Cardiac-specific norepinephrine mass transport and its relationship to left ventricular size and systolic performance

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Departments of 1Cardiology and 2Geriatric Medicine, Department of Internal Medicine, and the 3Horace H. Rackham School of Graduate Studies, University of Michigan; and 4Geriatric Research, Education, and Clinical Center and 4Cardiology Section of Veterans Affairs Ann Arbor Healthcare System, Ann Arbor, Michigan 48105

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Grossman, P. Michael, Oscar A. Linares, Mark A. Supiano, Hakan Oral, Rajendra H. Mehta, and Mark R. Starling. Cardiac-specific norepinephrine mass transport and its relationship to left ventricular size and systolic performance. Am J Physiol Heart Circ Physiol 287: H878–H888, 2004. First published April 8, 2004; 10.1152/ajpheart.00007.2003.—Objectives of this study were to develop a technique for quantifying cardiac-specific norepinephrine (NE) mass transport and determine whether cardiac NE kinetic modeling parameters were related to physiological variables of left ventricular (LV) size and systolic performance in nine patients with chronic mitral regurgitation. Biplane cineventriculograms were used to determine LV size and ejection fraction (EF), micromonometer LV pressures and radionuclide LV volumes from a range of loading conditions to calculate LV end-systolic elastance, and [3 H]NE infusions with LV and coronary sinus sampling for [3 H]NE and [3 H]mass transport. Total NE release rate into cardiac interstitial fluid (IF) averaged 859 ± 214 and NE released de novo into cardiac interstitial fluid (MIF) averaged 546 ± 174 pmol/min. Both MIF and MIF,0,0,0,0 correlated directly with LV end-systolic volume (r = 0.84, P = 0.005; r = 0.86, P = 0.003); inversely with LV EFs (r = −0.75, P = 0.02; r = −0.81, P = 0.008); and inversely with LV end-systolic elastance values, optimally fit by a nonlinear function (r = 0.89, P = 0.04; r = 0.96, P = 0.01). We conclude that total and newly released NE into interstitial fluid of the heart, determined by regional mass transport kinetic model, are specific measures of regional cardiac-specific sympathetic nervous system activity and are strongly related to measures of LV size and systolic performance. These data support the concept that this new model of organ-specific NE kinetics has physiological relevance.

mitral regurgitation; left ventricle; ejection fraction; contractility

In chronic mitral regurgitation (MR), it is known that left ventricular (LV) contractile impairment exists despite the presence of a preserved LV ejection fraction (EF). In an animal model of surgical MR, the sympathetic nervous system (SNS) appears to contribute to this pathophysiological process. Initially, SNS activation may represent a compensatory mechanism to maintain LV ejection and contractile performance to meet the requirements for tissue perfusion. Ultimately, because of the persistent stimulus of the LV volume overload, a positive feedback mechanism may be established, which leads to impaired LV contractility despite the preservation of LV EF due to impaired β-adrenergic receptor responsivity and force-frequency relations. β-Adrenergic blockade results in an improvement of LV contractile dysfunction in animals with MR, lending support for a pathophysiological role for the SNS in the development of impaired LV contractility.

In patients with chronic MR, we have previously demonstrated that systemic SNS activity is increased, but only weak relationships existed with LV size and EF and no relationship existed with LV contractility. Thus, the SNS is activated in a regional, organ-specific manner, cardiac-specific SNS activity may differ from measures of systemic SNS activity. Hence, systemic SNS activity may not be reflective of the cardiac-specific milieu. Although the systemic SNS has only a weak relationship with LV size and EF and none with LV contractility in chronic MR patients, whether cardiac-specific SNS activity has a relationship with these physiological variables (particularly LV contractility) and, therefore, represents the effector of changes in β-adrenergic receptor responsivity and myocardial contractile performance in chronic MR patients remains unknown.

Measurement of cardiac-specific SNS activity has been performed using organ-specific norepinephrine (NE) spillover. This technique provides a heterogeneous measure of organ-specific SNS activation and has become the main approach to the neurochemical assessment of regional organ-specific SNS activation. Nonetheless, this isotope dilution approach assumes that the organ-specific SNS can be described mathematically by the differential equation dy/dt = −ky, which has the single parameter k that describes a single exponential decay. The isotope dilution approach does not, therefore, incorporate the more complex geometry of the organ-specific SNS, which cannot be sampled directly. Moreover, a single differential equation is inadequate to describe the biexponential disappearance of whole body [3 H]NE from plasma and organ-specific [3 H]NE decay.

In the present study, we developed a model to quantify organ-specific NE mass transport for the assessment of cardiac-specific SNS activity in humans. We define a neurophysiologically based model using NE mass flux vector tracking that quantitatively describes cardiac NE mass transport, accurately fits the experimental data collected, and provides new dynamic measures of cardiac-specific SNS activity in humans. These measurements of cardiac-specific SNS activity were determined in a population of patients with chronic MR to test the hypothesis that this is a physiologically relevant model of...
cardiac-specific SNS that would yield modeling parameters that are related to an increase in LV size and reduction in LV ejection and contractile performance.

**Glossary**

- **NE** Norepinephrine
- **SNS** Sympathetic nervous system
- **M_C** Content of NE species of origin within the circulation (pmol)
- **M_IF** Content of NE species of interstitial fluid origin (pmol)
- **M_IF^r** Rate of neuronal uptake after release of NE mass species into the interstitial fluid (pmol/min)
- **M_IF^*e** Rate of extraneuronal uptake of NE mass species from the interstitial fluid (pmol/min)
- **M_CE** Spillover rate of NE mass species from the interstitial fluid into the circulation (pmol/min)
- **M_CE^r** Rate of total NE release into the interstitial fluid (pmol/min)
- **I^* [3H]NE tracer application level (dpm/min)**
- **M_IF^r,em** Sum of neuronal and nonneuronal uptake of NE mass species from the interstitial fluid (pmol/min)
- **M_CE^r,em** Rate of entry of NE mass species into the coronary circulation from outside the subsystem (pmol/min)
- **M_CE^r,em + M_CE^r** Rate of exit of NE mass species from the coronary circulation of the subsystem (pmol/min)
- **M_IF^r,C** Rate of uptake of NE mass species from the coronary circulation of the subsystem (pmol/min)
- **K** $n \times n$ matrix of elements $k_{ij}$ (min$^{-1}$)
- **SAf** Estimated cardiac tissue interstitial fluid specific activity of $[3H]$NE (dpm/pmol)
- **SAs** Arterial plasma specific activity of $[3H]$NE (dpm/pmol)
- **SAs,cs** Coronary sinus plasma specific activity of $[3H]$NE (dpm/pmol)
- **I^* [3H]NE tracer application level (dpm/min)**
- **uS** Systemic constant rate infusion of $[3H]$NE (dpm/min)
- **Ca** Arterial plasma $[3H]$NE concentration (dpm/ml)
- **F** Coronary sinus plasma flow measured by thermodilution (ml/min)
- **V_D** Subsystem cardiac dilution volume of NE (ml)
- **V_D** Whole body dilution volume of NE (ml)
- **M^T** Transpose of the diagonal matrix of compartmental mass sizes
- **SPNE** NE spillover rate (pmol/ml)
- **MR** Mitral regurgitation
- **LV** Left ventricle
- **ESV** End-systolic volume
- **EDV** End-diastolic volume
- **EF** Ejection fraction
- **Ees** End-systolic elastance

