Different relationships of spillover to release of norepinephrine in human heart, kidneys, and forearm

IRWIN J. KOPIN,1 BENGT RUNDQVIST,2 PETER FRIBERG,2 JACQUES LENDERS,3 DAVID S. GOLDSTEIN,1 AND GRAEME EISENHOFER1

1Clinical Neuroscience Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892; 2Department of Physiology, Göteborg University, Göteborg, S-413 45 Sweden; and 3Department of Internal Medicine, St. Radboud University Hospital, Nijmegen, 6523GA The Netherlands

Kopin, Irwin J., Bengt Rundqvist, Peter Friberg, Jacques Lenders, David S. Goldstein, and Graeme Eisenhofer. Different relationships of spillover to release of norepinephrine in human heart, kidneys, and forearm. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R165–R173, 1998.—Spillover of norepinephrine (NE) into plasma is used frequently as an index of NE release and therefore of sympathetic nerve activity. An important limitation of NE spillover is that it reflects not only release but also uptake processes that intervene before the transmitter reaches the circulation. To overcome this limitation, we developed a method for estimating NE release based on measurements of the specific activities of [3H]NE in plasma and interstitial fluid during intravenous infusion of [3H]NE. We applied this method to examine relationships among NE release, tissue uptake, and spillover in the human heart, kidneys, and forearm. The sum of uptake and spillover of released NE provided an estimate of NE release into the interstitial fluid. In the kidneys, NE release averaged three times NE spillover, in skeletal muscle, 12 times NE spillover, and in the heart, >20 times NE spillover. Thus NE release greatly and variably exceeds NE spillover from these organs, so that assessing regional sympathetic function requires an understanding of the relationship of NE spillover to NE release.

catecholamines; normetanephrine; metanephrine; kinetics; sympathetic nervous system

THE PLASMA CONCENTRATION OF NOREpinephrine (NE), the neurotransmitter released at sympathetic nerve terminals throughout the body, is commonly used as an index of sympathetic nerve activity (17, 26).

Several important caveats limit the validity of plasma NE levels as an index of sympathetic “tone.” First, both clearance of NE from the bloodstream and the rate of NE entry into the bloodstream (spillover) determine plasma NE levels. To avoid this problem, Esler et al. (10) used the specific activity (SA) of plasma [3H]NE measured during intravenous infusion of [3H]NE to estimate NE clearance from and NE spillover into the systemic circulation.

“Total body” NE spillover, however, also has limitations, because sympathetic outflows differ among tissues and organs. For example, sympathetic activity in the arm influences local NE spillover, and so changes in antecubital venous plasma NE levels may not reflect changes in sympathetic activity elsewhere in the body (15, 25).

To avoid this limitation, the tracer dilution method was applied subsequently to estimate regional NE spillover (12, 20). Measurements of regional NE spillover now constitute the main method for assessing local sympathetic activity neurochemically (8, 9, 12) in investigating effects of drugs, exercise (31, 34), meal ingestion (3), aging (14, 33, 34), and disease states (6, 10, 13, 30).

Regional NE spillover rates may also be misleading, however. Because most released NE undergoes neuronal reuptake before entering the bloodstream (4), small differences in NE reuptake may cause large proportional changes in NE spillover (6). This is especially important in organs such as the heart, where neuronal uptake figures prominently in the inactivation of released NE (18).

The present report introduces a method for estimating the rate of release of NE into the interstitial fluid in an organ and applies the method to examine differences among organs in the proportion of released NE that spills over into the venous outflow. Because NE in the interstitial fluid either undergoes removal by tissue uptake or else spills over into the venous outflow, the rate of NE release is the sum of NE spillover and the rate of local uptake of released NE. The same isotope dilution principle used to estimate rates of total body or regional NE spillover was used to estimate tissue uptake of released NE from the interstitial fluid. Just as endogenous NE dilutes [3H]NE in the bloodstream, it also dilutes [3H]NE in the interstitial fluid compartment; however, in contrast to the SA of arterial and venous plasma [3H]NE, which can be measured directly, the SA of [3H]NE in the interstitial fluid was estimated indirectly. Because the sole source of normetanephrine (NMN) production within an organ is NE entering nonneuronal cells from the interstitial fluid, the SA of [3H]NMN formed in the region was assumed to equal that of [3H]NE in the interstitial fluid. The rate of NE release was then calculated from the sum of regional spillover and local uptake of released NE. Using this approach, we estimated rates of NE release and examined relationships among NE release, uptake, and spillover in the human heart, kidneys, and forearm.

METHODS

Subjects. Data were obtained as part of ongoing protocols at the National Institutes of Health (Bethesda, MD), at the University of Göteborg (Göteborg, Sweden), and at St. Rad...
boud's University Hospital (Nijmegen, The Netherlands) as described previously (7). Informed consent was obtained from each subject, and all procedures were approved by the appropriate institutional review boards.

