Sympathetic Nervous Function in Human Heart as Assessed by Cardiac Spillovers of Dihydroxyphenylglycol and Norepinephrine

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Background. Measurement of cardiac norepinephrine spillover may indicate the amount of transmitter at neuroeffector sites but does not distinguish neuronal release or reuptake in determining this amount or provide information about other aspects of sympathetic function. This report examines how cardiacl spillover of the norepinephrine metabolite dihydroxyphenylglycol (DHPG) provides additional distinct information about cardiac sympathetic function.

Methods and Results. Arterial and coronary venous blood samples were taken during cardiac catheterization and intravenous infusion of [3H]norepinephrine in 57 subjects. Subjects were given intravenous yohimbine or underwent mental stress, handgrip exercise, and cycling exercise to activate sympathetic nerves or were given intravenous desipramine to block norepinephrine reuptake. Cardiac DHPG spillover (601±41 pmol/min) was eightfold greater than norepinephrine spillover (78±10 pmol/min) at rest and increased during sympathetic activation by 65% of the increase of norepinephrine. This and the desipramine-sensitive cardiac production of [3H]-labeled DHPG from [3H]norepinephrine indicated that 10.5 times more endogenous norepinephrine is recaptured than escapes into plasma; that more than 90% of recaptured norepinephrine is sequestered into storage vesicles; and that under resting conditions, most cardiacl spillover of DHPG and turnover of norepinephrine are from metabolism of transmitter leaking from vesicles; the latter process is independent of exocytotic transmitter release with a rate at rest over 100-fold that of norepinephrine spillover and over 10-fold that of norepinephrine reuptake.

Conclusions. Cardiac spillover of DHPG provides information about processes close to or within sympathetic nerve endings that cannot be provided by measurements of norepinephrine spillover alone. This includes quantitative information about the role of neuronal uptake in terminating the actions of norepinephrine at neuroeffector sites and the importance of vesicular–axoplasmic exchange of norepinephrine as a dynamic process contributing to norepinephrine turnover. (Circulation 1992;85:1775-1785)

KEY WORDS • uptake, neuronal • desipramine • dihydroxyphenylalanine

Because of the importance of the sympathetic nervous system in the regulation of cardiovascular performance in stress and disease, investigators have long sought experimental approaches to assess sympathetic activity in clinical settings. The introduction of sensitive and specific techniques to measure plasma concentrations of norepinephrine, the principal neurotransmitter of sympathetic nerves, appeared to herald the end of this quest.1,2 Development of radiotracer techniques to estimate spillover of norepinephrine into plasma from specific tissues provided a further refinement to assess regional sympathetic activity in organs such as the heart.3

Norepinephrine spillover into plasma is, however, influenced by factors other than sympathetic nerve traffic, including neuronal reuptake,4 displacement of vesicular stores,5 and prejunctional modulation of transmitter release.6 Sympathetic nerve traffic can be assessed directly by electronic recording of neural burst rate and amplitude,7 but this is impractical for clinical assessment of cardiac sympathetic function.

The terms sympathetic activity and release, spillover, or turnover of norepinephrine are often used synonymously, but they actually denote quite different processes that are only partly related. Most norepinephrine released by sympathetic nerves is recaptured and then sequestered from the axoplasm back into storage vesicles.8 The contribution of exocytotic release of norepinephrine to its turnover—the depletion of previously synthesized stores of transmitter—is therefore minimized and only depends on the fraction of released norepinephrine that escapes neuronal reuptake and vesicular sequestration and is metabolized intraneuronally and ex-
traneuronally or spills over into plasma. The influences of these processes on norepinephrine turnover are weakened by the additional contribution from metabolism of norepinephrine leaking from vesicles, a process independent of exocytotic transmitter release.8-12

Recognition that there is more to assessment of sympathetic function than measurement of sympathetic activity and that measurements of nerve traffic or norepinephrine spillover provide limited information indicates a need for additional approaches to assess sympathetic nervous function.

Dihydroxyphenylglycol (DHPG) is almost exclusively produced within sympathetic nerves from metabolism of norepinephrine leaking from storage vesicles or re-captured after exocytotic release.8,12 The sympathetic nerves of the heart produce large amounts of DHPG; this is reflected by much higher concentrations of DHPG in coronary venous plasma than in arterial plasma.8 Therefore, measurement of the cardiac production of DHPG may be particularly appropriate for providing regional information about the turnover, re-uptake, and intraneuronal disposition of norepinephrine in the heart.

This report is based on data derived from cardiac catheterization studies of norepinephrine spillover at two centers. Assays of plasma DHPG were also carried out to ascertain whether cardiac spillovers of this catechol may provide additional information about sympathetic function. In some subjects, the neuronal uptake-blocking drug desipramine was administered to determine the proportion of cardiac DHPG production derived from norepinephrine recaptured by cardiac sympathetic nerves. The influence of sympathetic activation on the cardiac spillover of DHPG was examined before and during mental stress, intravenous yohimbine, isometric handgrip exercise, and cycling exercise. Cardiac spillovers of the catecholamine precursor dihydroxyphenylalanine (DOPA) and of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also examined to provide additional information about cardiac norepinephrine synthesis.