**MATERIALS AND METHODS**

**Patient Selection**

The study group consisted of nine patients referred for an assessment of the severity of their chronic MR. Patients were excluded from this study if they had coronary artery disease, atrial fibrillation, significant aortic valve disease, or concomitant mitral stenosis or were unable to provide written informed consent. The group consisted of eight men and one woman aged 49 to 71 yr (mean 60 ± 8 yr). There were four patients in New York Heart Association clinical class I, four in class II, and one in class III. All β-adrenergic receptor antagonists, calcium channel antagonists, and diuretic medications were stopped 24–48 h before cardiac catheterization. All patients gave written informed consent on a form previously approved by the Institutional Review Boards at the University of Michigan or Veterans Affairs Healthcare Systems, Ann Arbor, MI.

**Cardiac Catheterization Protocol**

After documentation of normal coronary anatomy, each patient entered the protocol. A right heart catheterization was performed. A micromanometer-tipped catheter (Millar Instrument, Houston, TX) was then positioned for the measurement of LV pressure and the performance of blipane contrast cineventriculography. Subsequently, a thermodilution catheter (Cordis, Miami, FL) was placed in the coronary sinus (CS) for flow measurements and blood sampling. The CS catheter placement was confirmed by fluoroscopy by use of a contrast injection and measurement of the O$_2$ saturation of CS blood samples. The CS plasma flow was determined during the steady-state infusion of $[3H]$NE at 40, 50, and 60 min in triplicate by thermodilution. Finally, micromanometer LV pressures and radionuclide LV volumes were acquired during baseline and altered loading conditions to calculate LV $E_{es}$, as previously described by our laboratory (32).

**Cineventriculography**

Biplane contrast cineventriculograms of one of the first three beats after contrast injection were used for volume analysis (16, 33). The LV systolic pressure was defined as the maximum LV pressure, and LV end-diastolic pressure was defined as the pressure at the Z point immediately after the A wave on the LV pressure waveform (33). The cineventriculographic LV end-diastolic and end-systolic volumes were calculated from biplane cineventriculographic image pairs by use of a modified Simpson’s rule algorithm previously validated against human heart casts in our laboratory (35). The cineventriculographic LV end-diastolic and end-systolic volumes were defined as the maximum and minimum values on the cineventriculographic LV volume curve. LVEF was then calculated by dividing stroke volume by end-diastolic volume.

**LV Contractility Calculation**

To evaluate LV contractility, the LV $E_{es}$ concept was used. In the cardiac catheterization laboratory, during right atrial pacing, microanometer LV pressures and radionuclide ventriculograms for LV volumes were acquired over a range of LV loading conditions to calculate $E_{es}$ as previously described by our laboratory (32).

**NE Infusion Protocol**

The protocol adapted for this analysis was based on our previously published procedures (6, 9, 14, 20, 21, 27, 30, 37). The protocol consisted of an intravenous infusion of $[3H]$NE, approximate specific activity 40–60 Ci/mmol (1Ci = $2.2 \times 10^8$ Bq). New England Nuclear, Boston, MA), calculated to deliver 0.7 μCi·min$^{-1}$·m$^{-2}$ at a rate of 0.2 ml/min using an infusion pump (Harvard Apparatus, South Natick, MA). Ascorbic acid (1 mg/ml) was added to the infusion to prevent...
Cardiac Spillover of NE

Cardiac spillover of NE (SPNE) is defined as the rate of entry into the coronary venous drainage of the total NE released into the cardiac interstitial space. Previously, this value has been used to describe cardiac NE kinetics (5, 7). The cardiac SPNE into the plasma is derived from the arterial-CS difference in tracer and tracee mass transport and the CS plasma flow (F). The formula is

\[ \text{SP}_{\text{NE}} = F \cdot [\text{NE}_{\text{cs}} - \text{NE}_a] + (E \cdot \text{NE}_a) \]

where extraction = E = [(\text{[3H]}\text{NE})_{\text{cs}} - \text{[3H]}\text{NE}_{\text{a}},]/\text{[3H]}\text{NE}_{\text{a}}] (subscript a is arterial, and subscript cs is coronary sinus).

Model Development

NE mass flux vector tracking. Heat flux vector tracking (17) is used to study heat transfer mechanisms in a variety of media under various conditions. The link between tracking heat transfer mechanisms and NE mass transport is the law of conservation of mass. From a mathematical standpoint, the properties that govern the diffusion of heat are identical to those for the diffusion of mass. From this, a continuity equation for the conservation of cardiac NE mass was written, the uniquely identifiable components of the in vivo cardiac SNS need to be identified (15). The tool for system identification of NE mass transport mechanisms used in the present study is NE mass flux vector tracking.

NE mass flux vector tracking of cardiac NE mass species. Our model of the cardiac-specific SNS for the fate of NE after it is released from postganglionic sympathetic neurons is based on the known neurophysiology of the SNS, NE system information from the literature, the results of our experiments, and NE mass flux vector tracking and is represented in Fig. 1. Two species of NE mass are identified on the basis of their sites of origin within the cardiac SNS (the circulation and interstitial fluid): \( M_{\text{af}} \) and \( M_{\text{if}} \), respectively. A portion of the NE mass released from the neuron into the interstitial fluid of the sympathetic cleft is immediately transported back into the neuron via the mechanism of reuptake to release \( M_{\text{af}} \). Another portion of it is removed via extraneuronal mechanisms \( (M_{\text{if}}^{\text{ex}}) \). The portion of the NE mass flux that escapes these processes diffuses into the circulation \( (M_{\text{af}}) \). As illustrated in Fig. 1, only the sum of \( M_{\text{af}} \) and \( M_{\text{if}}^{\text{ex}} \) can be uniquely determined from the experiment (20).

Therefore, the rate of total neuronal release of NE into the interstitial fluid zone is given by

\[ M_{\text{if}} = M_{\text{af}} + M_{\text{if}}^{\text{ex}} \]

The NE mass flux that diffuses into the local cardiac circulation mixes with NE entering the cardiac circulation from the body \( (M_{\text{af}}) \). Therefore, \( M_{\text{af}} \) is the total rate of NE mass transport into the local cardiac circulation from all sources.

