Neuronal Reuptake of Norepinephrine and Production of Dihydroxyphenylglycol by Cardiac Sympathetic Nerves in the Anesthetized Dog

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Background. Reuptake of norepinephrine by cardiac sympathetic nerves before and during two levels of electrical stimulation of the left ansa subclavia was estimated in anesthetized dogs from the cardiac production of dihydroxyphenylglycol (DHPG), the intraneuronal metabolite of norepinephrine.

Methods and Results. The method depended on the effects of neuronal uptake blockade with desipramine on the cardiac production of [3H]DHPG from intravenously infused [3H]norepinephrine. The ratio of the desipramine-induced decrease in the cardiac extraction of [3H]norepinephrine to the production of [3H]DHPG was used to transform the cardiac production of DHPG from recaptured norepinephrine into a rate for norepinephrine reuptake. Cardiac spillover of norepinephrine into plasma increased from 49±12 to 205±40 and 451±118 pmol/min during sympathetic activation. Cardiac DHPG production increased from 108±18 to 166±34 and 240±47 pmol/min. Desipramine decreased resting cardiac DHPG production by 20% and completely blocked the stimulation-induced increase. Thus, most (80%) cardiac DHPG produced at rest was derived from norepinephrine leaking from storage vesicles. This amount remained constant, and that derived from recaptured norepinephrine increased during sympathetic activation. The cardiac extraction of [3H]norepinephrine (126,000 dpm/min) and production of [3H]DHPG (3,790 dpm/min) were decreased by 55–57% after desipramine. Thus, only 3% of the norepinephrine recaptured by cardiac sympathetic nerves appeared in plasma as DHPG. The remainder was sequestered into storage vesicles (more than 94%) or ultimately formed metabolites other than DHPG (less than 3%). Reuptake of norepinephrine by cardiac sympathetic nerves was 1,188±476 pmol/min and increased in parallel with cardiac norepinephrine spillover to 4,182±1,982 and 6,594±2,241 pmol/min during sympathetic stimulation.

Conclusions. Of the norepinephrine released by cardiac sympathetic nerves, 16-fold more was recaptured than entered plasma. Combined estimation of norepinephrine reuptake and spillover offers an approach to assess the efficiency of neuronal reuptake in disorders of cardiac function. (Circulation 1991;84:1354–1363)

Findings that the heart extracted a large percentage of perfused norepinephrine and that most of this was into sympathetic nerves have illustrated the importance of neuronal reuptake for inactivating norepinephrine within the cardiac neuroeffector junction. Reduced efficiency of neuronal reuptake may be responsible for increased cardiac intrasynaptic concentrations of norepinephrine in disorders of cardiac function such as heart failure and hypertrophic cardiomyopathy. The resulting increase in norepinephrine spillover into the coronary circulation could occur independent of any increase in norepinephrine release by cardiac sympathetic nerves. Thus, development of methodology with which to assess the efficiency of cardiac norepinephrine reuptake may be particularly appropriate to identify the basis of any involvement of the sympathetic nerves in disorders of cardiac function.

Available methodology for the examination of cardiac neuronal uptake of norepinephrine in vivo has been in large part limited to assessment of the uptake of circulating radiolabeled norepinephrine. How-
ever, neuronal uptake of norepinephrine from the synaptic cleft is more efficient than from plasma. Furthermore, extraneuronal uptake operates in series downstream from neuronal uptake and the synaptic cleft so that norepinephrine released into the cleft mainly undergoes neuronal uptake, whereas extraneuronal uptake tends to be more important for metabolism of circulating norepinephrine. To confound matters, the extraction of circulating catecholamines is dependent on both the density of innervation and hemodynamic factors (e.g., blood flow and arteriovenous shunts) that may influence the perfusion of tissues. Thus, assessment of the neuronal extraction of circulating \([H] \text{norepinephrine}\) does not reliably reflect the efficiency of reuptake of the endogenously released neurotransmitter. Ideally, this would be best assessed by the ratio of the amount of neurotransmitter recaptured to that released by sympathetic nerves. Cousineau, Rose, and colleagues devised a method of estimating norepinephrine release into and reuptake from the cardiac interstitial space. However, the method is limited by its complexity and requirement for a bolus injection of pharmacologically active amounts of \([H] \text{norepinephrine}\) into the coronary artery.

Findings that some of the norepinephrine recaptured by sympathetic nerves is metabolized to dihydroxyphenylglycol (DHPG) have provided the basis for an alternative method of estimating norepinephrine reuptake in vivo. This methodology requires the intravenous infusion of \([H] \text{norepinephrine}\) and examination of the effects of neuronal uptake blockade with desipramine on the neuronal extraction of \([H] \text{norepinephrine}\) and resulting production of its labeled DHPG metabolite. The amount of \([H] \text{norepinephrine}\) removed by neuronal uptake as a ratio of the amount of \([H] \text{DHPG}\) appearing in plasma provides a factor with which to transform the amount of endogenous DHPG derived from recaptured norepinephrine into a rate for norepinephrine reuptake. To date, this methodology has been limited to estimates of norepinephrine reuptake for the entire body, but the considerable arteriovenous step-up in plasma DHPG across the coronary circulation suggests that the method may be particularly suitable for estimation of norepinephrine reuptake by cardiac sympathetic nerves. In the present study, we used an anesthetized dog preparation to establish the usefulness of DHPG production by the heart for estimation of norepinephrine reuptake by cardiac sympathetic nerves.