Methods

Patients

Fifty-seven patients, 41 at the Baker Medical Research Institute (BMRI), Victoria, Australia, and 16 at the National Institutes of Health (NIH), Bethesda, Md., underwent cardiac catheterization and received intravenous infusions of [3H]norepinephrine. At the BMRI, subjects included 35 normal volunteers and six patients with left ventricular heart failure. At the NIH, subjects included four patients with hypertrophic cardiomyopathy, five patients with microvascular angina diagnosed according to previously defined criteria,13 and seven with chest pain and normal coronary angiograms. All procedures were approved by the appropriate institutional review committees, and all patients gave written informed consent before studies began.

Catheterization

A coronary sinus thermodilution catheter used to measure coronary blood flow and sample coronary venous blood was inserted via a forearm vein (in patients at the BMRI) or the right internal jugular vein (in patients at the NIH) and passed using fluoroscopic guidance into the coronary sinus. In patients at the NIH, the tip of the catheter was passed distally to the great cardiac vein. Correct positioning of the catheter was confirmed by manual injection of radiopaque contrast dye. Coronary venous blood samples were obtained simultaneously with samples taken from a radial or brachial artery by using a percutaneously placed cannula. The arterial cannula was also used to monitor systemic arterial pressure. An indwelling cannula in the antecubital or brachial vein was used for infusion of [3H]norepinephrine.

Radiotracer Infusion

At the NIH, [3H]norepinephrine ([L-2,5,6-3H]norepinephrine; New England Nuclear, Boston, Mass.) was prepared for human use in 50-μCi aliquots and stored at -70°C until used. For each study, a 50-μCi aliquot was diluted in 5% dextrose and infused at 1.25 μCi/min (1.5 ml/min). At the BMRI, [3H]norepinephrine ([L-7-[3H]norepinephrine; New England Nuclear) was prepared for human use in 50–100-μCi aliquots and stored in 0.05 M acetic acid at -70°C until used. For each study, an aliquot was diluted in 0.9% saline and 1 mM ascorbic acid and infused intravenously at 0.8 μCi/min (0.2 ml/min). The stability, purity, and preparation of radiotracers at both centers has been described in greater detail elsewhere.14

General Procedure

Subjects were studied in the morning after an overnight fast, having refrained from coffee and tobacco for at least 8 hours. All studies were performed in the supine position. Samples of arterial and coronary venous blood (8–10 ml) were collected simultaneously during infusions of [3H]norepinephrine, which in some subjects lasted up to 4 hours, but which in most subjects were terminated within 3 hours. A total of 235 pairs of arterial and coronary venous blood samples were collected from the 57 subjects included in this report. Blood samples were stored on ice, and plasma was separated by centrifugation at 4°C and stored at -70°C until assayed for catechol content.

To examine for the effects of the length of the radiotracer infusion on cardiac production of [3H]DHPG, data from 203 paired arterial and coronary venous plasma samples (patients with cardiac failure or hypertrophic cardiomyopathy excluded) were partitioned into 20-minute time periods according to the time after the start of the radiotracer infusion at which samples were collected. The time of collection varied between 15 and 224 minutes after the start of the radiotracer infusion. At least two and up to eight pairs of blood samples were taken from each subject. Samples included those collected before and after the procedures described below. Some patients participated in up to three successive procedures; handgrip exercise and mental stress were carried out initially in random order, and cycling or intravenous infusion of desipramine were always carried out last. Each intervention period was preceded by a rest period of at least 15 minutes, at the end of which baseline arterial and coronary venous blood samples were taken.
Mental Arithmetic

In this procedure described in detail elsewhere,15 34 normal subjects at the BMRI performed mental arithmetic over 10 minutes by serial subtractions of a range of numbers from large numbers. Subjects were verbally encouraged to perform and were distracted by frequent interruptions. Blood samples were taken during the last minute of the procedure.

Isometric Handgrip Exercise

Fifteen normal volunteers studied at the BMRI performed isometric handgrip exercise (Harpden Hand Grip, British Indicators, UK) at 20–30% of maximum strength for as long as possible up to 10 minutes. Blood samples were taken during the last minute of the exercise, which varied between 6 and 10 minutes.

Cycling Exercise

Nine normal volunteers studied at the BMRI performed cycling exercise in the supine position for 10 minutes at 50% of their maximum work capacity, according to a procedure described previously and shown to cause marked increases in cardiac norepinephrine spill-over.16 Blood samples were taken at the end of exercise.

Yohimbine Administration

At the NIH, yohimbine was administered intravenously to four patients with hypertrophic cardiomyopathy and seven with chest pain syndromes (four with microvascular angina and three with normal coronary angiograms). Yohimbine was administered initially as a 62.5 μg/kg intravenous bolus followed by a 15-minute infusion at 0.5 μg/kg/min; after absence of ill effects to the preliminary dose, another 62.5-μg/kg bolus was given followed by a 15-minute infusion at 1 μg/kg/min. The cumulative dose has been previously established to increase plasma norepinephrine significantly.9 Blood samples were taken 15 minutes after the start of the 1 μg/kg/min infusion.