Parts of the data for the present report were gleaned from the values in the previous report (7). Included are data for only those subjects in whom simultaneously collected arterial and venous samples were obtained and analyzed for epinephrine (Epi), NE, and their O-methylated metabolites. Arterial, coronary sinus, and renal venous blood was obtained from 11 male normal volunteers (aged 29-50) in Göteborg, and arterial and forearm venous blood from 10 subjects (5 normotensive and 5 hypertensive, 6 males and 4 females, aged 28-47) was obtained from subjects in Nijmegen.

Catheterization and isotope administration. In Göteborg, the clinical studies were conducted in a cardiac catheterization laboratory, in the morning, and at least 12 h after any medications, smoking, or ingestion of caffeinated beverages. Under local anesthesia, a cannula was inserted into a radial or brachial artery. Another catheter was advanced under fluoroscopic guidance via an internal jugular vein into the coronary sinus to sample coronary venous blood and measure coronary sinus blood flow or via a femoral vein into the right renal vein to obtain renal venous blood. Coronary sinus blood flow was measured by thermodilution immediately before each collection of blood, and renal blood flow was measured by the clearance of p-aminohippurate from arterial plasma.

[3H]NE (levo-2,5,6-[3H]NE, 40-60 Ci/mmol; New England Nuclear, Boston, MA) was infused via a forearm vein at a rate of 1.0-1.5 mCi/min in combination with a similar amount of [3H]Epi (levo-N-methyl-[3H]Epi, 65-75 Ci/mmol, also from New England Nuclear). Arterial and coronary venous blood samples (10-20 ml) were obtained at least 15 min after the start of radiotracer infusions and collected into ice-chilled tubes containing heparin or EDTA. Plasma was separated and stored as described in Analysis of blood samples.

In Nijmegen, studies of forearm NE kinetics were conducted in a patient observation room at an ambient temperature of 21-22°C. All subjects were studied in the supine position and had abstained from nicotine, alcohol, and caffeinated beverages for at least 12 h before the study. Forearm blood flow was measured in the limb opposite to that used for infusion of [3H]-labeled catecholamines, using venous occlusion strain-gauge plethysmography, with circulation to the hand excluded by inflation of a wrist cuff to 100 mmHg above systolic pressure for the duration of each blood flow determination. Blood samples were obtained simultaneously from a brachial artery and a deep antecubital vein of the limb in which blood flow was measured, with cutaneous venous blood excluded by inflation of the wrist cuff during the short interval of blood withdrawal to exclude blood from the hand, and the plasma was collected and stored as described below.

Analysis of blood samples. Plasma was separated by centrifugation at 4°C and kept below -80°C until assayed for catecholamines and their O-methylated metabolites. Plasma catecholamines and their O-methylated metabolites were assayed by liquid chromatography with electrochemical detection (7, 22, 27). Tritium contents of timed collections of the effluent leaving the electrochemical cell were measured by liquid scintillation spectroscopy. Interassay and intra-assay coefficients of variation for NE were 6.5 and 1.9%. Coefficients of variation were 12.2% for NMN and 11.2% for MN (inter assay) and 4.2% for NMN and 3.3% for MN (intra-assay). SA (dpm/pmol) were calculated from the ratios of the concentration of [3H]-labeled (dpm/ml) and unlabeled compound (pmol/ml) in each plasma sample.

Calculation of NE release into interstitial fluid. Intravenous infusion of a radiolabeled compound to determine the rate of endogenous production of the compound was introduced by Stetten et al. (36) and popularized by Steele et al. (35). The equation for the rate of entry (N) of an endogenous compound into the plasma compartment from sources not derived from the infused radioactive substance is

\[ \text{SA}_p \cdot (I + N) = \text{SA}_p \cdot I \quad (1a) \]

or

\[ N = I \cdot (\text{SA} \cdot \text{SA}_p - 1) \quad (1b) \]

where \( \text{SA}_p \) and \( \text{SA}_p \) are the SA of the infused and the mixture of infused with endogenous compound in the plasma, and \( I \) is the rate of infusion of the radioactive compound. If the infused compound has a high SA, \( I \) is negligible compared with \( N \), and the equation reduces to \( N = I \cdot \text{SA}_p \), where \( *I \) is the rate of infusion of the tracer radioactivity (\( \text{SA}_p \cdot I \)). This approach was first applied to NE by Esler et al. (10) to estimate the total \( N \) of endogenous NE into plasma (total body spillover, \( \text{SO}_T \)), as follows

\[ \text{SO}_T = *I \cdot \text{SA}_p \quad (2) \]

where \(*I\) is the rate of infusion of [3H]NE and \( \text{SA}_p \) is the specific activity of [3H]NE in arterial plasma.

