<th>NE Baseline</th>
<th>NE-25</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>107±5</td>
<td>133±5</td>
<td>0.001</td>
<td>111±5</td>
<td>137±6</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>58±3</td>
<td>64±5</td>
<td>0.006</td>
<td>60±3</td>
<td>70±4</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>74±3</td>
<td>87±3</td>
<td>0.001</td>
<td>77±3</td>
<td>92±4</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>63±3</td>
<td>58±3</td>
<td>0.008</td>
<td>67±3</td>
<td>55±3</td>
<td>0.003</td>
</tr>
<tr>
<td>FBF, mL/min per dL</td>
<td>3.08±0.2</td>
<td>3.6±0.4</td>
<td>0.4</td>
<td>3.4±0.3</td>
<td>4±0.4</td>
<td>0.18</td>
</tr>
<tr>
<td>Plasma NE, pg/mL</td>
<td>139±18</td>
<td>226±30</td>
<td>0.02</td>
<td>117±13</td>
<td>2658±700</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma Epi, pg/mL</td>
<td>8±1.5</td>
<td>27±15</td>
<td>0.2</td>
<td>15±2.5</td>
<td>17±2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Plasma DHPG, pg/mL</td>
<td>980±73</td>
<td>2245±206</td>
<td>0.001</td>
<td>1143±135</td>
<td>1000±125</td>
<td>0.09</td>
</tr>
<tr>
<td>Plasma DA, pg/mL</td>
<td>14±2.5</td>
<td>19±5</td>
<td>0.13</td>
<td>10±2</td>
<td>10±3</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma DOPA, pg/mL</td>
<td>1710±88</td>
<td>1660±92</td>
<td>0.6</td>
<td>1700±75</td>
<td>1570±90</td>
<td>0.09</td>
</tr>
<tr>
<td>Plasma DOPAC, pg/mL</td>
<td>1500±220</td>
<td>1740±300</td>
<td>0.1</td>
<td>1530±230</td>
<td>1500±210</td>
<td>0.7</td>
</tr>
<tr>
<td>DHPG/NE ratio</td>
<td>7±0.85</td>
<td>11±1.5</td>
<td>0.009</td>
<td>10±1.3</td>
<td>0.5±0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>DOPAC/DHPG ratio</td>
<td>1.57±0.26</td>
<td>0.8±0.15</td>
<td>0.001</td>
<td>1.38±0.19</td>
<td>1.6±0.25</td>
<td>0.03</td>
</tr>
</tbody>
</table>

F8B indicates forearm blood flow; NE, norepinephrine; Epi, epinephrine; DA, dopamine; DOPA, dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid.

Conversion factors to express catecholamines in mmol/L units: 169.2 for NE, 183.15 for Epi, 170.07 for DHPG, 153.1 for DA, 197.24 for DOPA, and 168.1 for DOPAC.

P values are those obtained by paired t test for the differences between baseline and drug.

Instrumentation and Measurements
Two large antecubital veins were catheterized: one for blood sampling and the other for drug infusion. Forearm blood flow was measured in the side ipsilateral to blood sampling using venous occlusion plethysmography as described previously. Basal cardiac output (CO) was measured by the acetylene rebreathing method as described previously.

Breath-by-breath gas analysis was conducted using an automated SensorMedics 2200 metabolic cart (SensorMedics). A thoracic bioimpedance measurement device (KIM4; Heine-Mann & Gregory) was used for continuous monitoring of stroke volume and to calculate relative changes in CO and systemic vascular resistance (SVR) produced during drug infusions.

Blood pressure (BP) was measured by an automated sphygmomanometer (Vital-Guard 450C; Ivi Biomedical Systems, Inc.). All signals were digitized at a sampling rate of 500 Hz using DL-720USB and Windaq Pro+ software (DATAQ Instruments) and processed with user software written in PV-Wave (Visual Numerics Inc).

Mean BP was calculated as diastolic BP+1/3 systolic BP—dias-tolic BP. The mean BP used for continuous CO determinations was derived from the integral of the finger BP waveform. Forearm vascular resistance was calculated as mean BP divided by forearm blood flow and expressed in units of mm Hg·mL−1·min−1·m−2. SVR was calculated as mean BP/CO×80 and expressed as dynes s/cm².

Effect of Drugs on Plasma Catecholamines and Hemodynamics
Subjects rested quietly for ≥30 minutes after instrumentation. Baseline measurements were then taken after a 5-minute saline infusion at 30 mL/h. Tyramine (lyophilized tyramine HCl, 80% free base, diluted in 0.9% saline) was then infused intravenously at increasing doses for 5 minutes each (0.25, 0.5, 0.75, 1.0, 1.25, and 2.0 mg/min) until a rise in systolic BP of ∼25 mm Hg was obtained. After a 45-minute rest period, baseline measurements were repeated. Then norepinephrine (norepinephrine bitartrate, 50% free base dilute in 5% dextrose in water) was then infused intravenously at increasing doses for 5 minutes each (0.5, 1.0, 2.0, 3.0, 4.0, and 8.0 μg/min) until a rise in systolic BP of ∼25 mm Hg was obtained.