Desipramine Administration

In nine normal subjects and six patients with heart failure studied at the BMRI, desipramine was infused into a forearm vein at 10–20 μg kg⁻¹·min⁻¹. Infusions of desipramine started between 100 and 199 minutes after the start of radiotracer infusions (mean, 157 minutes). Infusions lasted 25–30 minutes, so that the total dose of desipramine administered was 0.25–0.5 mg/kg. Blood samples were taken at the end of the infusion.

Assay of Plasma Catechols and Metabolites

Catechols (norepinephrine, DHPG, DOPA, and DOPAC) were extracted from 1 ml plasma with alumina adsorption, separated by liquid chromatography according to previously described methods, and amounts were quantified by electrochemical detection.17,18 Fractions of the eluant leaving the electrochemical cell were collected into scintillation vials for measurement of [³H]-labeled catechols by liquid scintillation spectroscopy. The contributions of [³H]-labeled DHPG or norepinephrine to total plasma DHPG and norepinephrine concentrations were less than 2%; therefore, endogenous levels of catechols were not corrected for the contribution from the exogenous [³H]-labeled catechols. Intra-assay and interassay coefficients of variation for the assays at both centers have been described previously.17,18

Plasma concentrations of HVA in arterial and coronary venous plasma were estimated by using liquid chromatography with electrochemical detection according a method developed at the BMRI.19 The intra-assay coefficient of variation for this assay was 2.8% (n=8), and the interassay coefficient of variation was 8.1% (n=23).

Data Analysis

The fractional cardiac extraction of [³H]norepinephrine was calculated by the formula

\[
F_x = \frac{[³H]N_{E_v} - [³H]N_{E_a}}{[³H]N_{E_v}} (1)
\]

where [³H]N_{E_v} is the concentration of [³H]norepinephrine in arterial plasma and [³H]N_{E_a} is the concentration of [³H]norepinephrine in coronary venous plasma.

The fractional cardiac extraction of [³H]norepinephrine attributable to neuronal uptake was estimated as described previously20

\[
F_{xU_1} = F_{xTOT} - F_{xU_2} (2)
\]

where F_{xTOT} is the total fractional cardiac extraction of [³H]norepinephrine and F_{xU_2} is the fractional cardiac extraction after neuronal uptake blockade with intravenous desipramine. The equation assumes complete inhibition of neuronal uptake, whereas past studies have indicated that greater than a 90% block is unlikely to be achieved.11 However, previous findings that cardiac extractions of [³H]isoproterenol, which is not a substrate for neuronal uptake, were similar to those of [³H]norepinephrine after desipramine20 indicated that the dose of desipramine used in the present study would be sufficient for near-maximum inhibition of neuronal uptake.

The rate of cardiac extraction of [³H]norepinephrine attributable to neuronal uptake (dpm/min) was calculated according to the formula

\[
[³H]N_{E_{EXT}} = F_{xU_1} \cdot [³H]N_{E_a} \cdot PF (3)
\]

where PF is the coronary plasma flow before administration of desipramine.

Cardiac norepinephrine spillover was estimated according to the established formula³

\[
N_{E_{SP}} = (F_x \cdot N_{E_a} + (N_{E_v} - N_{E_a})) \cdot PF (4)
\]

where N_{E_a} is the concentration of norepinephrine in arterial plasma and N_{E_v} is that in coronary venous plasma.

The use of plasma flow in Equation 4, an established convention3,8,15,16,21 based on the relative inability of norepinephrine to penetrate membranes, has been validated by examination of the recovery of exogenous norepinephrine added to whole blood.19

The cardiac spillover of endogenous or [³H]-labeled DHPG into plasma was estimated using the formula

\[
DHPG_{SP} = (DHPG_v - DHPG_a) \cdot BF (5)
\]

where DHPG_a is the concentration of endogenous or [³H]-labeled DHPG in arterial plasma, DHPG_v is that in coronary venous plasma, and BF is the coronary blood flow.
Because exogenous DHPG added to whole blood and stored on ice for 2 hours distributed equally between plasma and red cell compartments,\textsuperscript{19} blood flow was used to estimate cardiac DHPG or $[^3\text{H}]$DHPG spillover. Because extraction of DHPG by the heart is negligible (<7%) compared with the arteriovenous step-up (80%),\textsuperscript{22} estimation of cardiac spillover of DHPG without correction for extraction of DHPG underestimates spillover only slightly.

The cardiac spillovers of DOPA or DOPAC into plasma were estimated according to the formula described elsewhere\textsuperscript{23}

$$D_{SP}= (D_s-D_a) \cdot PF$$

(6)

where $D_s$ is the concentration of DOPA or DOPAC in arterial plasma and $D_a$ is that in coronary venous plasma.