**Methods**

**Animal Preparation**

Eight mongrel dogs (18–24 kg) of either sex were used in studies approved by the Baker Institute Animal Ethics Committee and carried out in accord with the guidelines set by the National Health and Medical Research Council of Australia. Animals were anesthetized using an intravenous infusion of ketamine (2 mg/kg·hr) and \(\alpha\)-chloralose (50 mg/kg·hr) and ventilated with room air supplemented with oxygen using a large animal respirator (Harvard Apparatus, South Natick, Mass.). Expired \(\text{CO}_2\) was monitored with a Normocap \(\text{CO}_2\) monitor (Datex, Finland) and maintained between 35 and 40 mm Hg. Blood gases were measured using a 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Italy), and any base deficits were corrected by intravenous injection of sterile sodium bicarbonate solution. Body temperature was maintained between 38°C and 40°C with a heated operating table.

The heart was exposed through a thoracotomy in the fourth left intercostal space. Two Teflon cannulas were inserted into the aorta and connected to polyvinyl catheters (i.d., 1 mm; o.d., 2 mm). Left and right vagosympathetic trunks were exposed in the neck through a midline incision and cut immediately before commencement of the experimental protocol. The right side of the heart was then exposed through a second thoracotomy in the fifth right intercostal space. Animals were heparinized (300 units/kg followed by 100 units/kg·hr), and coronary sinus outflow was diverted to the right atrium via an extracorporeal circuit consisting of a 16F cannula inserted into the coronary sinus orifice connected to a 20F catheter inserted into the right atrial appendage. The circuit contained a sampling port and 3-mm in-line electromagnetic flow probe (In Vivo Metric, Calif.) connected to a pulsed-logic flowmeter (model BL-610, Biotronex Laboratory, Md.). A catheter pressure transducer (model MPC-500, Millar Instruments, Houston, Tex.) was passed transmurally into the left ventricle for measurement of pressure and stroke work and calculation of \(dP/dt_{max}\), the maximal rate of rise of left ventricular pressure. The left stellate ganglion was then exposed and decentralized. The two limbs of the left ansa subclavia were retracted from surrounding tissue with an insulated bipolar platinum electrode.

**Experimental Protocol**

After surgical preparation was completed, \([H] \text{norepinephrine}\) (levo-[\(^H\)2,5,6]-norepinephrine, 42.1 Ci/mmol, New England Nuclear, Boston) was diluted with 0.9% saline to a strength suitable for infusion into a foreleg vein at a rate of 90 nCi/kg·min and 0.318 ml/min using a syringe pump (Harvard Apparatus). The radiotracer was stored with 100 \(\mu\)mol/l sodium ascorbate and 20 mmol/l acetic acid to minimize degradation before and after dilution for infusion. A sample of the infusate was stored at \(-80\)°C for estimation of radiochemical purity and assay of \([H] \text{norepinephrine}\) tritium content.

Baseline measurements of coronary blood flow and \(dP/dt_{max}\) were made. Blood samples (5 ml) were taken simultaneously from the aorta and coronary sinus 40–45 minutes after the start of the radiotracer infusion. The heparinized blood was immediately centrifuged to separate blood cells from plasma, which was stored at \(-80\)°C for assay of plasma catechols. All subsequently collected samples were treated similarly.
After baseline samples were taken, the left ansa subclavia was stimulated using a model SD9 stimulator (Grass Instruments, Quincy, Mass.) at two consecutive frequencies applied for 6 minutes each and titrated to cause 50% and 100% increases in dP/dt max. Frequencies of stimulation for each animal between 0.5 and 2 Hz for the 50% increase and between 2 and 10 Hz for the 100% increase were established before the radiotracer infusion began. Pulses were of 2-msec duration and applied at supramaximal voltage (between 12 and 24 V). Physiological measurements were made and blood samples were taken at the end of each 6-minute period of stimulation.

Desipramine HCl (Sigma, St. Louis, Mo.) was injected intravenously as a bolus (2 mg/kg salt) after sympathetic stimulation. Blood samples were taken and measurements of coronary blood flow and dP/dt max were made 30 minutes later. Cardiac sympathetic stimulation, blood sampling, and physiological measurements were repeated, after which the infusion of [3H]-labeled catecholamines was stopped.

Twenty minutes after the radiotracer infusion was stopped, the ansa subclavia was stimulated for 6 minutes at the highest frequency to determine whether cardiac release of [3H]norepinephrine could be detected. Coronary blood flow was measured and blood samples were collected at the end of the stimulation period.

Animals were then killed with sodium pentobarbital (150 mg/kg), and samples (40–60 mg wet wt) of tissue were immediately taken from the apical, mid, and basal regions of the left ventricle, the apical and basal regions of the right ventricle, and the right and left atria. Tissue samples were snap-frozen in liquid nitrogen, weighed, and then homogenized in 1 ml of 0.4 M perchloric acid containing 100 μmol/l EDTA and 100 μmol/l sodium ascorbate. Samples were centrifuged, and the supernatant was stored at −80°C until assayed for catecholamines.