Tyramine and norepinephrine were diluted immediately before each study and kept at 4°C until use. Bags and lines containing drug were covered with aluminum foil to avoid possible light-dependent oxidation. A total of 5 mL of infusate was obtained before and after the end of the infusion to measure tyramine and dopamine concentrations. These samples were handled in the same manner as blood samples for catecholamine determinations.

Plasma for catecholamines were stored at −70°C and assayed for norepinephrine, epinephrine, dopamine, dihydroxyphenylglycol (DHPG), dihydroxyphenylalanine, and dihydroxyphenylacetic acid (DOPAC) by high-performance liquid chromatography as described previously.

We confirmed that tyramine did not interfere with dopamine or other catecholamine assays.

Drugs and Statistical Analysis
Tyramine in lyophilized form was purchased from Clinalfa. Norepinephrine was obtained from our local pharmacy. Results are expressed as mean ± SEM. Paired or unpaired 2-sided t test were used for intraindividual and interindividual comparisons, respectively, as appropriate. One-way ANOVA for repeated measurements (ANOVA rm) was used to determine dose-response effect of drugs on the various parameters measured. Data were analyzed using Graph Pad Prism (version 4.03; GraphPad Software Inc). A P value <0.05 was considered statistically significant.

Results
Subjects’ mean body mass index was 26±1.5 kg m², and their weight and height were 78±7 kg and 1.72±0.035 m, respectively. The mean dose of tyramine and norepinephrine required to increase systolic BP by 25 mm Hg was 1.38±0.13 mg/min and 4.25±0.6 μg/min, respectively. Hemodynamic and neurohumoral changes are shown in the Table. No side effects were reported.

Hemodynamic Responses
Tyramine increased systolic and diastolic BP in a dose-response fashion (ANOVA rm; P<0.001 and P<0.003, respectively). Heart rate decreased by 6±1 bpm during the maximal dose (P<0.008; Table) but not in a dose-dependent fashion (ANOVA rm; P=0.6).

Forearm blood flow and forearm vascular resistance did not change significantly during intravenous infusion of tyra-
mine, despite a significant increase in mean BP and SVR (Figure 1; Table). Resting supine CO, measured by the acetylene rebreathing method, was 4.2 ± 0.5 L/min (measurements could not be obtained for technical reasons in 3 subjects). Tyramine did not alter CO determined by thoracic impedance (6.8 ± 0.6 and 6.2 ± 0.7 L/min for baseline and TYR-25, respectively), but SVR increased significantly by 30% during the TYR-25 dose (P < 0.005; Figure 1).

Systemic infusion of norepinephrine increased systolic and diastolic BP and decreased HR in a dose-dependent manner (ANOVA rm; P < 0.001, for all). The maximal decrease in HR (12 ± 2 bpm) was twice that produced by tyramine. Norepinephrine did not change forearm blood flow or local vascular resistance significantly (Figure 1; Table) but produced a significant increase in SVR (Figure 1).

Systemic infusions of tyramine and norepinephrine produced similar nonsignificant increases in forearm blood flow and forearm vascular resistance (Figure 2). For comparison purposes, we also include data reported previously1 using tyramine later found to be contaminated with dopamine. Tyramine contaminated with dopamine produced an increase in forearm blood flow and a decrease in resistance. This is in contrast to the increase in forearm vascular resistance produced by the cold pressor test (Figure 2).

Plasma Dopamine and Other Catecholamines
The concentration of tyramine in the infusate was 2500 μg/mL, and it contained 0.15 ± 0.04 μg/mL of dopamine (<0.01% contamination). Accordingly, the maximal dose of dopamine administered because of this contamination would be 0.082 μg/min. The concentrations of dopamine and tyramine at the beginning and end of the study were the same, indicating that there was no in vitro conversion of tyramine to dopamine during study period.

The effect of tyramine and norepinephrine on plasma catecholamines is depicted in the Table. Tyramine produced parallel and dose-dependent increases in plasma norepinephrine and DHPG (Figure 3). In contrast, intravenous norepinephrine did not increase plasma DHPG despite a significant increase in plasma norepinephrine (Figure 3). Plasma concentration of dopamine did not increase during tyramine or norepinephrine infusions (Figure 3). The monoamine oxidase (MAO) metabolite of dopamine DOPAC increased slightly with tyramine, but this did not reach significance (Table). The DOPAC/DHPG ratio, which reflects generation and release of dopamine intraneuronal metabolites in relation to norepinephrine metabolites, decreased significantly during tyramine infusion (Table).