The specific activity of $[^3\text{H}]$DHPG released into coronary venous plasma (disintegrations per minute per picomole) was calculated from the formula

$$SA= ([^3\text{H}]\text{DHPG}_s-[^3\text{H}]\text{DHPG}_a)/(\text{DHPG}_s-\text{DHPG}_a)$$

(7)

Statistical Methods

Because of the more selective regional area of the heart studied at the NIH than at the BMRI (great cardiac vein versus coronary sinus sampling), estimated cardiac spillovers of catechols tended to be lower for patients studied at the NIH. Therefore, analysis of data did not allow direct comparisons of absolute rates of cardiac norepinephrine, DHPG, and DOPA spillover between patients studied at the two institutes. All data are expressed as mean±SEM. Statistical analysis was by paired $t$ test. Relations between variables were examined by least-squares linear regression analysis, with the significance of relations determined after transformation of Pearson’s correlation coefficients to Fisher’s $z$ coefficients. Nonnormally distributed data were transformed logarithmically before statistical analysis. Statistical significance was defined as $p<0.05$.

Results

Arterial and Coronary Venous Plasma Concentrations of Catechols and Homovanillic Acid

Coronary venous plasma concentrations of endogenous DHPG were consistently higher than arterial concentrations in each of the 235 pairs of samples from the 57 subjects and were 81±5% greater ($p<0.001$) than arterial concentrations in normal subjects at rest (Figure 1). Coronary venous plasma concentrations of DOPA and DOPAC were also higher ($p<0.001$) than arterial concentrations in normal subjects at rest, but arteriovenous increments in these two catechols were not universally observed, and the magnitude of the increments for DOPA and DOPAC (17–18% increments) were less than for DHPG (Table 1). Plasma concentrations of norepinephrine and HVA did not differ between samples taken from the coronary sinus and artery. Steady-state concentrations of $[^3\text{H}]$norepinephrine in arterial plasma (680±30 dpm/ml) were considerably and consistently higher ($p<0.001$) than those in venous plasma (127±9 dpm/ml), indicating substantial extraction of norepinephrine during its passage through the coronary circulation.

Cardiac Spillovers of Catechols

In normal subjects at rest (n=34, BMRI subjects) cardiac spillover of DHPG averaged 601±41 pmol/min, much more than the cardiac spillovers of norepinephrine, DOPA, or DOPAC (Table 2). Cardiac spillover of DHPG increased ($p<0.01$) by 35±10% during mental stress, by 31±9% after intravenous yohimbine, and by

\[
\begin{array}{ccc}
\text{Arterial} & \text{Coronary Venous} & \text{Spillover increment} \\
\text{Concentrations (nmol/l)} & \text{Concentrations (nmol/l)} & \\
\text{Norepinephrine} & 1.31±0.09 & 1.25±0.09 & -0.06±0.07 \\
\text{DHPG} (n=42) & 5.8±0.2 & 10.5±0.3 & 4.7±0.2* \\
\text{DOPA} (n=42) & 6.4±0.24 & 7.5±0.24 & 1.1±0.1* \\
\text{DOPAC} (n=35) & 10.4±0.7 & 12.4±0.7 & 2.0±0.3* \\
\text{HVA} (n=13) & 66.7±8.4 & 67.3±8.3 & 0.6±1.5 \\
\text{MHPG} (n=10) & 13.9±1.2 & 16.6±1.3 & 2.7±0.4* \\
\end{array}
\]

Results are mean±SEM. Plasma concentrations of norepinephrine, dihydroxyphenylglycol (DHPG), and dihydroxyphenylalanine (DOPA) were measured in 42 normal subjects studied at the Baker Medical Research Institute (BMRI), Victoria, Australia, and the National Institutes of Health (NIH), Bethesda, Md. In 35 of these subjects (all at the BMRI), measurements of plasma dihydroxyphenylacetic acid (DOPAC) were carried out, and in 13 of these, measurements of plasma homovanillic acid (HVA) were also carried out. Plasma concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) represent those reported in a previous study at the NIH for 10 patients with chest pain but normal coronary arteries.\textsuperscript{24} $*p<0.002$, significant arterial–venous increment.
178±38% during cycling exercise, whereas isometric exercise was without effect (Figure 2). All stimuli increased (p<0.005) cardiac norepinephrine spillover (Table 3). Percentage increases in the cardiac spillover of norepinephrine were larger (p<0.001) than those in DHPG; therefore the ratio of DHPG to norepinephrine spillover decreased during sympathetic activation. During cycling exercise, the most potent stimulus, absolute increases in cardiac DHPG spillover were two thirds those of norepinephrine, whereas percentage increases were one tenth; therefore cardiac spillover of norepinephrine approached that of DHPG.