Equation 1b can be applied also to measure the rates at which endogenously released NE spills over into the venous outflow or is taken up in the tissue (Fig. 1). In Fig. 1, the rate of NE release (\( R \)) is the sum of the rates of tissue uptake (\( U_1 \) and spillover (\( \text{SO}_1 \)). The rate of inflow (pmol/min) of arterial [3H]NE is the plasma flow rate (F) (pmol/min), multiplied by the arterial plasma NE concentration (NEa) (pmol/ml). Because a fraction (\( E \)) of arterial NE is extracted during passage of blood through the tissue, the fraction of arterial NE that enters the venous outflow is \( 1 - E \). The fraction \( 1 - E \) is determined from the ratio of the venous to arterial concentrations of [3H]NE (\( \text{[3H]NE}_v / \text{[3H]NE}_a \)). SO is the rate of venous outflow of NE derived from endogenous NE released into the interstitial fluid (equivalent to \( N \) in Eq. 1b). SO was calculated from the SA of arterial and venous [3H]NE (\( \text{SA}_a \) and \( \text{SA}_v \), respectively).

![Fig. 1. Diagrammatic representation of plasma-interstitial fluid (IF) model for estimating norepinephrine (NE) release, uptake, and spillover.](http://apregu.physiology.org/Downloadedfrom)
and the entry rate of arterial NE (equivalent to I in Eq. 1b) into the venous outflow, \((1 - E) \cdot F \cdot NE_a\)

\[ SO = F \cdot NE_a \cdot (1 - E) \cdot [(SA_a / SA_v) - 1] \]  

(3)

An analogous equation, with \(SA\) instead of \(SA_v\), was used to estimate the \(U_t\) of NE from the interstitial fluid that was released derived from NE

\[ U_t = F \cdot NE_a \cdot E \cdot [(SA_a / SA_v) - 1] \]  

(4)

The total NE released into the interstitial fluid is the sum of \(U_t\) and \(SO\)

\[ R = F \cdot NE_a \cdot E \cdot [(SA_a / SA_v) - 1] + (1 - E) \cdot [(SA_a / SA_v) - 1] \]  

(5)

For comparisons among subjects, NE spillover rates were normalized for the total body NE spillover in each subject. Because hepatic removal of NE masks NE spillover and release in the splanchnic circulation (5), the total body NE release rate cannot be determined. Therefore, NE release rates in each of the tissues, like regional spillover rates, were expressed as percentages of the total NE spillover into the arterial plasma. The calculated rates of forearm muscle NE spillover and release were used to estimate total body skeletal muscle spillover and release (7).

The calculation of \(SA\). The SA of NE in the interstitial fluid \((SA_a)\) was measured indirectly by assuming all of the NMN formed in the tissue is derived from NE in the interstitial fluid. Thus the SA of \([3H]NE\) formed in the tissue is derived from NE in the interstitial fluid. The spillover rates of \([3H]NMN\) (dpm/min) and of NMN (pmol/min) were determined from the plasma F, from concentrations in arterial (\(NMNa\)) and venous (\(NMNv\)) plasma, and from an extraction fraction \((EM)\)

\[ SO_{NMN} = F \cdot [NMN_v - (1 - EM) \cdot NMN_a] \]  

(6)

The extraction fraction of metanephrine (MN) is similar to that of NE (7). Thus the extraction fraction for MN \((EM)\) was used in Eq. 6 as the extraction fraction of NMN. The values for \(EM\) were determined using the rates of MN formation from circulating endogenous Epi during a constant infusion of \([14C]Epi\) (7). The ratio of the spillover rate of \([3H]NMN\) (dpm/min) to the spillover of MN (pmol/min) provided an estimate of the SA of \([3H]NMN\) formed in the tissue (dpm/pmol) and therefore was assumed to equal \(SA\).

Statistical methods. Results are expressed as means ± SE. Differences were assessed by ANOVA or by paired or unpaired Student's t-tests as appropriate. Statistical significance was defined as \(P < 0.05\).

RESULTS

Plasma amine levels. In the forearm and kidney, venous plasma NE concentrations were higher than arterial, whereas in the heart, arterial and venous NE concentrations were similar (Table 1). Coronary sinus plasma contained higher MN concentrations than did arterial plasma, renal venous plasma contained lower NMN levels than arterial, and forearm venous plasma contained similar NMN concentrations to arterial. In all three organs, venous MN concentrations were significantly lower than arterial concentrations.

<table>
<thead>
<tr>
<th>Organ</th>
<th>n</th>
<th>Norepinephrine</th>
<th>Metanephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>11</td>
<td>87.0 ± 0.9</td>
<td>27.5 ± 1.8</td>
</tr>
<tr>
<td>Kidneys</td>
<td>11</td>
<td>55.9 ± 3.8*</td>
<td>43.4 ± 1.6*</td>
</tr>
<tr>
<td>Forearm</td>
<td>10</td>
<td>68.5 ± 3.2†</td>
<td>41.6 ± 4.5†</td>
</tr>
</tbody>
</table>

Values are means ± SE in percentages. Extraction fractions for \([3H]norepinephrine\) (NE) were determined from differences in arterial and venous concentrations of \([3H]NE\) divided by arterial concentrations. Extraction fractions for \([3H]metanephrine\) (MN) were determined from differences in arterial and venous concentrations of \([3H]MN\) (corrected for small amount of \([3H]MN\) formed from \([3H]Epi\) taken up in tissues) divided by arterial concentration of \([3H]MN\) as previously described (9). *\(P < 0.01\) compared with heart; †\(P < 0.01\) compared with kidneys.