**Liquid Chromatographic Assay of Catechols**

Plasma samples (1 ml), tissue supernatants (100 μl), and infusates (5 μl) were analyzed for concentrations of norepinephrine and DHPG by liquid chromatography with electrochemical detection based on a previously established method.18 Timed collections of the eluant leaving the electrochemical cell allowed fractionation of [3H]norepinephrine and [3H]DHPG into scintillation vials for counting by liquid scintillation spectroscopy. Plasma concentrations of [3H]-labeled and total (endogenous) norepinephrine and DHPG were corrected for loss during extraction using the recovery of a known amount of dihydroxybenzylamine internal standard added to plasma samples before extraction. Recovery of DHPG was 10–15% lower than that of the internal standard; an additional correction for the lower recoveries of DHPG was made using the recovery of an external DHPG standard. Intra-assay coefficients of variation determined from six repeated determinations of a single quality control plasma sample were 2.8% for norepinephrine, 3.7% for DHPG, 3.2% for [3H]norepinephrine, and 7.6% for [3H]DHPG. Interassay coefficients of variation determined from 16 consecutive extractions and assay runs during the study period were 2.7% for norepinephrine, 9.4% for DHPG, 4.3% for [3H]norepinephrine, and 10.9% for [3H]DHPG.

**Data Analysis**

The fractional extraction of [3H]norepinephrine ([3H]NE) across the heart was calculated according to the following formula:

\[ F_x = \left( \frac{[3H]NE_a - [3H]NE_v}{[3H]NE_a} \right) \]  

(1)

where [3H]NE a is the arterial plasma concentration of [3H]norepinephrine, and [3H]NE v is the concentration of [3H]norepinephrine in coronary sinus plasma.

The cardiac spillover of norepinephrine into plasma (pmol/min) was estimated according to the following formula, which has been described previously19:

\[ SP = \left( \frac{(F_x \cdot NE_a) + (NE_v - NE_a)}{PF} \right) \]  

(2)

where Fx is defined in Equation 1, NE a is the arterial plasma concentration of norepinephrine, NE v is the norepinephrine concentration in coronary sinus plasma, and PF is the coronary plasma flow.

The rate of uptake of [3H]norepinephrine into cardiac sympathetic nerves before desipramine (dpm/min) was estimated according to the following formula:

\[ [3H]NE_U = (F_x - F_{x0}) \cdot [3H]NE_a \cdot PF \]  

(3)

where Fx is the fractional extraction of [3H]norepinephrine across the heart before desipramine, F x0 is that after desipramine, [3H]NE a is the arterial plasma concentration of [3H]norepinephrine before desipramine, and PF is the plasma flow before desipramine.

The cardiac production of [3H]DHPG (dpm/ml) or endogenous DHPG (pmol/min) was estimated according to the following formula:

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

(4)

where DHPG a is the concentration of endogenous DHPG or [3H]DHPG in coronary sinus plasma, DHPG v is that in arterial plasma, and BF is blood flow. Previous studies in humans showed that intravenously infused DHPG was not extracted across the coronary circulation,20 indicating that examination of a possible cardiac extraction of DHPG was unlikely to be necessary for estimation of the cardiac production of DHPG in the present study.

Blood flow rather than plasma flow was used to estimate the cardiac production of DHPG (see Equation 4) based on results showing that exogenous DHPG added to whole blood and stored on ice for 1 or more hours was distributed equally between plasma and blood cell compartments. The use of plasma flow to estimate the cardiac spillover of norepinephrine (a standard practice for this purpose14,19) would have been less appropriate. The contribution of norepinephrine added to whole blood to the endothelial release of DHPG was small, however, because the norepinephrine concentration of this solution was very low (100 pM).

**Results**

The fractional extraction of [3H]norepinephrine ([3H]NE) across the heart was calculated according to the following formula:

\[ F_x = \left( \frac{[3H]NE_a - [3H]NE_v}{[3H]NE_a} \right) \]  

(1)

where [3H]NE a is the arterial plasma concentration of [3H]norepinephrine, and [3H]NE v is the concentration of [3H]norepinephrine in coronary sinus plasma.

The cardiac spillover of norepinephrine into plasma (pmol/min) was estimated according to the following formula, which has been described previously19:

\[ SP = \left( \frac{(F_x \cdot NE_a) + (NE_v - NE_a)}{PF} \right) \]  

(2)

where Fx is defined in Equation 1, NE a is the arterial plasma concentration of norepinephrine, NE v is the norepinephrine concentration in coronary sinus plasma, and PF is the coronary plasma flow.

The rate of uptake of [3H]norepinephrine into cardiac sympathetic nerves before desipramine (dpm/min) was estimated according to the following formula:

\[ [3H]NE_U = (F_x - F_{x0}) \cdot [3H]NE_a \cdot PF \]  

(3)

where Fx is the fractional extraction of [3H]norepinephrine across the heart before desipramine, F x0 is that after desipramine, [3H]NE a is the arterial plasma concentration of [3H]norepinephrine before desipramine, and PF is the plasma flow before desipramine.