Figure 1 also emphasizes that NE mass is transported into the cardiac SNS in arterial blood from the body \( (M_{\text{af}}) \), there is local mixing of NE mass species, and the mixed NE mass species exit the cardiac SNS in CS blood at a rate equal to \( M_{\text{af}} \) + \( M_{\text{if}} \). In addition, Fig. 1 highlights that the whole body NE dilution volume is bounded by a region contained within a large volume (solid-line rectangle) compared with the cardiac NE dilution space, which is a smaller volume (dashed-line rectangle).

By application of Reynolds' transport theorem (11) and the law of conservation of mass on a differential control volume of plasma fluid entering as the cardiac-specific inflow, the rate of entry of NE mass \( (M_{\text{af}}) \) is equal to the rate of exit of NE mass \( (M_{\text{af}} + M_{\text{if}}) \). The NE mass transport processes taking place locally via \( M_{\text{af}} \) and \( M_{\text{if}} \) represent, therefore, local interfacial surface NE mass transport phenomena (13).

Continuous equations for conservation of NE mass. Taking the limits of \( M_{\text{af}} \) and \( M_{\text{if}} \) as both \( M_{\text{af}} \) and \( M_{\text{if}}^{\text{ex}} \) and taking \( \Delta t \) to 0 gives

\[ M_{\text{af}} = (k_{\text{af}} \cdot C_{\text{af}}) \cdot M_{\text{C}} + (k_{\text{af}} \cdot C_{\text{af}}^{\text{ex}}) \cdot M_{\text{af}} + M_{\text{af}}^{\text{in}} \]

\[ M_{\text{if}} = (k_{\text{if}} \cdot C_{\text{if}}) \cdot M_{\text{C}} - (k_{\text{if}} \cdot C_{\text{if}}^{\text{ex}}) \cdot M_{\text{if}} + M_{\text{if}}^{\text{in}} \]

Simultaneous solution of Eqs. 1 for both \([\text{3H}]\text{NE} \) tracer and NE tracee mass was performed using the WinSAAM program for data analysis and graphics (39). Details of the NE mass transport calculations based on the negative inverse of the K matrix are provided in the Appendix. The basic theory for use of the negative inverse of the K matrix for species identification based on site of origin has been presented by several authors (3, 4, 12).

In the steady state

\[ \text{s}_{\text{af}} = \frac{1}{M_{\text{af}}^{\text{ex}} + M_{\text{af}}^{\text{in}}} \]

where \( \text{s}_{\text{af}} \) is the cardiac tissue interstitial fluid specific activity of \([\text{3H}]\text{NE} \) and \( 1^* \) is the constant input rate of \([\text{3H}]\text{NE} \) into the heart, which is equal to the arterial concentration of \([\text{3H}]\text{NE} \) times the measured CS plasma flow rate. The arterial and CS specific activities (\( S_a \) and \( S_c \), respectively) were calculated as the tracer NE concentration (in dpm/ml) divided by the tracee NE concentration (in pmol/ml) in arterial and CS plasma, respectively.

Model assumptions. First, the cardiac-specific \([\text{3H}]\text{NE} \) tracer and NE tracee mass transport systems are assumed to be indistinguishable, i.e., there is no isotope effect, and therefore the \([\text{3H}]\text{NE} \) transport rate coefficients describing the \([\text{3H}]\text{NE} \) tracer transport are equal to those of the NE tracee transport. Second, the kinetic model of NE mass transport phenomena (13).

The kinetic model for both \([\text{3H}]\text{NE} \) tracer and NE tracee mass transport is given by a set of simultaneous ordinary differential equations, where the unknowns are the fluxes of NE mass transport into and out of the cardiac tissue interstitial fluid and the cardiac tissue.

**References**

3. Reynolds, E. (1980) The kinetic model for both \([\text{3H}]\text{NE} \) tracer and NE tracee mass transport is given by a set of simultaneous ordinary differential equations, where the unknowns are the fluxes of NE mass transport into and out of the cardiac tissue interstitial fluid and the cardiac tissue.
Statistical Analysis

Paired t-tests were used to compare variables. Pearson correlation matrices were also used to establish relationships between model parameters and measures of LV size and systolic performance. A significant difference or relationship was established when the probability of rejecting the null hypothesis was 0.05 or less.

RESULTS

LV Size and Systolic Performance

The measures of LV size and systolic performance are shown in Table 1. The mean LV end-diastolic volume was $169 \pm 24$ ml, and the mean LV end-systolic volume was $70 \pm 24$ ml.

Table 1. LV size and systolic performance

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>EDV, ml</th>
<th>ESV, ml</th>
<th>EF</th>
<th>$E_{es}$, mmHg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>135</td>
<td>50</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>192</td>
<td>90</td>
<td>0.53</td>
<td></td>
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<tr>
<td>3</td>
<td>170</td>
<td>138</td>
<td>0.19</td>
<td>0.19</td>
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<tr>
<td>4</td>
<td>183</td>
<td>33</td>
<td>0.82</td>
<td>1.04</td>
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<tr>
<td>5</td>
<td>200</td>
<td>61</td>
<td>0.70</td>
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<td>6</td>
<td>181</td>
<td>75</td>
<td>0.59</td>
<td>0.73</td>
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<tr>
<td>7</td>
<td>151</td>
<td>56</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>132</td>
<td>43</td>
<td>0.67</td>
<td>1.96</td>
</tr>
<tr>
<td>9</td>
<td>178</td>
<td>82</td>
<td>0.54</td>
<td>0.79</td>
</tr>
<tr>
<td>Mean</td>
<td>169</td>
<td>70</td>
<td>0.59</td>
<td>0.94</td>
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<tr>
<td>SD (±)</td>
<td>24</td>
<td>31</td>
<td>0.17</td>
<td>0.65</td>
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LV, left ventricular; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; $E_{es}$, end-systolic elastance.
Fig. 2. NE infusion protocol. These represent NE kinetic data from one of the chronic mitral regurgitation (MR) patients in our study. Arterial and CS samples were drawn during the 60-min infusion at 40, 50, and 60 min to document a stable state. Then, after infusion termination at 60 min, blood samples were drawn frequently during the biexponential, tritiated NE decay. NEa, arterial NE level; NEcs, CS NE level. These levels remain constant throughout the study. NEa, arterial tritiated NE activity; NEcs, CS tritiated NE activity.