Discussion
In the present study, we demonstrated that intravenous administration of a tyramine preparation, documented to be
free of dopamine contamination, does not produce the increase in plasma dopamine or forearm vasodilation reported previously.\(^1\) Therefore, these putative effects of tyramine were caused by contamination of dopamine in commonly used tyramine solutions, as suggested by Goldstein and Holmes.\(^5\) We also found that the hemodynamic effects of intravenous administration of dopamine-free tyramine were similar to those of intravenous norepinephrine; both produced an increase in SVR but did not produce a significant forearm vasoconstriction.

These observations are important because tyramine is widely used as a pharmacological tool to evaluate the role of the sympathetic nervous system on human physiology and pathology. The underlying assumption is that tyramine mimics naturally occurring sympathetic activation because it induces the nonexocytotic release of norepinephrine from catecholamine-containing vesicles in sympathetic neurons. In agreement with this assumption, we observed parallel and dose-dependent increases in plasma norepinephrine and DHPG during tyramine infusion. DHPG is an intraneuronal metabolite of norepinephrine formed by MAO.\(^10\) Of interest, tyramine induced a greater increase in DPHG compared with norepinephrine. Tyramine displaces norepinephrine from neuronal vesicles into the axoplasm, and it is likely that some of it is converted to DHPG, and only a portion reaches the circulation.

We initially proposed that the increase in plasma dopamine observed in previous studies could be explained by tyramine-induced release of dopamine from sympathetic neurons. The results presented here prove this hypothesis to be wrong. We found only a small increase in plasma dopamine, and we did not observe an increase in plasma DOPAC, the intraneuronal metabolite of dopamine. These findings do not negate the possibility that tyramine releases dopamine from sympathetic nerve terminals, as reported in the literature.\(^3\) In a recent study, perfusion of tyramine through a microdialysis catheter into the nucleus accumbens of rats induced a 100-fold increase in dopamine dialyzate levels, and this effect was greatly reduced in rats fed a diet deficient in n-3 polyunsaturated fatty acids.\(^11\) These observations suggest that tyramine can induce the release of dopamine, assuming the same tyramine preparation was used in both groups of animals. On the other hand, our results suggest that dopamine release from sympathetic neurons plays a minor role in the overall hemodynamic effect of intravenous tyramine in humans.

It is noteworthy that neither tyramine nor norepinephrine produced a significant increase in forearm vascular resistance. A similar phenomenon is seen when other vasoconstrictors are given intravenously, including angiotensin II.\(^12\) Tyramine and norepinephrine increased SVR significantly, suggesting that other vascular beds have a greater sensitivity to these vasoconstrictors than the forearm. Alternatively, the forearm may be more sensitive to the buffering effects of baroreflexes than other vascular beds. Because of its ready access and ease of measurement, forearm blood flow is frequently used as a surrogate of the effects of vasoactive agents on systemic hemodynamics. However, our observation cast doubt about the usefulness of this approach.

The dopamine-free tyramine preparation used in the present study is commercially available in lyophilized form, which ensures its stability and prevents nonenzymatic oxidation to dopamine. We observed <0.01% contamination of dopamine in tyramine solutions freshly diluted from this lyophilized form compared with ~1% contamination in our previous preparations of tyramine stored as a saline solution containing sodium metabisulfite as preservative. Care should be taken to avoid dopamine contamination when using tyramine as a pharmacological tool to mimic the effects of naturally occurring sympathetic activation. Even then, we should consider that most stimuli do not elicit a homogeneous sympathetic activation, but a heterogeneous one, and that the pattern of activation depends on the stimuli and pathways recruited. For example, the patterns of sympathetic activation produced by the cold pressor test and by tyramine infusion.
are qualitatively different; the former produces significant forearm vascular resistance, and the latter does not.\textsuperscript{1}

**Perspectives**

We found that intravenous administration of a tyramine preparation free of dopamine contamination produces the expected increases in SVR, BP, and in plasma norepinephrine and its intraneuronal metabolite DHPG but not in plasma dopamine or its intraneuronal metabolite DOPAC. Previous observations of forearm vasodilation induced by tyramine infusions can now be explained by contamination of the infusate by dopamine. Tyramine is used often in clinical studies as a pharmacological tool to assess sympathetic function. The findings reported here are important because the interpretation of previously published studies, including our own, need to reconsider in light of the fact that results may be confounded by dopamine contamination. In future studies, care should be taken in using only dopamine-free tyramine preparations.

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**References**

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