The cardiac production of [3H]DHPG (dpm/ml) or endogenous DHPG (pmol/min) was estimated according to the following formula:

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

(4)

where DHPG a is the concentration of endogenous DHPG or [3H]DHPG in coronary sinus plasma, DHPG v is that in arterial plasma, and BF is blood flow. Previous studies in humans showed that intravenously infused DHPG was not extracted across the coronary circulation,20 indicating that examination of a possible cardiac extraction of DHPG was unlikely to be necessary for estimation of the cardiac production of DHPG in the present study.

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epinephrine uptake by nerves (see Equation 3) was validated by showing that 90% of exogenous norepinephrine added to whole blood was recovered in the plasma fraction.

The cardiac production of [3H]DHPG from [3H]norepinephrine removed by neuronal uptake but not sequestered into storage vesicles (dpm/min) or the cardiac production of endogenous DHPG from recaptured norepinephrine (pmol/min) was estimated according to the following formula:

\[ \text{DHPGprod} = \text{DHPGprod} - \text{DHPGprodDMI} \] (5)

where DHPGprod is the cardiac production of endogenous DHPG at the appropriate time point before desipramine or the cardiac production of [3H]DHPG immediately before desipramine, and DHPGprodDMI is the mean cardiac production of endogenous or [3H]-labeled DHPG after desipramine.

The rate of norepinephrine reuptake by cardiac sympathetic nerves was estimated from the following formula, which was based on that described for the entire body:15

\[ \text{NE reuptake} = \text{DHPGprod}_U \cdot \frac{[3H]\text{NEu}}{[3H]\text{DHPGprod}_U} \] (6)

where DHPGprodU is the cardiac production of DHPG from recaptured norepinephrine defined in Equation 5, [3H]NEu is the rate of uptake of [3H]norepinephrine into cardiac sympathetic nerves as defined in Equation 3, and [3H]DHPGprodU is the cardiac production of [3H]DHPG from [3H]norepinephrine removed by neuronal uptake but not sequestered into storage vesicles, as defined in Equation 5.

Statistical Methods

All data are expressed as mean±SEM. Statistical analysis was by one- or two-factor repeated analysis of variance. Post hoc tests were carried out using Dunnett’s method.21 All data were logarithmically transformed before statistical analysis. Relations between variables were examined by least-squares linear regression analysis. Statistical significance was defined as a probability of less than 0.05 unless otherwise indicated.

Results

Cardiac DHPG Production and Norepinephrine Spillover

The concentration of DHPG in coronary sinus plasma before sympathetic stimulation was 4.8±0.6 nmol/l, 67% more than the concentration in arterial plasma (2.9±0.4 nmol/l). During sympathetic stimulation, the arterial–coronary sinus step-up in plasma DHPG increased \( F=9.9, p<0.003 \) so that during the highest level of stimulation, DHPG was 102% more in coronary sinus plasma than in arterial plasma.

Cardiac DHPG production at rest was 108±18 pmol/min and increased \( F=18.1, p<0.001 \) by 54% and 122% during the two successive periods of sympathetic stimulation before intravenous desipramine (Figure 1). After intravenous desipramine, cardiac DHPG production at rest was 84±14 pmol/min. Desipramine blocked \( F=11.7, p<0.001 \) the stimulation-induced increase in cardiac DHPG production so that after desipramine, cardiac DHPG production was less \( F=66.9, p<0.001 \) than before desipramine.

The norepinephrine concentration in coronary sinus plasma before cardiac sympathetic stimulation was 3.0±0.9 nmol/l, 38% less than that in arterial plasma (4.8±1.6 nmol/l). During sympathetic stimulation, arterial plasma norepinephrine was unchanged, but the coronary sinus plasma concentration increased by 2.8-fold during the highest frequency of electrical stimulation.

Cardiac spillover of norepinephrine into plasma increased \( F=31.3, p<0.001 \) by 4.1-fold and 9.2-fold during the two successive periods of sympathetic stimulation before intravenous desipramine (Figure 1). Cardiac norepinephrine spillover also increased \( F=17.5, p<0.001 \) during sympathetic stimulation after intravenous desipramine, when spillovers at rest and during the lowest frequency of stimulation were 65–81% higher \( F=9.5, p<0.006 \) than before desipramine.

Cardiac production of DHPG was linearly and positively related to cardiac norepinephrine spillover (Figure 2). The slope of the regression line describing the relation indicated that the increase in cardiac DHPG production during sympathetic stimulation was one third of the increase in the cardiac spillover of norepinephrine. The regression line describing the relation intersected the y axis at a cardiac DHPG production of 94 pmol/min, which is close to the mean cardiac production of DHPG before and during sympathetic activation after intravenous desipramine (88 pmol/min).