Table 1. Concentrations of norepinephrine, normetanephrine, and metanephrine in arterial and venous plasma from heart, kidneys, and forearm

Table 2. Extraction fractions for \([3H]norepinephrine\) and \([3H]metanephrine\)

Amine extraction fractions. The extraction fraction of \([3H]NE\) was largest in the heart and lowest in the kidney (Table 2). The extraction fraction of MN was significantly greater in the kidney and muscle than in the heart.

SA. The SA of \([3H]NMN\) in arterial plasma was higher than that in cardiac venous plasma and lower than that in renal venous plasma (Table 3). In the forearm, there was no significant difference between the SA of arterial and venous \([3H]NMN\).

The SA of \([3H]NMN\) formed in the heart and forearm were significantly lower than those of \([3H]NMN\) in arterial plasma, whereas the SA of \([3H]NMN\) formed in the kidney was higher than that in arterial plasma. The SA of \([3H]NMN\) in the venous plasma from each organ was between that of arterial \([3H]NMN\) and \([3H]NMN\) formed in the organ (Table 3).

Values for \(SA_a\) for the heart and kidneys differed slightly (Fig. 2), because arterial blood was obtained at different times, to correspond with the coronary sinus and renal venous sampling. In the heart, \(SA_a\) was 23-fold greater than \(SA_v\); in the forearm, \(SA_v\) was 21.5-fold greater, and in the kidney, \(SA_v\) was 5.7-fold greater than \(SA_a\). In each tissue, \(SA_v\) was less than \(SA_a\) but higher than the corresponding \(SA_a\).

NE spillover and release rates. Total body NE spillover averaged 2,430 ± 284 pmol/min. The mean NE spillover from the kidneys was over tenfold greater...
Table 3. Specific activities of arterial and venous plasma [3H]normetanephrine

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Kidney</th>
<th>Forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>119.2 ± 10.2</td>
<td>118.3 ± 10.1</td>
<td>83.7 ± 9.0</td>
</tr>
<tr>
<td>Venous</td>
<td>88.1 ± 6.6†</td>
<td>142.3 ± 12.3‡</td>
<td>71.9 ± 9.7</td>
</tr>
<tr>
<td>SAi</td>
<td>42.5 ± 3.2‡</td>
<td>177.3 ± 27.7‡</td>
<td>60.5 ± 15.5‡</td>
</tr>
</tbody>
</table>

Values are means ± SE in dpm/pmol; n = 11 in heart and kidney and 10 in forearm. Heart and kidney measurements were taken from same subjects. Interstitial fluid specific activity (SAi) was estimated from SA of [3H]normetanephrine formed in tissues (see Calculation of SAi). *P < 0.025, †P < 0.01 vs. arterial NE; ‡P < 0.01 vs. venous and arterial NE.

than from the heart (Fig. 3), representing 29 vs. 2.6% of the total body NE spillover. In contrast, the mean NE release rate from the kidneys was only two times that in the heart. NE spillover was a much smaller proportion of NE release in the heart than in the kidney (4.8 vs. 34%).

Rates of NE spillover and release into interstitial fluid in the forearm averaged 0.90 ± 0.16 and 10.4 ± 1.31 pmoles/min·100 g⁻¹. The proportion of released NE that spilled over into the venous outflow was 33% in the kidneys, 8.4% in the forearm, and 4.8% in the heart (Fig. 3). NE spillover from skeletal muscle represented 20.5 ± 4.1% of total body NE spillover. The release rate of NE in total body skeletal muscle averaged 2.5-fold greater than in the kidneys and about fivefold greater than in the heart (Fig. 2).

**DISCUSSION**

The present report introduces a method for estimating rates of NE release into and uptake from interstitial fluid. Separately quantifying NE release, uptake, and spillover provides a more precise assessment of regional sympathetic function.

The rate of uptake of NE released into the interstitial fluid (U, in Fig. 1), added to the regional spillover, is the release rate of NE into the interstitial fluid. Measurement of regional spillover is based on the difference between the SA of [3H]NE in arterial and venous plasma. Analogously, measurement of regional uptake of the NE released into interstitial fluid is based on the difference in SA of [3H]NE in the arterial plasma and of [3H]NE taken up into the tissue from the interstitial fluid.

The key new independent variable in this approach is SAi. Techniques that could be used to sample [3H]NE in the interstitial fluid directly, such as microdialysis and collecting lymph, cannot be applied easily in parenchymal organs of humans. A third possibility, used in the present study, is estimation of SAi from the SA of [3H]MN in the tissues.

Although it may not be intuitively obvious, when SAi is determined from uniform sampling of interstitial fluid [3H]NE, the calculated rate of NE release is valid whether or not there are gradients in NE and [3H]NE concentrations within the interstitial fluid (see Appendix).