**NE Activity**

The mean arterial and CS plasma [3H]NE and NE levels during the infusion protocol are illustrated in Fig. 2. The arterial and CS plasma [3H]NE achieved a plateau during the [3H]NE infusion and then disappeared rapidly after the infusion was stopped. The arterial [3H]NE levels were higher than the CS levels. Arterial and CS plasma NE levels were stable throughout the sampling periods as measured by repeated-measures ANOVA (time-effect P values of 0.72 and 0.17, respectively). The disappearance of [3H]NE from the CS was not fit well by a single exponential. When a second exponential was added, an excellent fit of the decay of [3H]NE was observed for each subject. The goodness of fit of the biexponential model was superior to the monoexponential model for each subject, both in terms of the Akaike (range of values 11.9 to 32.6 biexponential and 0.1 to 4.1 monoexponential) and Schwarz (range of values -12.5 to 35.5 biexponential and 6 to 12.8 monoexponential) criteria (15).

Table 2. Cardiac and arterial NE and [3H]NE2 specific activity

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>u3, dpm/min</th>
<th>[3H]NEa, dpm/ml</th>
<th>[3H]NEcs, dpm/ml</th>
<th>NEa, pmol/ml</th>
<th>NEcs, pmol/ml</th>
<th>SAa, dpm/pmol</th>
<th>SACa, dpm/pmol</th>
<th>SACs, dpm/pmol</th>
<th>F, ml/min</th>
<th>SPNE, pmol/ml</th>
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<td>2,468,303</td>
<td>1,258</td>
<td>639</td>
<td>1.68</td>
<td>1.56</td>
<td>747</td>
<td>409</td>
<td>95</td>
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<td>308</td>
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<td>3,760</td>
<td>3,167</td>
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<td>187</td>
<td>83</td>
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<td>884</td>
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<td>772</td>
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<td>132</td>
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<td>1,651</td>
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<td>1.78</td>
<td>1.84</td>
<td>930</td>
<td>498</td>
<td>118</td>
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<td>1,674</td>
<td>994</td>
<td>1.87</td>
<td>2.18</td>
<td>884</td>
<td>428</td>
<td>136</td>
<td>71</td>
<td>76.7</td>
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<td>SD (±)</td>
<td>408,464</td>
<td>800</td>
<td>841</td>
<td>0.61</td>
<td>0.78</td>
<td>152</td>
<td>188</td>
<td>53</td>
<td>20</td>
<td>34.5</td>
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</table>

u3, Constant infusion rate of [3H]norepinephrine ([3H]NE) into circulation; [3H]NEa, arterial plasma [3H]NE concentration (conc); [3H]NEcs, coronary sinus (CS) plasma [3H]NE conc; NEa, arterial plasma NE conc; NEcs, CS plasma conc; SAa, arterial [3H]NE specific activity; SACa, CS [3H]NE specific activity; SACs, tissue interstitial [3H]NE specific activity; F, measured CS plasma flow rate; SPNE, NE spillover = cardiac NE spillover rate into the plasma derived from the levels from the difference in tracer and tracee concs and the CS plasma flow. The formula is SPNE = F [([NEcs - NEa] + (E - NEa))], where extraction (E) = ([3H]NEcs - [3H]NEa)/[3H]NEa.

**Experimental Database**

The experimentally derived steady-state [3H]NE tracer and NE tracee data collected for each individual are presented in Table 2. The mean arterial plasma NE level was significantly lower than the mean CS plasma level (P < 0.001). The mean arterial plasma [3H]NE level was significantly higher than the mean CS plasma [3H]NE level (P < 0.001). The arterial [3H]NE specific activity (SAa) was significantly higher than the CS [3H]NE specific activity (SAcs) (P < 0.001). The tissue interstitial [3H]NE specific activity (SAt) was significantly less than both SAa and SAcs (P < 0.001).

**Input Function**

The mean arterial plasma NE level was 1.87 ± 0.61 pmol/ml. The mean arterial plasma [3H]NE level was 1.674 ± 800 pmol/ml. The mean measured CS plasma flow rate was 71 ± 20 ml/min. Thus the calculated mean constant [3H]NE tracer input rate into compartment 1 was I* = CsaF, equal to 125,550 ± 92,400 dpm/min, where I* is the [3H]NE tracer input rate (in dpm/min),
Cₐ is the arterial [³H]NE concentration (in dpm/ml), and F is the measured CS plasma flow rate (in ml/min) (Table 2).

**NE Mass Transport**

In Table 3, the estimated elements of the K matrix and the dilution volume for Mₐ (Vₐ) for the NE mass transport model (Fig. 1) are tabulated along with their measures of estimability. The whole body NE system dilution volume (VᵢF) is also shown for comparison. This value was derived from the two-compartment model for systemic NE kinetics, using only the arterial plasma NE tracer and tracee data as previously described (8). The NE mass transport modeling parameters were estimated with very good accuracy with the exception of two values for kᵢF, and one value for kᵢF, which had a coefficient of variation in excess of 50% (see subjects 4 and 7). The cardiac-specific NE dilution volume for Mₐ represented ~12% of the systemic NE dilution volume. The elements of the K matrix were used to quantify the NE mass transport rate constants for the NE tracers as functions of the elements of the K matrix and the transverse of the diagonal matrix MᵢF = [Mⁿ, MᵢF] (see APPENDIX).

The cardiac-specific NE mass transport model estimates of NE species mass contents and transport rates are presented in Table 4. The values are tabulated with their corresponding achievable accuracy for each individual. The model NE molecule mass transport rates were estimated with very good accuracy with the exception of one value for MᵢF, which almost exceeded our criterion for estimability, i.e., a coefficient of variation > 50% (subject 7 at 49%). Mₐ was significantly lower than MᵢF (P < 0.001), and the ratio of Mₐ to MᵢF was ~53:1. MᵢF, corresponded to 94.5 ± 2.6% of MᵢF, Only 5.5 ± 2.6% of MᵢF, exited via MᵢF, + MᵢF, MᵢF, comprised 39.2 ± 8.8% of MᵢF, and MᵢF, constituted 63.1 ± 8.5%. As shown in Table 3, the total rate of NE release into the interstitial fluid from all sources is equal to MᵢF = MᵢF, + MᵢF, Therefore, the difference between the total release of NE into the interstitial fluid (MᵢF) and the amount of it that is taken up from the circulation (MᵢF, + MᵢF, + MᵢF, = MᵢF) gives the rate of locally released NE into the cardiac interstitial fluid, which is equivalent to MᵢF, + MᵢF, Therefore, as shown in Table 3, uptake of NE from the cardiac circulation (MᵢF, + MᵢF, contributes significantly to the content of NE in the interstitial fluid (MᵢF) and the total rate of NE release into the interstitial fluid.