Relations Between Cardiac Spillovers of Dihydroxyphenylglycol and Norepinephrine

In normal subjects, there was no relation between cardiac spillovers of DHPG and norepinephrine at rest, whereas there was a positive relation (r=0.71, p<0.001) during sympathetic activation (Figure 3). The increase in cardiac DHPG spillover during sympathetic activation was also positively correlated (r=0.77, p<0.001) with the increase in norepinephrine spillover. The slopes of the linear regression lines describing the relations between cardiac DHPG and norepinephrine spillovers during sympathetic activation (y=0.65x+629) or the increase in cardiac DHPG and norepinephrine spillovers (y=0.64x+74) indicated that for every 1-pmol/min increase in the rate of cardiac norepinephrine spillover, there was a 0.64–0.65-pmol/min increase in the rate of cardiac DHPG spillover.

Table 2. Cardiac Spillovers of Dihydroxyphenylglycol, Norepinephrine, Dihydroxyphenylalanine, and Dihydroxyphenylacetic Acid at Rest

<table>
<thead>
<tr>
<th>Substance</th>
<th>Spillover (pmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydroxyphenylglycol</td>
<td>601±41</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>78±10</td>
</tr>
<tr>
<td>Dihydroxyphenylalanine</td>
<td>81±9</td>
</tr>
<tr>
<td>Dihydroxyphenylacetic acid</td>
<td>156±26</td>
</tr>
</tbody>
</table>

Results represent mean±SEM spillovers (pmol/min) determined in 34 normal subjects studied at the Baker Medical Research Institute, Victoria, Australia. Multiple determinations were made at rest in each subject so that mean spillovers for the group were determined from averaged spillovers in each subject.

Table 3. Cardiac Spillovers of Dihydroxyphenylglycol and Norepinephrine Before and During Mental Arithmetic, Isometric Handgrip Exercise, Intravenous Infusion of Yohimbine, and Cycling Exercise

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dihydroxyphenylglycol Spillover (pmol/min)</th>
<th>Norepinephrine Spillover (pmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest (BMRI patients)</td>
<td>595±42</td>
<td>76±9</td>
</tr>
<tr>
<td>Mental arithmetic (n=34)</td>
<td>762±62*</td>
<td>136±18*</td>
</tr>
<tr>
<td>Rest (BMRI patients)</td>
<td>644±76</td>
<td>87±17</td>
</tr>
<tr>
<td>Handgrip exercise (n=15)</td>
<td>664±94</td>
<td>149±40*</td>
</tr>
<tr>
<td>Rest (NIH patients)</td>
<td>321±42</td>
<td>40±8</td>
</tr>
<tr>
<td>Yohimbine (i.v.) (n=11)</td>
<td>417±58*</td>
<td>84±20*</td>
</tr>
<tr>
<td>Rest (BMRI patients)</td>
<td>639±90</td>
<td>58±14</td>
</tr>
<tr>
<td>Cycling exercise (n=9)</td>
<td>1,543±154*</td>
<td>1,400±163*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BMRI, Baker Medical Research Institute, Victoria, Australia; NIH, National Institutes of Health, Bethesda, Md.

$p<0.05$, significantly different from rest.

Cardiac Production of [3H]Dihydroxyphenylglycol From [3H]Norepinephrine

Concentrations of [3H]DHPG in coronary venous plasma were higher (p<0.001) than concentrations in arterial plasma. The percentage increment (63±4%) did not change with time of the radiotracer infusion and was less (p<0.001) than that for endogenous DHPG. Steady-state plasma concentrations of [3H]norepinephrine were reached within 20 minutes after the start of the radiotracer infusion, whereas concentrations of [3H]DHPG in arterial and coronary venous plasma increased progressively (p<0.001) with time after the start of the infusion (Figure 4, upper panel). At 206 minutes after the start of the radiotracer infusion, arterial and coronary venous plasma concentrations of [3H]DHPG were 3.4-fold greater than concentrations at 20 minutes.

The time-dependent increase in plasma [3H]DHPG was associated with a significant increase (p<0.001) in the arterial-venous increment in plasma [3H]DHPG concentrations across the coronary circulation. Because there was no time-dependent change in the arterial-venous increment in the plasma concentration of endogenous

Figure 2. Graphs show percentage increases in cardiac spillovers in dihydroxyphenylglycol (DHPG) for individual subjects during mental stress (n=34), during isometric handgrip exercise (n=15), after intravenous infusion of yohimbine (n=11), and during cycling exercise (n=9).
DHPG, the specific activity of [3H]DHPG released into coronary venous plasma increased (p<0.001) with time after the start of the radiotracer infusion (Figure 4, lower panel).

The regression line describing the relation between time of infusion and the specific activity of [3H]DHPG released by the heart at normalized arterial plasma concentrations of [3H]norepinephrine of 1,000 dpm/ml had a y-intercept value of 5.1 dpm/pmol. Comparison of this value with the cardiac spillover of DHPG in normal subjects at rest (601 ± 41 pmol/min, Table 2) indicated a significant difference from values before desipramine (p<0.001).