There were striking differences among tissues in the relationships of NE release, uptake, and spillover. NE release averaged three times NE spillover in the kidneys, ~12 times spillover in the forearm, and >20 times spillover in the heart. Spillover therefore greatly and variably underestimates release. Esler et al. (11) estimated that the kidneys contribute ~25% of the SORF of NE and the heart contributes only ~3%. The present findings confirm this disparity but also show that it results from large differences among these organs in the proportion of released NE that undergoes local reuptake or spills over into plasma. Although the combined weights of the kidneys and the heart are about equal, blood flow to the kidneys is ~1,100 ml/min compared with 200 ml/min for the heart (24). Because NE spillover increases with plasma flow (21), the much greater proportion of released NE that spills over into the venous outflow from the kidneys (SO/R) than in the heart (34.4 vs. 4.9%) may in part result from the extraordinarily high blood flow through the kidneys. The mean rate of NE release into interstitial fluid in the kidneys was only about two times that in the heart. Because the content of NE in the kidneys is ~30% of that in the heart (29), the greater estimated NE release in the kidneys indicates that a larger proportion of NE stores is released per unit time in the kidneys than in the heart.

Forearm NE spillover was determined initially in units of picomoles per minute per 100 g of tissue and then extrapolated to total body skeletal muscle (7). The estimated rate of NE spillover from total body skeletal muscle was lower than that from the kidneys (20.5 ± 4.1 vs. 28.8 ± 2.7% of total body NE spillover). These values agree with previous estimates that the kidneys and muscle each contribute ~25% of the spillover of NE into systemic plasma (11). Because a much greater proportion of released NE is removed by uptake in the skeletal muscle than in the kidneys, regional spillovers fail to reflect the much greater NE release in the total body skeletal muscle than in the kidneys (Fig. 3).

Blood flow to skeletal muscle as a function of mass is much lower than to the heart (4 vs. 70 ml·min⁻¹·100
Despite the higher rate of blood flow to the heart, the ratio of spillover to release was remarkably small. The high density of cardiac sympathetic innervation may account for this by more efficient neuronal removal of NE from the interstitial fluid in the heart than in the skeletal muscle.

Other approaches have attempted to avoid the limitations of spillover as an index of NE release. Chang and colleagues (1) proposed the “plasma appearance rate,” defined as \( \frac{S}{R(1 - E)} \). In their model, a portion of interstitial fluid NE, together with plasma NE within the capillaries, is considered a single “plasma compartment.” This is a special case of Eq. 5, where \( S_A \) (for the plasma compartment) is equal to \( S_{A_{\infty}} \) because arterial NE inflow and unlabeled NE released into the plasma compartment mix completely before entering the venous outflow. The plasma appearance rate as defined by Chang et al. (1) is the minimum value for release of NE into the interstitial fluid (see Eq. 5). Thus, whereas Chang et al. (1) found that spillover was 25% of the plasma appearance rate, in the present study, only 8.4% of released NE spilled over into forearm venous plasma. Because the model proposed by Chang et al. (1) ignores diffusion barriers to NE, plasma appearance rate underestimates NE release.

To take into account diffusion barriers for NE entry into the interstitial fluid, Cousineau et al. (2) and Rose et al. (32) applied a multiple indicator technique introduced by Ziegler and Goresky (38). In this method, the concentrations of test tracer substances in the capillary and interstitial fluid compartments are assumed to vary as functions of distance along the capillary and of time after injection of the bolus containing the tracers. This is called a “distributed” model of capillary plasma-interstitial fluid exchange. An alternative (“tissue homogeneity”) model for kinetic analysis of transport with multiple indicators, as proposed by Johnson and Wilson (23), presumes a “well-mixed” interstitial fluid compartment. If the capillary entrances and exits were distributed randomly within the tissue and if there were diffusional interactions between adjacent capillaries, then complete mixing in the interstitial compartment would be simulated, as in the tissue homogeneity model (28). From the histological study by Wearn (37), the tissue homogeneity model may more closely represent cardiac capillary plasma-interstitial fluid exchange than does the distributed model.