### Table 3. Elements of the K matrix

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>kᵢF, min⁻¹</th>
<th>kᵢF, min⁻¹</th>
<th>kᵢF, min⁻¹</th>
<th>kᵢF, min⁻¹</th>
<th>VᵢF, ml</th>
<th>VᵢF, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0240</td>
<td>0.7349 (12)</td>
<td>0.0104 (26)</td>
<td>0.0214 (30)</td>
<td>194.8</td>
<td>2,343</td>
</tr>
<tr>
<td>2</td>
<td>0.0350</td>
<td>0.7298 (21)</td>
<td>0.0364 (24)</td>
<td>0.0403 (13)</td>
<td>482.9</td>
<td>2,114</td>
</tr>
<tr>
<td>3</td>
<td>0.0523</td>
<td>0.3816 (16)</td>
<td>0.0149 (34)</td>
<td>0.0320 (28)</td>
<td>338.3</td>
<td>1,835</td>
</tr>
<tr>
<td>4</td>
<td>0.0240</td>
<td>0.5563 (11)</td>
<td>0.0080 (30)</td>
<td>0.0132 (53)</td>
<td>287.5</td>
<td>1,708</td>
</tr>
<tr>
<td>5</td>
<td>0.0822</td>
<td>0.4952 (10)</td>
<td>0.0162 (32)</td>
<td>0.0164 (47)</td>
<td>350.3</td>
<td>3,468</td>
</tr>
<tr>
<td>6</td>
<td>0.0474</td>
<td>0.7018 (16)</td>
<td>0.0148 (27)</td>
<td>0.0180 (34)</td>
<td>428.9</td>
<td>2,005</td>
</tr>
<tr>
<td>7</td>
<td>0.0372</td>
<td>0.9940 (21)</td>
<td>0.0194 (59)</td>
<td>0.0247 (71)</td>
<td>173.6</td>
<td>1,306</td>
</tr>
<tr>
<td>8</td>
<td>0.0323</td>
<td>0.5705 (5)</td>
<td>0.0064 (13)</td>
<td>0.0065 (46)</td>
<td>275.9</td>
<td>2,729</td>
</tr>
<tr>
<td>9</td>
<td>0.0389</td>
<td>0.8214 (10)</td>
<td>0.0091 (24)</td>
<td>0.0227 (27)</td>
<td>152.7</td>
<td>1,438</td>
</tr>
<tr>
<td>Mean</td>
<td>0.0355</td>
<td>0.6651 (13)</td>
<td>0.0140 (30)</td>
<td>0.0217 (39)</td>
<td>298.3</td>
<td>2,161</td>
</tr>
<tr>
<td>SD (±)</td>
<td>0.0098</td>
<td>0.1847 (5)</td>
<td>0.0095 (12)</td>
<td>0.0100 (17)</td>
<td>113.8</td>
<td>618.1</td>
</tr>
</tbody>
</table>

Values in parentheses are achievable accuracy of parameter estimate expressed as percent coefficient of variation. kᵢF, is an a priori calculated parameter for which entered parameter estimation is known. (See GLOSSARY for additional definitions.)

### Table 4. NE species contents and mass transport rates

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>MᵢF, pmol</th>
<th>MᵢF, pmol</th>
<th>MᵢF, pmol/min</th>
<th>MᵢF, pmol/min</th>
<th>MᵢF, pmol/min</th>
<th>MᵢF, pmol/min</th>
<th>MᵢF, pmol/min</th>
<th>MᵢF, pmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>328.02 (11)</td>
<td>23,231 (23)</td>
<td>248.91 (5)</td>
<td>7.86 (11)</td>
<td>241.05 (5)</td>
<td>496.21 (8)</td>
<td>729.40 (8)</td>
<td>1,012.85 (4)</td>
</tr>
<tr>
<td>2</td>
<td>674.36 (15)</td>
<td>13,516 (16)</td>
<td>515.73 (8)</td>
<td>23.60 (15)</td>
<td>492.13 (8)</td>
<td>544.32 (4)</td>
<td>1,012.85 (4)</td>
<td>603.55 (28)</td>
</tr>
<tr>
<td>3</td>
<td>1,122.12 (12)</td>
<td>28,670 (12)</td>
<td>486.95 (5)</td>
<td>58.71 (12)</td>
<td>428.23 (7)</td>
<td>916.89 (4)</td>
<td>1,286.41 (4)</td>
<td>603.55 (28)</td>
</tr>
<tr>
<td>4</td>
<td>418.19 (10)</td>
<td>28,950 (26)</td>
<td>424.62 (5)</td>
<td>10.03 (10)</td>
<td>232.65 (5)</td>
<td>380.93 (5)</td>
<td>603.55 (28)</td>
<td>603.55 (28)</td>
</tr>
<tr>
<td>5</td>
<td>528.93 (9)</td>
<td>42,068 (28)</td>
<td>276.89 (4)</td>
<td>14.94 (9)</td>
<td>261.95 (4)</td>
<td>688.35 (20)</td>
<td>935.36 (20)</td>
<td>935.36 (20)</td>
</tr>
<tr>
<td>6</td>
<td>620.79 (14)</td>
<td>29,425 (21)</td>
<td>465.07 (6)</td>
<td>29.42 (14)</td>
<td>435.65 (6)</td>
<td>529.34 (13)</td>
<td>935.36 (20)</td>
<td>935.36 (20)</td>
</tr>
<tr>
<td>7</td>
<td>382.33 (16)</td>
<td>19,548 (49)</td>
<td>394.46 (10)</td>
<td>15.22 (16)</td>
<td>379.24 (10)</td>
<td>482.85 (22)</td>
<td>846.87 (22)</td>
<td>846.87 (22)</td>
</tr>
<tr>
<td>8</td>
<td>551.81 (4)</td>
<td>19,095 (12)</td>
<td>332.61 (2)</td>
<td>17.80 (10)</td>
<td>314.82 (2)</td>
<td>320.18 (35)</td>
<td>617.20 (34)</td>
<td>617.20 (34)</td>
</tr>
<tr>
<td>9</td>
<td>251.06 (10)</td>
<td>24,416 (21)</td>
<td>233.24 (1)</td>
<td>10.56 (8)</td>
<td>222.65 (4)</td>
<td>553.35 (7)</td>
<td>935.56 (13)</td>
<td>935.56 (13)</td>
</tr>
<tr>
<td>Mean</td>
<td>544.18</td>
<td>28,769.78</td>
<td>355.17</td>
<td>20.90</td>
<td>334.26</td>
<td>545.82</td>
<td>859.18</td>
<td>859.18</td>
</tr>
<tr>
<td>SD (±)</td>
<td>255.07</td>
<td>10,941.81</td>
<td>113.04</td>
<td>15.74</td>
<td>101.94</td>
<td>174.24</td>
<td>214.32</td>
<td>214.32</td>
</tr>
</tbody>
</table>

Values in parentheses are achievable accuracy of estimate in percentage. MᵢF, content of NE species of origin within the circulation; MᵢF, content of NE species of interstitial fluid origin; MᵢF, rate of entry of NE mass species into the coronary circulation from outside the subsystem; MᵢF, + MᵢF, rate of exit of NE mass species from the coronary circulation of the subsystem; MᵢF, = MᵢF, spillover rate of NE mass species from the interstitial fluid into the circulation = rate of uptake of NE mass species from the circulation into the interstitial fluid; MᵢF, contained by neuronal and nonneuronal uptake of NE mass species from the interstitial fluid; MᵢF, rate of total NE release into the interstitial fluid.
of NE release or “appearance rate” in the interstitial fluid (MIF) at a ratio of ~2:1.