Cardiac Extraction of [3H]Norepinephrine and Production of [3H]DHPG

At rest, 69±3% of the [3H]norepinephrine entering the coronary circulation was extracted. This proportion decreased \( F=22.1, p<0.001 \) to 63±3% and 53±5% during successive periods of sympathetic stimulation. After desipramine, the cardiac extraction of [3H]norepinephrine decreased \( F=104.1, p<0.001 \) to 31±2% at rest and 20±2% and 17±2% during sympathetic stimulation. The difference in the cardiac extraction of [3H]norepinephrine before and after desipramine was unaltered by activation of cardiac sympathetic nerves and indicated that 39±4% of the [3H]norepinephrine entering the coronary circulation was removed by neuronal uptake (Figure 3). The rates of removal of [3H]norepinephrine by neuronal uptake into cardiac sympathetic nerves did not differ before and during sympathetic activation (average, 69,200±13,000 dpm/min).

The concentration of [3H]DHPG in arterial plasma was 1.7±0.2% of the concentration [3H]norepinephrine (Table 1). The coronary sinus plasma concentration of [3H]DHPG was higher than the arterial
plasma concentration before ($F=172, p<0.001$) and after ($F=83, p<0.001$) desipramine. After desipramine, concentrations of $[^3H]$DHPG were decreased in arterial ($F=7.9, p<0.02$) and coronary sinus ($F=47, p<0.001$) plasma and remained stable during the remainder of the $[^3H]$norepinephrine infusion. Cardiac production of $[^3H]$DHPG was 3,785±999 dpm/min immediately before desipramine and decreased ($F=18.9, p<0.001$) by 57% to 1,622±429 dpm/min after desipramine (Figure 3). The reduced rate of cardiac $[^3H]$DHPG production after desipramine remained stable during the remainder of the radiotracer infusion.

**Norepinephrine Reuptake by Cardiac Sympathetic Nerves**

Norepinephrine reuptake by cardiac sympathetic nerves at rest was 1,186±476 pmol/min, 20-fold more than the cardiac spillover of norepinephrine into plasma (Figure 4). During the two successive periods of sympathetic stimulation, reuptake of norepinephrine by cardiac sympathetic nerves increased ($F=10.1, p<0.005$) by 3.5-fold and 5.6-fold in parallel with the increases in cardiac spillover of norepinephrine into plasma. The combined estimates of cardiac norepinephrine reuptake and spillover at rest and during sympathetic activation indicated that 16±5-fold more norepinephrine was recaptured by cardiac sympathetic nerves than escaped neuronal reuptake and spilled over into the coronary circulation. The combined estimates also indicated that less than 6% of the norepinephrine released into the cardiac neuroeffector junction escaped neuronal and extraneuronal uptake and diffused into plasma.

**Neuronal Accumulation and Release of $[^3H]$Norepinephrine**

Tissue concentrations of $[^3H]$norepinephrine were highest in the right atrium, lowest in the right ventricle, and intermediate in the left ventricle and atrium (Table 2). The specific activity of $[^3H]$norepinephrine did not differ among the three regions of the left ventricle but

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**Figure 1.** Bar graphs of cardiac production of dihydroxyphenylglycol (DHPG) (top panel) and cardiac spillover of norepinephrine (bottom panel) at rest and during electrical stimulation of left ansa subclavia at two consecutive frequencies of stimulation before (cross-hatched bars) and after (solid bars) intravenous desipramine. Desipramine blocked the increase in cardiac DHPG production during sympathetic activation. Difference in DHPG production before and after desipramine represented amount of DHPG entering coronary sinus plasma that was produced from recaptured norepinephrine. *p<0.05, significantly different from resting values. †p<0.05, significantly different from values before desipramine.
was higher (F=17.1, p<0.001) in each left ventricular region than the specific activity of \(^3\text{H}\)norepinephrine in the right ventricle or right and left atrium.

During the final period of sympathetic stimulation after the radiotracer infusion was stopped, the concentration of \(^3\text{H}\)norepinephrine in coronary sinus plasma (401±53 dpm/ml) was higher (p<0.01) than that in arterial plasma (243±24 dpm/ml). The ratio of cardiac spillovers of \(^3\text{H}\)-labeled and endogenous norepinephrine—estimated assuming extractions similar to those measured during the radiotracer infusion—showed that the specific activity of \(^3\text{H}\)norepinephrine released from the heart was not different from that of \(^3\text{H}\)DHPG released from the heart after desipramine or the specific activity of \(^3\text{H}\)norepinephrine in left ventricular tissue (Table 3).

![Bar graphs of differences in cardiac fractional extractions of \(^3\text{H}\)norepinephrine before and after desipramine (left panel) reflecting proportion of \(^3\text{H}\)norepinephrine removed into cardiac sympathetic nerves. Proportion of \(^3\text{H}\)norepinephrine extraction by neuronal uptake was used to estimate rate of \(^3\text{H}\)norepinephrine removal into cardiac sympathetic nerves (middle panel). Comparison of rate of \(^3\text{H}\)norepinephrine removal into cardiac sympathetic nerves (middle panel, 69,200 dpm/min) with difference in cardiac production of \(^3\text{H}\)dihydroxyphenylglycol (\(^3\text{H}\)DHPG) before and after desipramine (right panel, 2,160 dpm/min) indicated that for every 32 molecules of norepinephrine removed into cardiac sympathetic nerves, one molecule of DHPG was produced that entered coronary sinus plasma. This proportion was used to transform cardiac production of DHPG from recaptured norepinephrine (see Figure 1) into a rate for reuptake of norepinephrine by cardiac sympathetic nerves.](image)