Both models assume negligible NE concentration gradients between the region of NE release and the capillary-interstitial fluid barrier. If there were NE concentration gradients, release rates would be greater than calculated using either the distributed or tissue homogeneity model, both of which would then underestimate NE release. In humans and in experimental animals, there is about a threefold concentration gradient for NE concentration between the vascular neuroeffector junctions and the plasma (16, 19). This can explain why cardiac NE spillover was 13% of apparent NE release in the study reported by Rose et al. (32) compared with 4.9% in the present study. Another method for estimating cardiac NE spillover and release is based on effects of desipramine on arterial and coronary venous plasma levels of \(^3\text{H}\)-labeled and unlabeled dihydroxyphenylglycol (6). Results from studies using this independent method indicated that NE spillover in humans was 4.5% of estimated NE release, which agrees well with the value of 4.9% obtained in the present study. This agreement suggests that almost all NE released from the sympathetic nerves in the heart enters the interstitial fluid before it is recaptured. All the various methods available for assessing sympathetic nerve activity have limitations, including this one. The regional spillover method by Esler (8) and Esler et al. (11) and the plasma appearance method by Chang (1) underestimate NE release. The plasma appearance method provides an estimate of the minimal rate of NE release. The procedure used by Rose et al. (32) requires injection of a bolus of blood containing \(^{131}\text{I}\)-albumin, \(^{3}\text{H}\)glucose, and physiologically active amounts of \(^{3}\text{H}\)NE into the coronary artery as well as multiple rapid sampling of carotid sinus venous outflow and computer processing of the time-activity curves in the venous plasma. The distributed model also underestimates NE release because there are concentration gradients for NE in the interstitial fluid. Estimation of NE release based on desipramine-induced decrements in plasma levels of \(^3\text{H}\)-labeled and unlabeled dihydroxyphenylglycol (6) requires administration of desipramine to inhibit NE uptake and accurate measurements of \(^3\text{H}\)-labeled and unlabeled dihydroxyphenylglycol lev-
Perspectives

Biochemical assessment of sympathetic nerve activity has generally depended on measurements of NE released into the circulation. Although regional differences in NE spillover have been used to indicate the level of sympathetic activity, it was suspected that there may be differences among tissues in the proportion of released NE that reaches the effluent venous plasma. This report introduces a method for assessing regional NE release into the interstitial fluid based on determination of \( \text{SA} \), which in turn is estimated from the spillover of \(^3\text{H}\)-labeled and unlabeled MN and NMN formed in the tissue. The results show that the proportion of released NE that spills over from the interstitial fluid into venous plasma differs substantially among tissues; the proportion is lowest in the heart and highest in the kidneys.

APPENDIX

The tissue homogeneity and distributed models of capillary plasma-interstitial fluid exchange (Fig. 4) are the bases of two sets of equations (A and B, respectively) describing release of NE into the interstitial fluid.

Tissue homogeneity model. In the tissue homogeneity model, during a steady state, concentrations of NE are a function of distance (x) from the entry point into the exchanging portion of the capillary length, but the concentration of NE in the interstitial fluid is uniform. The concentration of NE \( \left( [\text{NE}]_i \right) \) in a plug of plasma flowing through the capillary is defined by

\[
F \cdot [\text{NE}]_a + P \cdot dS \cdot [\text{NE}] = F \cdot [\text{NE}]_a + P \cdot dS \cdot [\text{NE}]_b (A1)
\]

where \([\text{NE}]_a\) = interstitial NE concentration, \(P = \text{permeability (cm/s)}, S = \text{surface area (cm}^2\), \(L = \text{length of the capillary (cm)}, \) and \(F = \text{flow (cm}^3\text{/s)}, \) and all are constant. Also, \(dS/dx = S/L\). Therefore

\[
F \cdot \frac{d[\text{NE}]_b}{dx} = P \cdot \frac{dS}{x} \cdot (\text{[NE]}_a - [\text{NE}]_b)
\]

Thus

\[
[\text{NE}]_b = [\text{NE}]_a - \left( [\text{NE}]_b - [\text{NE}]_i \right) \cdot e^{-P \cdot S \cdot L/F} x (A2)
\]

The solution to this differential equation is

\[
[\text{NE}]_b = [\text{NE}]_a - \left( [\text{NE}]_b - [\text{NE}]_i \right) \cdot e^{-P \cdot S \cdot L/F} x (A3)
\]

where \([\text{NE}]_a\) = arterial NE concentration. The NE concentration at the venous end of the capillary \((x = L)\) is then

\[
[\text{NE}]_b = [\text{NE}]_a - [\text{NE}]_b \cdot e^{-P \cdot S \cdot L/F} + [\text{NE}]_b (1 - e^{-P \cdot S \cdot L/F}) (A4)
\]

or

\[
[\text{NE}]_b = [\text{NE}]_b e^{-P \cdot S \cdot L/F} + [\text{NE}]_b (1 - e^{-P \cdot S \cdot L/F}) (A5)
\]

In the steady state, the total rate of NE entry into the capillary-interstitial fluid unit volume must equal the total rate of NE exit. Thus

\[
R + F \cdot [\text{NE}]_a = Cl_n \cdot [\text{NE}]_b + F \cdot [\text{NE}]_b (A6)
\]

where \(R = \text{release and } Cl_n = \text{clearance (cm}^3\text{/s)} \) of interstitial fluid NE by tissue sequestration or metabolism. Combining Eq. A5 and A6

\[
[\text{NE}]_b = [\text{NE}]_a \cdot e^{-P \cdot S \cdot L/F} + [\text{NE}]_b (1 - e^{-P \cdot S \cdot L/F}) (A7)
\]

\[
R + F \cdot [\text{NE}]_b = Cl_n \cdot [\text{NE}]_b + F \cdot [\text{NE}]_b (1 - e^{-P \cdot S \cdot L/F}) (A8)
\]

\[
[\text{NE}]_b = R + F \cdot [\text{NE}]_b (1 - e^{-P \cdot S \cdot L/F}) \]

\[
Cl_n + F \cdot (1 - e^{-P \cdot S \cdot L/F}) (A9)
\]