Cardiac-Specific NE Activity and LV Size and Systolic Performance

There was no relationship between MIF or MIFR and the LV end-diastolic volumes. In contrast, the LV end-systolic volumes correlated with the MIF (r = 0.86, P = 0.003) and MIFR values (r = 0.84, P = 0.005) (Fig. 3). The LVEF values were highly but inversely correlated with the MIF (r = -0.81, P = 0.008) and the MIFR values (r = -0.75, P = 0.02) (Fig. 4).

The values for the cardiac SPNE are provided in Table 2. In contrast to the relationships observed with the values for cardiac-specific NE release derived from the mass transport model, there was no relationship between the NE spillover fraction (SPNE) and LV end-systolic volume (r = 0.035, P = 0.98) or LV EF (r = -0.105, P = 0.79).

The LV Ees calculations were available from five chronic MR patients to compare with the cardiac-specific NE modeling parameters but unavailable from four because of technical data acquisition problems. The Ees values demonstrated an inverse relationship with the MIF (r = -0.96, P = 0.06) and MIFR values (r = -0.86, P = 0.10). These data were, however, best fit by a nonlinear function (r = 0.96, P = 0.01; and r = 0.89, P = 0.04, respectively; Fig. 5).

DISCUSSION

The purposes of this investigation were to develop a new model of cardiac-specific NE kinetics and determine whether the cardiac-specific NE kinetic parameters obtained from this new model were related to LV size and systolic performance. The model we developed provides a measure of newly released NE into the interstitial fluid of an organ, which may afford a more specific measure of regional SNS activity in humans. The present study demonstrates that the cardiac-specific NE mass transport model (Fig. 1) imparts a good description of the dynamics of distribution and metabolism of organ-specific NE kinetics in humans. The present study design also allowed us to differentiate between the total NE release rate into the inter-

Fig. 3. Cardiac-specific NE activity relates directly to left ventricular (LV) end-systolic volume (ESV). At left, MIF correlates directly with LVESV. At right, MIFR also correlated directly with LVESV.

Fig. 4. Cardiac-specific NE activity relates inversely with LV ejection fraction (EF). At left, MIF correlates inversely with LVEF. At right, MIFR also correlates inversely with LVEF.
stital fluid of the heart and the rate of newly released NE into
the interstitial fluid of the heart ($M_{IF}^{F}$ and $M_{IF}^{F,rel}$, respectively). Our results further show that reuptake of NE from the circulation ($M_{IF}^{F,c}$) contributes significantly to the NE content in the interstitial fluid and thus the total NE release rate into this site. By separating out the components of total NE release, i.e., newly released NE and reuptake of NE from the circulation, more specific insight into the dynamics of NE in the interstitial space of the heart is gained, which may provide more specific indexes of cardiac-specific SNS activity in humans.

Neurohormonal activation occurs as part of the cardiac response to disease and hemodynamic challenge (10, 19). Therefore, the development of a model system for cardiac-specific NE mass transport must have physiological relevance to provide insight into the response of the model system in physiological terms. In previous studies of chronic MR patients, we demonstrated weak relationships between systemic SNS activity and cineventriculographic and echocardiographic measures of LV size and EF (22, 23). There was, however, no relationship demonstrated between systemic SNS activity and measures of LV contractility (unpublished data). Thus, because cardiac-specific SNS activity may differ from other organ systems and the systemic circulation and may play integral compensatory and pathophysiological roles in patients with chronic MR, we developed the cardiac-specific NE mass transport model and found that the NE system modeling parameters of total cardiac NE released into the interstitial fluid of the heart ($M_{IF}^{F}$) correlated directly with LV end-systolic volume and inversely with LVEF. Similarly, the rate of newly released NE into the interstitial fluid of the heart ($M_{IF}^{F,rel}$) correlated directly with LV end-systolic volume and inversely with LV EF. In contrast, SPNE did not correlate significantly with any of these hemodynamic parameters. The mean LV end-systolic volume was increased in the MR patients in this study, whereas the mean LVEF remained within the normal range. An increase in LV size before overt LV systolic dysfunction is a well-described phenomenon in MR (32, 40). The correlation between LV size and cardiac-specific SNS activity, using our model of NE mass transport in these chronic MR patients, has two possible physiological implications. First, an increase in cardiac-specific SNS activity may occur relatively early in the disease process as a compensatory mechanism to accommodate for the LV volume overload (22). Second, chronic activation of the cardiac-specific SNS may, over time, contribute to the process of LV dilation and subsequent systolic dysfunction in patients with chronic MR through a positive-feedback system (1). In support of the latter possibility, animal studies have suggested a central role for activation of the SNS in the development of LV contractile dysfunction associated with experimental MR (25, 38). In addition, in a subset of the chronic MR patients in our study, we were able to calculate $E_{es}$, an index of LV contractile performance. In contrast to the absence of a relationship between a systemic SNS modeling parameter (NE2) and LV contractility (unpublished data), there was a significant inverse relationship between $E_{es}$ and cardiac-specific NE release rates. These data were optimally fit by a nonlinear function. This suggests that, in the wide range of normal $E_{es}$ values (>1.0 mmHg/ml), cardiac-specific NE modeling parameters may be relatively narrow, whereas the opposite occurs in the relatively narrow depressed LV contractility range, where this relationship steepens as the cardiac-specific SNS modeling parameters sharply increase below an $E_{es}$ value of 1.0 mmHg/ml. Preliminary data from our laboratory suggest that there is a strong relationship between impaired β-adrenergic receptor responsivity and depressed LV contractility as measured by $E_{es}$ in patients with chronic MR (34). Thus, cardiac-specific but not systemic SNS activity may, through an ongoing positive-feedback mechanism (1), drive the development of LV contractile dysfunction in this state of chronic LV volume overload through a β-adrenergic receptor mechanism.

