Does Regional Norepinephrine Spillover Represent Local Sympathetic Activity?

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Regional spillover of norepinephrine (NE), based on isotope dilution and single-compartment steady-state kinetics, is considered one of the best parameters for estimating organ sympathetic activity. However, the effects of local changes in clearance of NE on the spillover have not yet been investigated. We studied local NE kinetics and clearance in the forearm of 10 healthy subjects using intra-arterial infusions of NE, tritiated NE, the neuronal uptake inhibitor desipramine, and tyramine, which competes with NE for the neuronal uptake carrier. Before and during complete blockade of neuronal uptake by desipramine the venous concentration-time curves for tritiated NE and for NE released by tyramine were biexponential, consistent with the presence of (at least) two compartments for circulating tritiated NE and for locally released NE. The time constants for tyramine-induced release of NE and, in the same subjects during desipramine infusion, for tritiated NE were almost equal at the same level of forearm blood flow. This argues against possible diffusion or transport differences for NE to and from the circulation and the synapse. The regional intrinsic clearance capacity (a measure of the maximal ability of an organ to irreversibly remove drug by all pathways in the absence of any flow limitations) for NE decreased in the forearm by 65% (p<0.01) during neuronal uptake blockade by desipramine; the forearm clearance decreased by 59% (p<0.001), whereas the spillover rate of NE increased from 33±5 to 63±11 pmol min⁻¹ (p<0.05). Nitroprusside-induced increments in blood flow increased the spillover of NE from 18±4 to 35±6 pmol min⁻¹ (p<0.01); the clearance of circulating NE also increased (by 58%, p<0.05), and the intrinsic clearance capacity remained unchanged. This demonstrates that regional spillover of NE is markedly influenced by local changes in clearance and flow. The new parameter plasma appearance rate of NE is proposed. Although also derived from isotope dilution, this parameter may better approximate the regional entry of NE into the blood pool than spillover. This is corroborated by the nonsignificant changes of plasma appearance rate of NE during our desipramine and nitroprusside infusions. (Hypertension 1991;18:56–66)

Sympathetic outflow may occur in a differentiated rather than in a global pattern. For instance, in the early stages of essential hypertension indications for a preferential increase in sympathetic nerve activity to the heart and kidneys have been found.¹ Therefore, adequate measures for organ sympathetic activity are needed. Venous plasma norepinephrine can provide only limited information of organ sympathetic nerve activity¹,² because it reflects the balance between the sum of the input of norepinephrine (NE) into the organ via 1) the arterial inflow of NE and 2) the local release of NE and the organ clearance of locally released and arterially inflowing NE.³-⁶ The introduction of the organ spillover rate of NE, with the use of radiotracer methodology,⁷ clearly provided a better measure of regional sympathetic activity. However, regional spillover of NE does not account for local metabolism of released NE, and in previous studies we found indications that the regional spillover of NE from the forearm may be influenced by local changes in blood flow.³,⁸ The flow dependence of spillover and clearance of NE is further substantiated by a recent study and by pharmacological literature.⁹-¹¹

Recently, compartmental modeling has been applied to estimate the total body release of NE, and it seems to provide a way to isolate total NE release from the other processes that determine plasma levels of NE.¹² When applying compartmental modeling to organ kinetics of NE the appropriate model (i.e., based on physiological and anatomic knowledge the simplest model that adequately describes the system) has to be determined.
Relevant for applying compartmental modeling is that transport or diffusion of NE from the blood vessel lumen to the sites of metabolism do not differ for NE originating from the circulation compared with that from local release. Kopin et al. found no evidence for such a difference in pithed rats. However, in the endothelial lining of peripheral arteries an active transport mechanism for catecholamines may be present, and in canine myocardium a diffusion barrier has been found. In humans, the time lag between hemodynamic changes and the appearance of NE into the circulation during certain interventions suggest the presence of a diffusion barrier for NE between the synapse and the vascular space.