Substituting this in Eq. A5 (multiplied by \(F\))

\[
F \cdot [\text{NE}]_b = F \cdot [\text{NE}]_b \cdot e^{-P \cdot S \cdot L/F} (A10)
\]

\[
\frac{F \cdot (1 - e^{-P \cdot S \cdot L/F})}{Cl_n + F \cdot (1 - e^{-P \cdot S \cdot L/F})} = R + F \cdot [\text{NE}]_b (1 - e^{-P \cdot S \cdot L/F}) (A11)
\]

or

\[
F \cdot [\text{NE}]_b = F \cdot [\text{NE}]_b (1 - a + b) (1 - b)(R + F \cdot [\text{NE}]_b) (A12)
\]

where \(a = 1 - e^{-P \cdot S \cdot L/F}\) and \(b = Cl_n/(Cl_n + F \cdot (1 - e^{-P \cdot S \cdot L/F})\).
For tracer \[^3H\]NE (*NE), \(R = 0\) and the extraction fraction \((E)\) is
\[
E = a \cdot b
\] (A13)

The first term in Eq. A12 represents arterial NE that is not extracted during passage of blood through the capillary, and the second term, \((1 - b) \cdot R\), is SO. The SA of venous NE \((SA_v)\) is
\[
SA_v = \frac{F \cdot [*NE_a] \cdot (1 - a \cdot b)}{F \cdot [*NE_a] \cdot (1 - a \cdot b) + (1 - b) \cdot R}
\]
\[
= \frac{SA_a}{1 + \frac{1 - b \cdot R}{1 - a \cdot b \cdot F \cdot [*NE_a]}}
\] (A14)

\[
R = \frac{(1 - a \cdot b) \cdot (SA_a - SA_v)}{(1 - b) \cdot SA_v} \cdot F \cdot [*NE_a]
\]
\[
= (1 - E) \left(\frac{SA_a - SA_v}{SA_v}\right) \cdot F \cdot [*NE_a]
\] (A15)

From Eq. A9, the SA of interstitial NE is
\[
SA_i = \frac{F \cdot [*NE_a] \cdot (1 - e^{-P \cdot S \cdot F})}{F \cdot [*NE_a] \cdot (1 - e^{-P \cdot S \cdot F}) + R \cdot F \cdot [*NE_a] \cdot a}
\]
\[
= \frac{SA_a \cdot a}{C_n + F \cdot (1 - e^{-P \cdot S \cdot F})}
\]
\[
= (1 - E) \left(\frac{SA_a - SA_v}{SA_v}\right) \cdot F \cdot [*NE_a] \cdot a
\]
\]
\[
R = \frac{(SA_a - SA_v)}{SA_i} \cdot F \cdot [*NE_a] \cdot a
\] (A16)

From Eqs. A15 and A17
\[
R = \frac{(SA_a - SA_v)}{SA_i} \cdot F \cdot [*NE_a] \cdot a = \frac{1 - E \cdot (SA_a - SA_v)}{1 - b} \cdot \frac{F \cdot [*NE_a] \cdot a}{SA_v}
\]
\[
= \frac{(SA_a - SA_v)}{SA_i} \cdot a - \frac{(SA_a - SA_v)}{SA_i} \cdot E = (1 - E) \left(\frac{SA_a - SA_v}{SA_v}\right)
\]
\[
a = E + \frac{(SA_a - SA_v)}{SA_v} \cdot \frac{SA_i}{SA_a - SA_v} \cdot (1 - E)
\] (A19)

Substituting \(a\) in Eq. A17
\[
R = \frac{(SA_a - SA_v)}{SA_i} \cdot F \cdot [*NE_a]
\]
\[
= \left[ E + \frac{(SA_a - SA_v)}{SA_a - SA_v} \cdot \frac{SA_i}{SA_a - SA_v} \cdot (1 - E) \right]
\]
\[
= \frac{(SA_a - SA_v)}{SA_i} \cdot E + \frac{(SA_a - SA_v)}{SA_v} \cdot (1 - E) \cdot F \cdot [*NE_a]
\] (A20)

This equation is identical to Eq. 5 in METHODS.