**Figure 2.** Plot of relations between cardiac dihydroxyphenylglycol (DHPG) production and cardiac norepinephrine spillover before (○) and after (●) desipramine. Data points represent mean values before and during electrical stimulation of left ansa subclavia at two consecutive frequencies of stimulation. Slope of the linear regression line for relation before desipramine indicated that there was 3.3-fold more norepinephrine released into the coronary circulation than DHPG appearing in coronary sinus plasma from recaptured norepinephrine. y intercept of regression line for relation before desipramine (94 pmol/min) was similar to mean cardiac production of DHPG after desipramine (88 pmol/min), both reflecting rate of leakage of DHPG from vesicles.

**Figure 3.** Bar graphs of differences in cardiac fractional extractions of \(^3\text{H}\)norepinephrine before and after desipramine (left panel) reflecting proportion of \(^3\text{H}\)norepinephrine removed into cardiac sympathetic nerves. Proportion of \(^3\text{H}\)norepinephrine extraction by neuronal uptake was used to estimate rate of \(^3\text{H}\)norepinephrine removal into cardiac sympathetic nerves (middle panel). Comparison of rate of \(^3\text{H}\)norepinephrine removal into cardiac sympathetic nerves (middle panel, 69,200 dpm/min) with difference in cardiac production of \(^3\text{H}\)dihydroxyphenylglycol (\(^3\text{H}\)DHPG) before and after desipramine (right panel, 2,160 dpm/min) indicated that for every 32 molecules of norepinephrine removed into cardiac sympathetic nerves, one molecule of DHPG was produced that entered coronary sinus plasma. This proportion was used to transform cardiac production of DHPG from recaptured norepinephrine (see Figure 1) into a rate for reuptake of norepinephrine by cardiac sympathetic nerves.
TABLE 1. Plasma Concentrations of [3H]Norepinephrine and [3H]DHPG Before and After Desipramine

<table>
<thead>
<tr>
<th></th>
<th>Before desipramine</th>
<th>After desipramine</th>
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<tbody>
<tr>
<td>[3H]Norepinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>4,345±381</td>
<td>6,187±534†</td>
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<tr>
<td>Coronary sinus</td>
<td>1,573±93*</td>
<td>4,913±459*†</td>
</tr>
<tr>
<td>[3H]DHPG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>76±16</td>
<td>63±11†</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>134±31*</td>
<td>84±14*†</td>
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Plasma concentrations of [3H]norepinephrine (mean±SEM dpm/ml) represent values of averaged data for each animal before and during sympathetic stimulation. Concentrations of [3H]dihydroxyphenylglycol ([3H]DHPG) (mean±SEM dpm/ml) represent those immediately before desipramine and averaged concentrations after desipramine.

*p<0.001 compared with arterial.
†p<0.02 compared with before desipramine.

Discussion

Increased rates of cardiac spillover of norepinephrine into plasma in disorders such as heart failure22 indicate increased concentrations of the neurotransmitter at myocardial neuroeffector junctions. High neuroeffector concentrations of norepinephrine may in turn reflect increased sympathetic activity or decreased efficiency of neuronal reuptake, the main means by which to terminate the actions of released norepinephrine. Available methods of examining cardiac sympathetic activity rely heavily on regional measurements of norepinephrine spillover into plasma.10,14,19,22 Interpretation of these measurements is confounded by the dependence of norepinephrine

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Top panel: Bar graph of cardiac norepinephrine reuptake (cross-hatched bars) or spillover into plasma (solid bars) before and during electrical stimulation of left ansa subclavia at two consecutive frequencies of stimulation. Bottom panel: Plot of relation between cardiac norepinephrine reuptake and spillover determined from mean values indicated that cardiac reuptake of norepinephrine was 16-fold greater than cardiac spillover of norepinephrine into plasma. *p<0.05, significantly different from resting values.
spillover on norepinephrine reuptake as well as release. The above considerations have led to a need for appropriate strategies to assess reliably the efficiency of norepinephrine reuptake and thereby determine whether increased spillover of norepinephrine is due to impaired reuptake or increased release.

Existing strategies for examination of neuronal uptake that depend solely on measurements of the tissue extraction of intravenously infused \(^{3}H\)norepinephrine\(^7\) are limited by the dependence of extraction on factors other than the efficiency of neuronal uptake. For example, rather than reflecting impaired neuronal uptake, decreased cardiac extraction of \(^{3}H\)norepinephrine in heart failure\(^8\) and hypertrophic cardiomyopathy\(^10\) may simply reflect greater extraneuronal uptake, degeneration of nerve endings, lower density of innervation, arteriovenous shunting, or higher blood flow. The strategy outlined here has been developed to circumvent these shortcomings by providing a measurement of the actual rate of norepinephrine reuptake by cardiac sympathetic nerves.