Cardiac spillover of [3H]DHPG of 3,065 dpm/min. The normalized arterial plasma concentration of [3H]norepinephrine (1,000 dpm/ml) and the cardiac plasma flow in the same subjects (79 ± 6 ml/min) indicated a corresponding delivery of [3H]norepinephrine to the heart of 79,000 dpm/min. The desipramine-sensitive cardiac extraction of [3H]norepinephrine in normal subjects indicated that 60 ± 3% of the [3H]norepinephrine delivered was removed by neuronal uptake. Comparison of the rate of [3H]norepinephrine removal by neuronal uptake (47,400 dpm/min) with the corresponding cardiac spillover of [3H]DHPG (3,065 dpm/min) indicated that at the beginning of radiotracer infusions, 6.5% of the [3H]norepinephrine extracted by desipramine-sensitive uptake appeared in plasma as [3H]DHPG.

**Effects of Desipramine**

Desipramine administration increased (p<0.001) arterial and coronary venous steady-state plasma concentrations of [3H]norepinephrine and substantially reduced (p<0.001) the arterial–venous decrement in plasma [3H]norepinephrine across the coronary circulation (Table 4). The above changes were associated with significant decreases in arterial (p<0.02) and coronary venous (p<0.001) plasma concentrations of [3H]DHPG and the arterial–venous increment in plasma [3H]DHPG (p<0.001). Desipramine also reduced arterial (p<0.001) and coronary venous (p<0.001) plasma concentrations of endogenous DHPG as well as the arterial–venous increment in plasma DHPG across the coronary circulation (p<0.001).

Among all subjects who received desipramine, 72 ± 3% of the [3H]norepinephrine in plasma was extracted during passage through the coronary circulation before desipramine, whereas after desipramine, the extraction was substantially reduced to 21 ± 3% (p<0.001) (Figure 5, upper panel). The corresponding rate of cardiac removal of [3H]norepinephrine by desi-
pramine-sensitive uptake was 42,696±7,190 dpm/min (from Equation 3 in “Methods”).

The cardiac spillover of [3H]DHPG into plasma before desipramine was 8,796±1,243 dpm/min, whereas after desipramine, it was reduced (p<0.005) to 6,148±1,009 dpm/min (Figure 5, middle panel), the difference indicating a cardiac-pramine-sensitive cardiac spillover of [3H]DHPG into plasma of 2,648±942 dpm/min. Comparison of the desipramine-sensitive cardiac extraction of [3H]norepinephrine (42,696±7,190 dpm/min) with the desipramine-sensitive cardiac spillover of [3H]DHPG (2,648±942 dpm/min) indicated that 6.2% of the [3H]norepinephrine extracted by desipramine-sensitive uptake and not sequestered into storage vesicles appeared in plasma as [3H]DHPG. This result was close to the cardiac spillover of [3H]DHPG derived from the initial neuronal uptake of [3H]norepinephrine (6.5%) calculated in normal subjects by using the y-intercept of the relation between time of radiotracer infusion and the specific activity of [3H]DHPG produced by the heart (Figure 4, lower panel).

In contrast to the partial but significant inhibitory effect of desipramine on cardiac production of [3H]-labeled DHPG, the cardiac spillover of endogenous DHPG was not significantly different before (802±120 pmol/min) and after (690±81 pmol/min) desipramine (Figure 5, lower panel).

**Discussion**

Disordered sympathetic nervous function is associated with several diseases of the heart, but in most instances, the exact nature of the abnormality is ill defined. Measurements of cardiac norepinephrine spillover may point to an abnormality in the amount of transmitter present at myocardial neuroeffector sites but cannot distinguish altered neuronal release from a difference in removal by neuronal reuptake or provide information about other aspects of sympathetic function. The present report highlights how cardiac sympathetic function may be examined more comprehensively by combined measurements of cardiac spillovers of DHPG and norepinephrine than by the latter alone.

Consistent and substantial arterial-venous increments in plasma DHPG across the coronary circulation (Figure 1) and the eightfold greater cardiac spillover of DHPG than of norepinephrine (Table 2) illustrate the importance of local deamination as a major determinant of norepinephrine turnover. DHPG may be O-methylated to 3-methoxy-4-hydroxyphenylglycol (MHPG), the other major cardiac norepinephrine metabolite.24-27 An arterial-venous increment in plasma MHPG across the human heart, 54% that of DHPG,24 indicates as in rats, cats, and rabbits25-27 that DHPG is also the principal cardiac norepinephrine metabolite in humans. Thus, combined cardiac spillover of norepinephrine and its deaminated metabolites, most notably DHPG, better reflects local transmitter turnover than does cardiac norepinephrine spillover alone.

DHPG is produced by intraneuronal metabolism of norepinephrine after either leakage of transmitter into the axoplasm from storage vesicles or reuptake of transmitter after exocytic release; the contribution of the latter process is reflected by the decrease in DHPG production after neuronal uptake blockade.9,11 In the present study, adequacy of the dose of desipramine to inhibit norepinephrine reuptake was confirmed by the 71% decrease in cardiac [3H]norepinephrine extraction (Figure 5). The insignificant decrease in cardiac spillover of endogenous DHPG after desipramine indicates that at rest, little of the DHPG produced by the heart is from recaptured norepinephrine. Thus, the major determinant of DHPG production and norepinephrine turnover in the human heart at rest is leakage of transmitter from vesicles.