The model structure (Fig. 1) imposes the distinction between cardiac-specific NE release that does not include NE uptake from the arterial inflow (newly released NE) and NE release that includes NE uptake from the arterial inflow (total NE release). The model structure imposes this distinction because renewal of NE in the interstitial fluid of a given cardiac sympathochromaffin tissue unit can take place in three ways. First, NE can be released directly into the interstitial fluid de
nov, i.e., newly released. Second, NE can be released somewhere else and arrive in the interstitial fluid, e.g., via uptake from the arterial inflow to the heart. Finally, NE released into the interstitial fluid that spills over into the circulation may find its way back into the interstitial fluid (uptake due to spillover). So, consistent with our results, total NE release is greater than newly released NE because total NE release represents a composite measure of NE release rates from all sources, including newly released NE.

As reported by Kopin et al. (18), a key independent variable in the study of regional NE kinetics is the specific activity of $[^{3}H]$NE in the interstitial fluid (SA$_{T}$). The approach utilized by Kopin et al. (18) required the simultaneous infusion of $[^{3}H]$NE and $[^{3}H]$epinephrine and measurement of their O-methylated metabolites. The SA$_{T}$ was estimated using the assumptions that the extraction fraction for metanephrine is comparable to that for normetanephrine and that all of the normetanephrine formed in tissue derives from NE in the interstitial fluid. Our study demonstrates that SA$_{T}$ may be estimated using the mass transport model. In addition, the NE mass transport model correctly predicted the expected profile for $[^{3}H]$NE specific activity, i.e., SA$_{a} > SA_{cs} > SA_{T}$, where SA$_{a}$ is the arterial, SA$_{cs}$ is the CS, and SA$_{T}$ is the interstitial fluid tissue specific activity (26). From the paper by Kopin et al. (18), the values for total NE release into the interstitial fluid of the heart ranged from 500 to 2,500 pmol/min, with a mean value of $\sim 1,500$ pmol/min. This value is comparable to our result for $M_{o}^{b}$, where the NE mass transport model total NE released into the cardiac interstitial fluid averaged 859 pmol/min, with a range from 617 to 1,286 pmol/min. Moreover, the NE mass transport model was able to separate total NE released into two components, one that was due to NE species from all sources (circulation and interstitial fluid) and one that was due to NE species of origin within the interstitial fluid. Finally, as noted by Kopin et al. (18), the cardiac SP$_{NE}$ into plasma derived from the arterial-CS difference in tracer and tracee concentrations and the CS plasma flow is misleading, as it grossly underestimates the rate of NE release into the interstitial fluid derived from estimates of SA$_{T}$.

In the study by Rose et al. (26), the apparent cardiac NE spillover was reported in two patients with isolated MR. The published data for these two subjects were 0.026 and 0.055 pmol·mL$^{-1}$·s$^{-1}$. In equivalent dimensional units from the cardiac-specific NE mass transport model in our chronic MR patients, the mean value corresponded to 0.025 ± 0.014 pmol·mL$^{-1}$·s$^{-1}$, which is a comparable value. Because the cardiac NE kinetic system model connectivity provided by the model of Rose et al. (26) is superior to that provided by the single-compartment model representation, i.e., it incorporates a reversible exchange pathway between the tissue capillary-interstitial surface interface like our NE mass transport model, there was close agreement between the apparent cardiac NE spillover using their model and our model.

There are potential limitations to this investigation. One of these is the utilization of LVEF as a measure of LV systolic performance. It is well appreciated that in the setting of chronic MR, the low-impedance left atrium maintains ejection (28, 32) and therefore LVEF, whereas myocardial performance may deteriorate. This deterioration in myocardial performance can be characterized by utilizing more sophisticated indexes of contractility such as $E_{es}$. This is a relatively load-independent index of contractility (29, 36) that can characterize the state of the myocardium in patients with chronic MR and a normal LV EF and predict individual LV systolic performance response characteristics to successful mitral valve surgery (31). These kinds of data suggest that more sophisticated indexes of contractility are better indicators of the status of LV systolic performance than is LVEF. Consequently, in addition to LV end-systolic volume and EF, we compared our indexes of cardiac-specific sympathetic tone to $E_{es}$ and found an inverse relationship that was best characterized by a nonlinear function. Although the numbers of patients are small, the relationship is strong, and it is in stark contrast to the lack of relationship between systemic sympathetic tone and comparable indexes of contractility. In addition to the assessment of LV systolic performance in patients with chronic MR, another limitation pertains to the assumptions implicit in the model development, particularly that the cardiac NE mass transport system is in comparable steady state to the systemic NE mass transport system, and net cardiac NE production is negligible in the time frame of our experimental protocol. It is important to emphasize that our method assesses NE release rate into the interstitial fluid, whereas other methods have been developed to assess neuronal NE release, including an examination of the exchange between NE storage vesicles and the synapse. Eisenhofer et al. (5) have estimated rates of cardiac neuronal NE synthesis, release, reuptake, and production using an experimental approach that involves concurrent infusions of $[^{3}H]$NE and $[^{3}H]$epinephrine with measurements of NE metabolites over a 3-h period. Despite the methodological differences between these two approaches, as well as that of Kopin et al. (18), the estimates obtained for cardiac NE release rate into the interstitial compartment are comparable.

In conclusion, this study introduces a new method for assessing cardiac-specific NE release into the interstitial fluid of an organ in general and the heart in particular. The method was shown to provide measures of both total and newly released NE into the interstitial fluid of the heart. The total NE release rate into the interstitial fluid obtained using this method is comparable to the result obtained by Kopin et al. (18) using an entirely different model and experimental technique. The key parameter that links the two approaches is SA$_{T}$. However, our study presents an alternative approach utilizing an infusion of a single radioisotope to estimate the “unknown” specific activity of $[^{3}H]$NE in the interstitial fluid of an organ. Our study extends the previous methods by providing a measure of newly released NE into the interstitial fluid of an organ, which may provide a more specific measure of cardiac-specific SNS activity in humans. Moreover, in contrast to SP$_{NE}$, our new cardiac-specific NE kinetic modeling parameters were strongly related to measures of LV size and systolic performance in patients with chronic MR, producing data to support the concept that they also have physiological relevance.

APPENDIX

Basic Analytic Theory

The general form of the continuous-time state-space equation governing the evolution of the state of variables in the cardiac-specific NE mass transport system model (Fig. 1 and Eq. 1) is defined by

$$M = KM + Bu$$
where \( K \) is the system state or evolution matrix, \( B \) is the input or control matrix, and \( C \) is the output or observation matrix. The \( p^{th} \) element of the \( n \times 1 \) vector \( M \) contains the contents of NE mass of species \( i \) at time \( t \) \((i = M_i, \text{ and } j = M_j)\). Equation A3 is solved subject to specification of the initial conditions of the experiment. For the initial condition \( M_i(0) = u.d(t) \), i.e., a bolus injection of magnitude \( u \) into the organ inflow at time \( 0 \), the \( p^{th} \) element of the \( n \times 1 \) vector inputs \( [u(t)] \) will be zero if there is no input into the \( p^{th} \) zone; otherwise, it will contain a Dirac delta function multiplied by a constant that depends on the magnitude of the input. The vector \( u(t) \) can also contain time-independent constants describing the rate of infusion of labeled NE into the system.