Substantial production of DHPG by the canine heart is in agreement with results in the isolated perfused cat, rabbit, and rat heart\(^6,23,24\) as well as in the human heart during cardiac catheterization,\(^7,10\) showing that DHPG is a major cardiac metabolite of norepinephrine. In most isolated tissues, DHPG is exclusively an extraneuronal metabolite of norepinephrine (see review by Kopin\(^11\)). In vivo studies in animals\(^16,17,25\) or humans\(^26,27\) also indicated that plasma DHPG is predominantly produced within sympathetic nerves. However, the contribution of the heart to the plasma pool of DHPG is small,\(^28\) and there may be species differences. Production of DHPG by the cat or rabbit heart is exclusively dependent on neuronal uptake,\(^6\) whereas some DHPG may be formed extraneuronally in the rat heart.\(^23\)

Significant cardiac production of \(^{3}H\)-labeled and endogenous DHPG remaining after intravenous desipramine may suggest an extraneuronal source of DHPG in the canine heart, but other considerations indicate otherwise. In the presence of an extraneuronal source, cardiac production of DHPG would be expected to increase during sympathetic activation independent of neuronal reuptake. Because desipramine completely blocked the increase in cardiac DHPG production during sympathetic activation, an extraneuronal source must have been negligible. The observed results are consistent with a neuronal source of most, if not all, of the DHPG produced by the canine heart.

The cardiac production of DHPG remaining after desipramine, together with its intraneuronal source, indicated that 80% of the DHPG produced by the canine heart at rest was derived from norepinephrine metabolized within the axoplasm after its leakage from storage vesicles. The responses of cardiac DHPG production to sympathetic stimulation before and after desipramine indicated that during sympathetic activation, the amount of DHPG produced from recaptured norepinephrine increased, whereas that produced from norepinephrine leaking from vesicles remained constant. Thus, when cardiac norepinephrine spillover had increased more than nine-fold, the proportion of cardiac DHPG produced from recaptured norepinephrine (62%) exceeded that derived from norepinephrine leaking from vesicles (38%), a reversal of the situation at rest. The regression line for the relation between cardiac norepinephrine spillover and DHPG production intersected the y axis close to the mean cardiac production of DHPG after desipramine, which is consistent with a source of DHPG formed from norepinephrine leaking from storage vesicles and independent of exocytotic release.

In the reserpinized cat and rabbit heart, blockade of neuronal uptake with cocaine caused complete cessation of \(^{3}H\)DHPG formation from perfused \(^{3}H\)norepinephrine within 30 minutes.\(^6\) Incomplete inhibition of cardiac \(^{3}H\)DHPG production by desipramine in the heart preparation used in the present study could be attributed to loading of storage vesicles with \(^{3}H\)norepinephrine before desipramine administration, a process that is not possible in the reserpinized heart. The similar specific activities of \(^{3}H\)DHPG released by the heart after desipramine and of left ventricular stores of \(^{3}H\)norepinephrine were consistent with the latter as a source of \(^{3}H\)DHPG. Thus, vesicular sequestration and storage of \(^{3}H\)norepinephrine before desipramine with subsequent production of \(^{3}H\)DHPG from \(^{3}H\)norepinephrine leaking from vesicles accounted for the resulting sustained cardiac production of \(^{3}H\)DHPG after desipramine.

The similar specific activities of \(^{3}H\)norepinephrine in tissue stores and released into plasma or metabolized to DHPG did not support a heterogeneous distribution of \(^{3}H\)-labeled and endogenous norepinephrine within left ventricular tissue and indicated that each had an equal propensity for exocytotic release or metabolism to DHPG. The above conclusions contrast with those of in vitro studies in which \(^{3}H\)norepinephrine was preferentially released and exhibited a heterogeneous distribu-

### Table 3. Specific Activities of \(^{3}H\)Norepinephrine and \(^{3}H\)DHPG

<table>
<thead>
<tr>
<th>Activity</th>
<th>Specific activity (dpm/pmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{3}H)Norepinephrine released by heart</td>
<td>23.5 ± 6.4</td>
</tr>
<tr>
<td>(^{3}H)DHPG released by heart</td>
<td>18.4 ± 3.2</td>
</tr>
<tr>
<td>(^{3}H)Norepinephrine in left ventricular tissue</td>
<td>18.5 ± 1.6</td>
</tr>
</tbody>
</table>

Specific activities (mean ± SEM) were estimated as described in Table 2. Specific activity of \(^{3}H\)norepinephrine released by heart was estimated from cardiac spillovers \(^{3}H\)-labeled and endogenous norepinephrine after radiotracer infusion was stopped. Specific activity of \(^{3}H\)dihydroxyphenylglycol (\(^{3}H\)DHPG) released by heart was estimated from averaged data for each animal after intravenous desipramine, whereas that for \(^{3}H\)norepinephrine in left ventricular tissue was estimated from averaged data in Table 2.
tion among different storage pools. The heterogeneous distribution was subsequently shown to be a result of uneven access of [3H]norepinephrine from the incubation medium to different layers of the isolated tissue preparation. Presumably, in the present study, [3H]norepinephrine could penetrate evenly from the circulation to storage sites throughout left ventricular tissue.