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**FIGURE 4.** Graphs show concentrations (mean±SEM) of [3H]dihydroxyphenylglycol (DHPG) in arterial (○) and coronary venous (●) plasma (upper panel) and specific activity of [3H]DHPG released into the coronary circulation (lower panel) as functions of time after the start of [3H]norepinephrine infusions. Data were derived from 203 paired samples (samples from patients with heart failure, hypertrophic cardiomyopathy, and after desipramine excluded) taken at different times after the start of the infusion and partitioned into 20-minute collection periods. Concentrations of [3H]DHPG were normalized to arterial plasma concentrations of [3H]norepinephrine of 1,000 dpm/ml. The difference in normalized arterial and coronary venous plasma concentrations was divided by the arteriovenous increment in endogenous DHPG (data not shown) to estimate the specific activity of [3H]DHPG produced by the heart. The regression line describing the relation between specific activity of [3H]DHPG and time of infusion (y=0.071x+5.1) indicated two components to cardiac [3H]DHPG production: a component (B) reflecting constant production of [3H]DHPG from [3H]norepinephrine removed into nerves and not sequestered into vesicles and a time-dependent component (A) representing production of [3H]DHPG from an accumulating pool of [3H]norepinephrine leaking from vesicles. NE, norepinephrine.
5.4-fold increase in combined DHPG and norepinephrine spillover, illustrating considerable divergence between neuronal turnover and exocytotic release of norepinephrine. Thus, compared with norepinephrine spillover, cardiac spillover of DHPG is an indirect and insensitive index of exocytotic norepinephrine release but provides other information that cannot be provided by measurements of norepinephrine, including the extensive contribution of vesicular leakage to norepinephrine turnover. This extensive contribution enables stores of norepinephrine to be maintained during sympathetic activation by percentage increases in norepinephrine synthesis much smaller than those in exocytotic transmitter release; thus, during cycling exercise, a 5.4-fold increase in the rate of norepinephrine synthesis would be sufficient to offset the depletion in cardiac transmitter stores associated with the 24-fold increase in norepinephrine spillover.

The above considerations are relevant to interpretation of measurements of norepinephrine turnover using the disappearance of cardiac tissue stores of radioactive norepinephrine. To draw valid inferences from these measurements about sympathetic activity, it is necessary to isolate the contributions of exocytotic release and vesicular leakage of norepinephrine to transmitter turnover.

In view of findings that inhibition of neuronal uptake with desipramine blocked sympathetic activation-induced increases in cardiac DHPG production in dogs and arterial plasma DHPG in humans, it appears likely that increased cardiac spillover of DHPG during mental arithmetic, intravenous yohimbine, and cycling exercise (Figure 2) in the present study was secondary to an increase in the amount of recaptured transmitter available for intraneuronal metabolism. Thus, the slope of the relation between cardiac spillovers of DHPG and norepinephrine during sympathetic activation (Figure 3) indicated that the amount of DHPG appearing in plasma from recaptured norepinephrine was 65% the amount of norepinephrine escaping local removal to spillover into plasma. This indicated that at rest, when norepinephrine spillover averaged 78±10 pmol/min, cardiac DHPG spillover derived from recaptured transmitter was 51 pmol/min. This amount represented only 8.4% of the total cardiac spillover of DHPG (601±41 pmol/min), providing an explanation for the insignificant nature of the decrease in cardiac spillover of DHPG after desipramine.

Similar to the present results (Figure 4), it was previously shown in cat and rabbit hearts perfused with [3H]norepinephrine that [3H]DHPG production increased bimodally, with an initial rapid increase followed by a slower, more sustained increase. In reserpinized hearts, in which vesicular sequestration was blocked, [3H]DHPG production showed only the initial rapid increase and was totally blocked by inhibition of neuronal uptake. Thus, it can be inferred that the sustained increase in cardiac [3H]DHPG production observed in the present study reflected labeling of cardiac sympathetic stores, resulting in a time-dependent increase in the proportion of [3H]DHPG produced from [3H]norepinephrine leaking from vesicles. Failure of neuronal uptake blockade to cause more than a 31% decrease in cardiac [3H]DHPG spillover was consistent with a remaining source of [3H]DHPG produced from
[3H]norepinephrine that had accumulated in storage vesicles during the 2.5 hours of radiotracer infusion before desipramine administration.

Comparison of the rate of [3H]norepinephrine removal by sympathetic nerves with the desipramine-sensitive cardiac spillover of [3H]DHPG indicated that only 6.2% of the norepinephrine removed by cardiac sympathetic nerves and not sequestered into vesicles appeared in plasma as DHPG. This proportion was similar to that calculated alternatively from the y-intercept of the relation between specific activity of [3H]DHPG produced by the heart and time of [3H]norepinephrine infusion (6.5%).