Distributed model. The second set of equations (B) describes the distributed model of capillary plasma-interstitial fluid exchange (Fig. 4). During the steady state, the concentrations of both the capillary and interstitial fluid NE are independent of time but vary with distance along the length of the capillary. At a distance \(x\) from the entry point, \([NE]_x\), in a plug of plasma flowing through the capillary is defined by the following
equation
\[
F \cdot [NE]_x + P \cdot dS \cdot [NE]_x = F \cdot [NE]_x - [NE]_x
\]
\[
= F \cdot [NE]_x - [NE]_x
\] (B1)

where \(P =\) permeability \((cm/s)\), \(dS =\) surface area \((cm^2)\) of a length of capillary \(dx\), \(dCln =\) clearance \((cm^3/s)\) by tissue sequestration or metabolism of NE in the microvolume of interstitial fluid in equilibrium with the corresponding capillary segment, and \(F =\) flow \((cm^3/s)\), and all are constants. This equation differs from that in the well-mixed interstitial fluid model (depicted in Fig. 3) in that the interstitial NE concentration, \([NE]_x\), at the distance \(x\) along the capillary varies with distance along the capillary (total length \(L\), in cm). Because \(dS/dx = S/L\)

\[
F \cdot d[NE]_x/dx = P \cdot dS/dx \cdot [NE]_x = P \cdot S \cdot [NE]_x
\]
\[
= \frac{P \cdot S \cdot [NE]_x}{L}
\]
\[
[NE]_x = \frac{P \cdot S \cdot [NE]_x}{P \cdot S + Cln}
\]
\[
[NE]_x = \frac{P \cdot S \cdot R}{P \cdot S + Cln}
\]

Because the differentials are constants, i.e.
\[
dR/dx = R / L, \quad dS/dx = S / L, \quad \text{and} \quad dCln/dx = Cln / L
\]
\[
d[NE]_x/dx = \frac{P \cdot S \cdot [NE]_x}{P \cdot S + Cln}
\]
\[
= \frac{P \cdot S \cdot [NE]_x}{F \cdot L \cdot P \cdot S + Cln}
\]

When \(x = 0\), \([NE]_x = [NE]_b\). Thus the solution to this differential equation is
\[
[NE]_x = \frac{R}{Cln} + [NE]_b - \frac{R}{Cln} e^{-(P\cdot S / F \cdot L \cdot (P \cdot S + Cln))} \cdot x
\] (B6)

At the venous end of the capillary, \(x = L\) and the rate of NE outflow is
\[
F \cdot [NE]_v = F \cdot [NE]_b \cdot e^{-(P\cdot S / F \cdot Cln)}
\]
\[
+ \frac{F \cdot R}{Cln} \cdot (1 - e^{-(P\cdot S / F \cdot Cln)})
\]
\[
= \frac{F \cdot R}{Cln} \cdot (1 - e^{-(P\cdot S / F \cdot Cln)})
\] (B7)

where the first term represents arterial NE that is not extracted during passage of blood through the capillary and the second term represents SO. Because \(R = 0\) for tracer \[^3H\]NE (*NE), the extraction fraction \((E)\) is
\[
E = 1 - e^{-(P\cdot S / F \cdot Cln)}
\] (B8)
Thus Eq. B7 can be expressed in terms of $E$

$$F \cdot [NE]_b = F \cdot [NE]_a \cdot (1 - E) + F \cdot \frac{R}{C_l} \cdot E \quad (B9)$$

The SA of NE in the venous outflow is

$$SA_v = \frac{F \cdot ([*NE]_b \cdot (1 - E))}{F \cdot [NE]_b \cdot (1 - E) + \frac{R}{C_l} \cdot E} \quad (B10)$$

which can be rearranged to

$$\frac{R}{C_l} = \frac{SA_a - SA_v \cdot (1 - E)}{E \cdot [NE]_b} \quad (B11)$$

From Eqs. B3 and B6, the concentration of NE in the interstitial fluid at distance $x$ along the capillary is

$$[NE]_x = \frac{R}{P \cdot S + Cl_0} + \frac{P \cdot S}{P \cdot S + Cl_0} \quad (B12)$$

or

$$[NE]_x = \frac{R}{Cl_0 \cdot (P \cdot S + Cl_0)} \cdot \left(1 - \frac{R}{Cl_0} \cdot e^{-([P \cdot S] \cdot (F - L)) \cdot (Cl_0 + P \cdot S) \cdot x}\right) \quad (B13)$$

The mean NE concentration of NE in the interstitial fluid is

$$\langle [NE] \rangle = \frac{1}{L} \int_0^L [NE]_x \cdot dx \quad (B14)$$

The SA of interstitial NE ($SA_i$) is the mean concentration of $[^3H]NE$ divided by the mean concentration of unlabeled NE. Because for $[^3H]NE$, $R = 0$

$$SA_i = \frac{F \cdot ([*NE]_b \cdot E)}{R + F \cdot \left(\frac{[NE]_b - [NE]_a}{Cl_0}\right) \cdot E} \quad (B17)$$

and from Eq. B11

$$R = \frac{SA_a - SA_v \cdot (1 - E)}{E \cdot [NE]_b} + \frac{SA_a - SA_v \cdot (1 - E)}{E \cdot [NE]_b} \cdot F \cdot [NE]_b \quad (B20)$$

This equation is identical to Eq. A22 obtained for the well-mixed interstitial fluid model and to Eq. 5 in METHODS.

Address for reprint requests: I. J. Kopin, Clinical Neuroscience Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bldg. 10, Rm. 4D-20, 10 Center Drive, MSC 1424, Bethesda, MD 20892-1424.

Received 7J January 1998; accepted in final form 13 March 1998.

REFERENCES