So, important questions for the study of organ NE release with compartmental modeling are what type of model adequately describes organ NE kinetics and whether transport of NE from the blood vessel lumen into the synaptic cleft differs from that in the opposite direction. In fact, these questions may also be relevant for other systems where local production and metabolism determine the resultant plasma concentrations. In the present study, we investigated the kinetics of circulating and locally released NE in the forearm, and since we used radiolabeled NE, we compared the removal of unlabeled and radiolabeled NE from the circulation in a range of blood flow.

Methods

Subjects

Ten healthy male volunteers, with a mean age of 26 years (range 21–31) and a mean body weight of 75 kg (range 62–95), participated after giving informed consent. In all subjects medical history, physical examination, and routine laboratory tests showed no evidence of cardiovascular or other diseases. None of the subjects took any medication either at the time of the study or in the previous 2 weeks. For at least 12 hours before the experiment all subjects refrained from smoking and from ingesting alcohol or caffeine-containing beverages. The protocol of the study was approved by the Medical Ethics Committee of the Leiden University Hospital.

Procedures

The methods used have been described in detail previously. All experiments were performed with the subjects in the supine position in a quiet room with a constant temperature of 22°–23°C. The brachial artery of the nondominant side was cannulated for intra-arterial monitoring of systolic and diastolic blood pressure and for blood sampling; in the same arm the brachial or basilar vein was cannulated for blood sampling. Forearm blood flow (FBF) was measured by computerized, R wave triggered, strain-gauge plethysmography. Intra-arterial blood pressure, heart rate, and FBF were determined four times per minute, using a microcomputer for analysis of the digitalized blood pressure and electrocardiographic and plethysmographic signals. The blood pressure and electrocardiographic and plethysmographic signals were also registered on a polygraph. Hand blood flow was not excluded during the experiments because of possible spurious effects of prolonged hand ischemia. Forearm volume was determined by water displacement. The measurements started at least 30 minutes after the cannulation procedures.

Study Protocol

The study protocol is depicted in Figure 1. All infusions were given intra-arterially.

Extraction of Radiolabeled and Unlabeled Norepinephrine

An intra-arterial infusion of 1-[2,5,6]-3H-norepinephrine ([3H]NE) (20 nCi-min⁻¹) was given for 48 minutes. During the 12-48-minute period, unlabeled NE (2.5 pmol·kg⁻¹·min⁻¹) was added. To increase local blood flow the nonspecific vasodilator sodium nitroprusside was added (15 pmol·kg⁻¹·min⁻¹ during the 24–36-minute period and 45 pmol·kg⁻¹·min⁻¹ during the 36–48-minute period). Hemodynamic parameters were measured immediately before and at the end of each 12-minute period. Venous blood samples were taken 2 minutes before and at the end of each 12-minute period, and arterial samples were taken at −2, 0, and 48 minutes.
Kinetics of Circulating Versus Endogenous Norepinephrine

In all 10 subjects two infusions of [3H]NE (50 nCi·min⁻¹) were given intra-arterially for 20 minutes, one before and one during neuronal uptake inhibition by desipramine (0.6 nmol·kg⁻¹·min⁻¹ i.a.). After both [3H]NE infusions, a 20-minute decay curve was made. The desipramine infusion was started 30 minutes before the second [3H]NE infusion. Venous blood samples were taken at 0, 1, 2, 4, 8, 16, and 20 minutes during and at 1, 2, 3, 4, 6, 8, 12, and 20 minutes after the end of the [3H]NE infusions. Arterial blood samples were taken before and at the end of each infusion.

In six of these subjects two intra-arterial infusions of tyramine (7.2 nmol·kg⁻¹·min⁻¹) were given for 20 minutes, one before and one during continued neuronal uptake inhibition by desipramine. After each tyramine infusion, a 20-minute decay curve was made. The tyramine infusions were given 20 minutes after the [3H]NE infusions. Blood samples were taken after the [3H]NE infusions. Plasma samples were taken before and at the end of each infusion.