Because 6.2% of recaptured norepinephrine appeared in plasma as DHPG and because this amount was 65% the amount of norepinephrine escaping reuptake to spill over into plasma, the rate of norepinephrine reuptake by sympathetic nerves could be estimated to be 10.5-fold (65/6.2) greater than the cardiac spillover of norepinephrine into plasma. Thus, from the cardiac spillover of norepinephrine in normal subjects at rest (78 pmol/min), the corresponding rate of norepinephrine reuptake by cardiac sympathetic nerves was 819 pmol/min (Figure 6). This finding that only a small amount of norepinephrine released by cardiac sympathetic nerves (<9%) escaped reuptake and spilled over into plasma agrees with findings in the dog heart, in which 16-fold more norepinephrine was recaptured than escaped into plasma,8 and in the sympathetic nervous system as a whole, in which between fourfold and 10-fold more norepinephrine was recaptured than escaped into plasma.12,30–32

Because axoplasmic norepinephrine is either sequestered into vesicles or metabolized to DHPG, the proportion of recaptured norepinephrine sequestered into vesicles can be estimated by subtraction of the cardiac production rate of [3H]DHPG from the neuronal removal rate of [3H]norepinephrine. Some DHPG is metabolized to MHPG before entry into plasma; therefore, cardiac spillover of [3H]DHPG underestimates total cardiac [3H]DHPG production. In the human heart, MHPG spillover is 54% that of DHPG spillover,24 so that the cardiac spillover of DHPG could underestimate the cardiac production of DHPG by up to one third. Because the cardiac spillover of [3H]DHPG from the immediate metabolism of neurally removed [3H]norepinephrine was 6.2% of the neuronal removal rate of [3H]norepinephrine, at most 93.8% (100–6.2) and at least 90.5% (100–[6.2–1.54]) of the norepinephrine recaptured by sympathetic nerves was estimated to be sequestered into vesicles. In turn, from the corresponding maximal and minimal amounts of DHPG produced by cardiac sympathetic nerves (926 pmol/min, all MHPG from DHPG; 601 pmol/min, no MHPG from DHPG), the rate of vesicular sequestration of norepinephrine was estimated to be between 8,821 (926·0.95/9.5) and 9,092 (601· 0.93·6.2) pmol/min (Figure 6).

Sequestration of norepinephrine into vesicles or metabolism to DHPG can be regarded as processes of axoplasmic transmitter loss that must be balanced by transmitter input from neuronal reuptake or vesicular leakage. Thus, subtraction of the reuptake rate (819 pmol/min) from the sum of vesicular sequestration and DHPG production rates (9,693–9,747 pmol/min) indi-
icated that the rate of leakage of norepinephrine from storage vesicles was between 8,874 and 8,928 pmol/min (Figure 6). Alternatively, because at rest only 8.4% of the total production of DHPG was from metabolism of recaptured norepinephrine, most (91.6%) from norepinephrine leaking from vesicles, the rate of norepinephrine leakage could be estimated from the reuptake rate to be 8,931 pmol/min (91.6/8.4 = 819), a result close to that calculated above.

Norepinephrine turnover in the heart can be estimated from the summed rates of loss of norepinephrine and its metabolites into coronary venous plasma. To offset loss from cardiac spillovers of norepinephrine, DHPG, and MHPG, synthesis of norepinephrine in normal subjects at rest was estimated to be 1,004 pmol/min (Figure 6). However, because the contribution to turnover from extraneuronal uptake and metabolism to normetanephrine was not included, this estimate represents the minimal rate of synthesis required to maintain cardiac transmitter stores. A previous study using an open-chest anesthetized dog heart preparation indicated that the contribution of extraneuronal uptake and metabolism to norepinephrine turnover was similar to the contribution of norepinephrine spillover.33

To estimate the rate of synthesis of dopamine, the cardiac spillovers of dopamine and its metabolites DOPAC and HVA must be considered. Cardiac spillover of HVA could not be detected; that of dopamine is negligibly small, but that of DOPAC indicated that less than 13% of the dopamine produced in the heart escaped vesicular sequestration and further metabolism to norepinephrine (Figure 6). Thus, similar to norepinephrine, vesicular sequestration of dopamine is a highly efficient process, responsible for removing over 87% of the dopamine in the axoplasm. Further comparison of the rate of cardiac DOPA spillover (81 pmol/min) with the minimum rate of dopamine synthesis (1,160 pmol/min) indicated that less than 6.5% of the DOPA produced in the heart by the action of tyrosine hydroxylase escaped further metabolism to spill over into plasma.

Summary

The present report shows how combined measurements of cardiac spillovers of DHPG and norepinephrine allow examination of processes close to or within the sympathetic nerve ending. In particular, the present results have shown that vesicular–axoplasmic exchange of norepinephrine is a dynamic process, contributing importantly to norepinephrine turnover, with a rate at rest of over 100-fold more than that of norepinephrine spillover and over 10-fold more than that of norepinephrine reuptake.

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