The \( n \times n \) evolution matrix of transfer rate coefficients (\( K \)) has the form

\[
K = \begin{bmatrix} -k_{i1} & k_{i2} \\ k_{j1} & -k_{j2} \end{bmatrix} \tag{A2}
\]

where \( k_{i1} = k_{i01} + k_{i12} \) and \( k_{j2} = k_{j02} + k_{j12} \) and, in correspondence with Eq. A1, \( k_{i01} = k_{i}\) CE, \( k_{j12} = k_{jF}\), and \( k_{j02} = k_{j}\) n. Physiological reality imposes restrictions on the elements of the evolution matrix so that all \( k_{ij} \) are nonnegative, the diagonal terms \(-k_{ij}\) are nonpositive, and the column sums are nonpositive.

The evolution matrix \( K \) governs the mass transport of NE within the system and is used to determine the solution to Eq. A3. The solution is given by

\[
M = e^{KM}(0) + \int_0^t e^{K(t-\tau)}U(\tau)d\tau \tag{A3}
\]

where \( M(0) \) is the \( n \times 1 \) vector of initial conditions; \( e^{KM} \) is obtained by computing the eigenvalues \( (A_1, A_2) \) by solving the characteristic polynomial \( \Delta(\lambda) = \det(K - \lambda I) \) = 0 and the corresponding \( n \times n \) matrix of eigenvectors

\[
A = \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{bmatrix}
\]

as the solution of \( KA = \lambda A \) and using the relationship

\[
e^{KA} = e^{AA_1^{-1}} = A e^{A_1^{-1}} \tag{A4}
\]

where \( e^{A} \) is the \( n \times n \) diagonal matrix with elements \( e^{A_{ij}} \).

The average time that a molecule of species \( i \) of NE remains in a given zone is intimately related to the negative inverse of the matrix \( K \). From the elementary theory of infinite series, the exponential function \( \exp(t) = e^t \) has the series expansion

\[
e^t = \sum_{n=0}^{\infty} \frac{t^n}{n!} \tag{A5}
\]

The integral can be obtained by integrating the matrix infinite series term by term

\[
\int_0^t e^{K(t-\tau)}U(\tau)d\tau = \left[ K + \frac{K^2}{2!} + \ldots \right] dt = \int_0^t e^{K(t-\tau)}U(\tau)d\tau = \left[ K + \frac{K^2}{2!} + \ldots \right] dt
\]

\[
= K + \frac{K^2}{2!} + \ldots
\]

\[
= K^{-1}[e^{Kt} - I] \tag{A7}
\]

Hence, if \( e^{Kt} \rightarrow 0 \), as \( t \rightarrow \infty \)

\[
\int_0^t e^{K(t-\tau)}U(\tau)d\tau = -K^{-1} \tag{A8}
\]

Consequently

\[
- K^{-1} = \begin{bmatrix} k_{i1}^{-1} & k_{i2}^{-1} \\ k_{j1}^{-1} & k_{j2}^{-1} \end{bmatrix} \tag{A9}
\]

where the elements of the \( n \times n \) matrix of NE species mean residence times are the \( k_{ij}^{-1} \). Given a known input function \( u(t) \) into compartment \( j \) and the corresponding response \( f_j(t) \) in compartment \( i \), the \( p^{th} \) column elements of \( -K^{-1} \) can be obtained for a constant input rate \( u_j(t) \) corresponding steady-state \( [\text{H}] \) NE tracer activity level \( f_j(\infty) \) in zone \( j \)

\[
k_{ij}^{-1} = \frac{f_j(\infty)}{u_j(t)} \tag{A10}
\]

So the elements of \( -K^{-1} \) have a simple interpretation: \( -k_{ij}^{-1} \) is the mean residence time that a molecule of NE mass of \( i \) due to a unit steady-state input rate \( u_j(t) \) resides in zone \( j \). Alternatively, \( -k_{ij}^{-1} \) equals the average number of NE molecules of species \( i \) generated by a unit steady-state input rate \( u_j(t) \) of NE molecules of species \( j \).

### NE Species Mass Transport Rates

Let \( M_i \) be the NE mass associated with species \( j \), and let \( M \) be a diagonal \( n \times n \) matrix of the NE masses, \( \text{diag}(M_1, M_2) \). Here, \( M_1 \) represents \( M_{CE} \), and \( M_2 \) represents \( M_{DF} \). The matrix of NE mass transport rates (\( M \)) is defined as

\[
M = KM = \begin{bmatrix} k_{i1}M_1 & k_{i2}M_2 \\ k_{j1}M_1 & k_{j2}M_2 \end{bmatrix} = \begin{bmatrix} M_1 & M_2 \\ M_1 & M_2 \end{bmatrix} \tag{A11}
\]

The elements \( M_i \) are the steady-state NE mass transport rates of species \( j \) to the zone of origin of species \( i \). Let \( M_{ij}^{-1} \) be the elements of the inverse of the \( M \) matrix. Because \( M = KM \), the elements of the inverse of \( M \) can be defined in terms of NE mass and NE species mean residence times

\[
M_i^{-1} = M_{ij}^{-1}K_{ji}^{-1} = \begin{bmatrix} k_{i1}^{-1} & k_{i2}^{-1} \\ M_1 & M_2 \end{bmatrix} \tag{A12}
\]

Defining \( M_{ij}^{-1} = 1/k_{ij}^{-1}M_{ij} \) and its reciprocal as \( M_{ij}^{-1} = M_{ij}/k_{ij}^{-1} \)

\[
M_{ij}^{-1} = \begin{bmatrix} \frac{1}{a_{11}} & \frac{1}{a_{12}} \\ \frac{1}{a_{11}} & \frac{1}{a_{22}} \end{bmatrix} = \begin{bmatrix} M_{ij}^{-1} & M_{ij}^{-1} \tag{A13}
\]

Because each element of \( M_{ij}^{-1} \) is defined as a ratio of mean residence time to steady-state NE mass, it has units of \((\text{mass/time})^{-1}\). The reciprocal \( (M_{ij}^{-1}) \) has units of mass/time. Thus \( M_{ij}^{-1} \) is the rate at which NE mass of species \( i \) would have to appear in zone \( j \) to account for the total NE mass of species \( i \) in zone \( j \).

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