Drug Solutions, Sample Collection, and Assay

[1]Norepinephrine, prepared according to the Dutch Pharmacopeia and l-[2,5,6]-3-H-norepinephrine (New England Nuclear, Boston, Mass.), specific activity 38.7 Ci·mmol⁻¹, radiochemical purity 95% tested by high-performance liquid chromatography (HPLC) with fractionated sampling, were diluted in 5% glucose on the morning of each investigation. Blood samples were collected into ice-chilled, heparinized tubes containing EGTA and reduced glutathione to yield final concentrations of 5 mM. Samples were centrifuged at 4°C and 3,500g for 15 minutes. Plasma was separated for determination of plasma NE by a single isotope, radioenzymatic assay and for determination of plasma [3H]NE. In 4-ml plasma samples 50 µl of 100 nM unlabeled NE was added, and catecholamines were extracted with a specific liquid extraction method, according to Smedes et al. In each extracted sample the recovery of the added unlabeled NE was determined by HPLC, and [3H]NE was determined by liquid scintillation counting. Plasma was stored at −75°C until assayed. Inter-assay coefficient of variation of the radioenzymatic assay was less than 5%, determined in plasma samples of approximately 0.5 nmol/l. After each experiment the NE and [3H]NE infusates were checked by HPLC and liquid scintillation counting.

Analysis

The hemodynamic values used for analysis represent the mean of six measurements made within 1.5 minutes. In the first infusion experiment the plasma catecholamine levels in the two blood samples taken at the end of each dose step were averaged because steady-state conditions had been reached.3,20

The fractional extraction of [3H]NE in the forearm was calculated using

\[
E = \frac{1 - Q \cdot V}{I}
\]

where E is fractional extraction; I is intra-arterial infusion rate of [3H]NE; Q is forearm plasma flow derived from forearm volume, FBF, and hematocrit; and V is venous plasma [3H]NE.

The E of intra-arterially infused NE in the forearm was calculated using

\[
E_{\text{of NE}} = \frac{I - Q \cdot V}{I}
\]

where V and V₀ are the venous plasma NE during and just before the NE infusion, and I is intra-arterial infusion rate of NE.

The intrinsic clearance (Clₜₐₐ), a measure of the maximal ability of an organ to irreversibly remove drug by all pathways in the absence of any flow limitations that is based on the Kₙ and Vₘₐₓ values of the eliminating processes (1 to n), is defined as

\[
Cl_{\text{intr}} = \frac{Q \cdot E}{1 - E}
\]

For the Clₜₐₐ of NE the fractional extraction is the fractional extraction of [3H]NE. The spillover rate of NE was calculated using

\[
SO = Q \cdot V - Q \cdot A + Q \cdot A \cdot E
\]

where SO is spillover rate, A is the arterial concentration of NE, and E is the fractional extraction of [3H]NE.

The newly proposed parameter for organ sympathetic activity, plasma appearance rate of NE, is formulated as

\[
PA = \frac{SO}{1 - E}
\]

where PA is plasma appearance rate. The derivation of this parameter, from the isotope-dilution method, is given in the "Discussion" section.

When unlabeled NE was infused, the calculations of spillover and plasma appearance rate were corrected for the infused NE by subtracting the nonextracted part of the NE infusate, that is, I(1 - E).

The individual concentration–time curves, using both infusion and decay parts of the curves, were used for nonlinear, least-squares curve fitting to the monoexponential model

\[
C_t = \frac{A}{\alpha \cdot T} (1 - e^{-\alpha \cdot t}) + C_0
\]

when t≤T (infusion part), and
TABLE 1. Clinical Data of 10 Healthy Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.3±1.0</td>
<td>21-31</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.3±3.2</td>
<td>62-95</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>126.9±3.5</td>
<td>113-152</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>58.0±1.4</td>
<td>52-65</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>61.9±1.7</td>
<td>55-73</td>
</tr>
<tr>
<td>Forearm blood flow (ml-dl⁻¹-min⁻¹)</td>
<td>4.9±0.9</td>
<td>1.8-10.5</td>
</tr>
<tr>
<td>Arterial plasma norepinephrine (nmol/l)</td>
<td>1.06±0.16</td>
<td>0.40-2.53</td>
</tr>
<tr>
<td>Venous plasma norepinephrine (nmol/l)</td>
<td>1.11±0.10</td>
<td>0.72-1.91</td>
</tr>
</tbody>
</table>