Estimation of norepinephrine reuptake requires determination of the production of [3H]DHPG from [3H]norepinephrine removed into nerves, independent of any subsequent storage. Exclusive and steady-state formations of [3H]DHPG from [3H]norepinephrine removed into sympathetic nerves are not necessary assumptions of the method. The necessary assumptions are that some [3H]DHPG is formed from [3H]norepinephrine metabolized immediately after its removal into nerves and that in proportion to the amount of [3H]norepinephrine removed, this is the same as the proportion of endogenous DHPG produced from recaptured norepinephrine.

Lack of a readily achievable steady state in the total production of [3H]DHPG during intravenous infusion of [3H]norepinephrine, resulting from vesicular accumulation of [3H]norepinephrine, limits the precision of these measurements for estimation of norepinephrine reuptake. In the present study, the effects of neuronal uptake blockade offered an approach to overcome this limitation. After the rapid desipramine-induced decrease, production of [3H]DHPG reflected leakage of [3H]norepinephrine from stores, with any subsequent decrease dependent on the half-life of stores. The difference in productions of [3H]DHPG immediately before and after desipramine was therefore used to estimate the amount of [3H]DHPG metabolized from the [3H]norepinephrine removed into nerve endings and not sequestered into vesicles.

Comparison of the uptake of [3H]norepinephrine by cardiac sympathetic nerves with the desipramine-sensitive production of [3H]DHPG indicated that only 3.1% of the norepinephrine recaptured by cardiac sympathetic nerves appeared in plasma as DHPG. This compares closely with a previous estimate in humans that 3% of the [3H]norepinephrine removed by cardiac sympathetic nerves appeared in plasma as [3H]DHPG. The low rate of conversion indicates why cardiac production of DHPG from recaptured norepinephrine at rest was minimal (20% of total cardiac DHPG production) and why the increase in cardiac DHPG production during sympathetic activation was only one third of the increase in cardiac norepinephrine spillover. Presumably, the remaining 97% of recaptured norepinephrine not appearing in plasma as DHPG either was sequestered into storage vesicles or ultimately formed metabolites other than DHPG. Studies in isolated rabbit, rat, and cat heart preparations indicated that cardiac production of the combined sum of norepinephrine metabolites other than DHPG never exceeded the production of DHPG.

Assuming a similar situation in the dog would indicate that at the most, only 6% of recaptured norepinephrine was metabolized and that at least 94% was sequestered into storage vesicles. This high rate of sequestration of norepinephrine into storage vesicles accounts for why as much as 20% of cardiac stores of norepinephrine are derived from the uptake of circulating norepinephrine compared with local synthesis.

Use of the 32-fold difference in the neuronal removal of [3H]norepinephrine and production of [3H]DHPG to transform endogenous DHPG production from recaptured norepinephrine into a rate for neuronal reuptake indicated that 16-fold more norepinephrine was recaptured by cardiac sympathetic nerves than escaped neuronal reuptake and spilled over into plasma. The parallel increases in cardiac norepinephrine reuptake and spillover during sympathetic activation indicated that increased norepinephrine release by cardiac sympathetic nerves did not saturate neuronal reuptake and that the efficiency of reuptake was not altered by the short-term increase in cardiac sympathetic activity. Thus, although cardiac norepinephrine spillover into plasma represented less than 6% of total neuronal release, the percent increase in cardiac norepinephrine spillover provided a close reflection of the percent increase in neuronal release.

However, recognition of the substantial contribution of neuronal reuptake to removal of neurotransmitter from the synaptic cleft indicates how minor changes in the efficiency of reuptake may be responsible for major changes in norepinephrine spillover when neuronal release remains unaltered. Thus, the observation that cardiac norepinephrine spillover showed only a modest or small increase after desipramine indicated that the drug markedly reduced norepinephrine release by cardiac sympathetic nerves, a conclusion consistent with previous studies in the anesthetized dog.

Summary

Neuroeffector concentrations of norepinephrine and its subsequent spillover into plasma are determined by the efficiency of neuronal reuptake as well as the neuronal release of transmitter. Thus, complete examination of sympathetic function and interpretation of norepinephrine spillover as an index of sympathetic activity should take into account the efficiency of neuronal reuptake. The present study shows how cardiac norepinephrine reuptake may be estimated from local production of [3H]-labeled and endogenous DHPG during intravenous infusion of [3H]norepinephrine. The precision of the method is limited by the high efficiency of vesicular sequestration, but to date the method offers one of the few approaches to estimate neuronal reuptake of norepinephrine in vivo.

References


23. Fiebig ER, Trendelenburg U: The neuronal and extraneuronal uptake and metabolism of 3H-(-)-noradrenaline in the perfused rat heart. Naunyn Schmiedebergs Arch Pharmacol 1978;303:21–35


**KEY WORDS** • desipramine • plasma • sympathetic nervous system • heart
Neuronal reuptake of norepinephrine and production of dihydroxyphenylglycol by cardiac sympathetic nerves in the anesthetized dog.
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_Circulation._ 1991;84:1354-1363
doi: 10.1161/01.CIR.84.3.1354

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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