When t>T (decay part), or the biexponential model

\[ C_t = \frac{A}{\alpha} \cdot (1-e^{-\alpha \cdot t}) \cdot e^{-\alpha \cdot (t-T)} + C_0 \]

When t≤T, and

\[ C_t = \frac{A}{\alpha} \cdot (1-e^{-\alpha \cdot t}) + \frac{B}{\beta} \cdot (1-e^{-\beta \cdot t}) + C_0 \]

During the combined infusion of \[^{3}H\]NE and NE and the addition of the two doses of sodium nitroprusside, a good correlation was found between the fractional extraction of NE and the fractional extraction of \[^{3}H\]NE when the average of the three measurements per subject was taken (y = -0.02 + 1.0x, r = 0.85, p<0.05) were not significantly different from one and zero, respectively.

The values for the FBF, and the spillover rate, plasma appearance rate, clearance, and intrinsic clearance of NE before and during the sodium nitroprusside infusion are given in Table 2. During the addition of the two doses of sodium nitroprusside, the systolic blood pressure decreased 12.3±4.6 mm Hg (p<0.05), the diastolic blood pressure changed 0.7 ±1.5 mm Hg (NS), the heart rate went up 2.8±4.3 beats/min (NS), and FBF increased from 3.7±0.5 to 6.9±0.9 ml-dl⁻¹-min⁻¹ (p<0.01). The decrease in systolic blood pressure, without significant changes in diastolic blood pressure and heart rate, is probably best explained by the local vasodilating effect of sodium nitroprusside resulting in a Bernoulli phenomenon at the catheter tip that will be most pronounced at the highest flow (i.e., during systole). Even when infused directly into a vein the highest dose of sodium nitroprusside (45 pmol/kg/min, which is equivalent to 0.014 μg/kg/min) is unlikely to produce systemic effects. In addition, the fact that the reverse was found during the desipramine infusion supports this notion.

Results

Clinical data of the 10 subjects are summarized in Table 1.

Extraction of Radiolabeled and Unlabeled Norepinephrine

Because of errors in the NE infusates in three and \[^{3}H\]NE infusate in one, the fractional extraction of NE could be compared with the fractional extraction of \[^{3}H\]NE in only six subjects.
TABLE 2. Spillover, Plasma Appearance Rate, and Intrinsic Clearance Capacity of Norepinephrine and Forearm Blood Flow

<table>
<thead>
<tr>
<th>Infusion</th>
<th>FBF (ml·dl⁻¹·min⁻¹)</th>
<th>SO (pmol·min⁻¹)</th>
<th>PA (pmol·min⁻¹)</th>
<th>CI (ml·dl⁻¹·min⁻¹)</th>
<th>Cl_intrinsic (ml·dl⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[³H]NE infusion combined with NE and NIP (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[³H]NE</td>
<td>4.8±0.9*</td>
<td>28±2</td>
<td>114±12</td>
<td>3.3±0.5</td>
<td>13±2</td>
</tr>
<tr>
<td>[³H]NE+NE</td>
<td>3.7±0.6</td>
<td>18±4</td>
<td>90±23</td>
<td>2.6±0.3</td>
<td>11±3</td>
</tr>
<tr>
<td>[³H]NE+NIP dose I</td>
<td>5.0±0.8</td>
<td>28±7</td>
<td>102±29</td>
<td>3.2±0.5</td>
<td>11±3</td>
</tr>
<tr>
<td>[³H]NE+NIP dose II</td>
<td>6.9±0.9†</td>
<td>35±6†</td>
<td>126±40</td>
<td>3.9±0.6*</td>
<td>13±4</td>
</tr>
<tr>
<td>[³H]NE infusion before and during DMI (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[³H]NE</td>
<td>4.9±0.9</td>
<td>33±5</td>
<td>103±15</td>
<td>3.0±0.4</td>
<td>9.1±1.3</td>
</tr>
<tr>
<td>[³H]NE+DMI</td>
<td>2.3±0.4‡</td>
<td>63±11§</td>
<td>150±28</td>
<td>1.1±0.1†</td>
<td>2.7±0.5§</td>
</tr>
</tbody>
</table>

FBF, forearm blood flow; SO, spillover rate; PA, plasma appearance rate; CI, clearance; Cl_intrinsic, intrinsic clearance capacity; NE, norepinephrine; NIP, sodium nitroprusside; DMI, desipramine.

* p<0.05, † p<0.01 compared with [³H]NE+NE infusion.
§ p<0.05, ‡ p<0.01 compared with single [³H]NE infusion before DMI.

Disappearance of Circulating Norepinephrine and Appearance of Endogenous Norepinephrine

The kinetics of [³H]NE in the forearm fitted best to a biexponential curve with a first half time (t₁) of 1.4±0.2 minutes and a second half time (t₂) of 14.3±7.0 minutes. During neuronal uptake inhibition circulating [³H]NE kinetics were also biexponential with a t₁ of 2.8±0.3 (p<0.001 compared with the t₁ before neuronal uptake inhibition, see Figure 3) and a t₂ of 24.4±8.6 minutes (NS, see Figure 3). Changes in blood pressure, heart rate, and FBF during the two [³H]NE infusions, before and during neuronal uptake inhibition, were insignificant.

The appearance of endogenously released NE in venous plasma during the first tyramine infusion also fitted best to a biexponential curve, with a t₁ of 3.3±0.8 minutes and a t₂ of 19.6±6.2 minutes (see Figure 4). FBF decreased markedly from 7.4±1.9 to 3.6±1.0 ml·dl⁻¹·min⁻¹ (p<0.01). In the same six subjects who received tyramine, the FBF at the end of the [³H]NE with desipramine infusion was 3.4±0.7 ml·dl⁻¹·min⁻¹, with a t₁ of 2.8±0.3 minutes and a t₂ of 19.1±1.3 minutes. The second tyramine infusion, during neuronal uptake inhibition by desipramine, had no appreciable effect on the release of NE as venous plasma NE and FBF did not change significantly (see Figure 4). During both tyramine infusions the arterial plasma NE (see Figure 4), BP, and heart rate did not change significantly, indicating absence of systemic effects of tyramine.

During the infusion of desipramine the appearance of endogenously released NE in the venous effluent was found to fit best to a monoexponential curve, with a half time of 20.2±3.8 minutes (see Figure 5). Systolic blood pressure increased 9.2±1.7 mm Hg (p<0.001) and FBF decreased from 4.9±1.0 to 2.3±0.4 ml·dl⁻¹·min⁻¹ (p<0.001), whereas diastolic blood pressure and heart rate did not change significantly. The arterial plasma NE also did not change significantly, and taking into account the absence of significant changes in diastolic blood pressure and heart rate, the increase in systolic blood pressure may have been due to the decrease in FBF, following the same token as the decrease in systolic blood pressure readings during the sodium nitroprusside-induced vasodilatation as discussed above. During the desipramine infusion the fractional extraction of [³H]NE decreased from 0.65±0.05 to 0.50±0.08 (p<0.05).

[Figure 3. Line graph shows venous plasma [³H]norepinephrine during intra-arterial infusion of [³H]norepinephrine (50 nCi·min⁻¹) from 0 to 20 minutes before and during neuronal uptake inhibition in the forearm by desipramine (DMI) (0.6 nmol·kg⁻¹·min⁻¹). First (t₁) and second (t₂) half times in minutes. The t₁ increased during neuronal uptake inhibition (p<0.001), but the change in t₂ was not significant.]
and, as could be expected from blockade of one of the major metabolic pathways for locally released NE in the forearm, decreased the intrinsic clearance of NE from \(9.1 \pm 1.3\) to \(2.7 \pm 0.5\) ml·dl\(^{-1}\)·min\(^{-1}\) \((p<0.001)\), whereas the spillover rate of NE increased from \(33 \pm 5\) to \(63 \pm 11\) pmol·min\(^{-1}\) \((p<0.05)\) and the plasma appearance rate from \(103 \pm 15\) to \(150 \pm 28\) pmol·min \(\) \((\text{NS}, \text{see Table 2})\).

### Discussion

In the present study we found that a two-compartmental model applies for the regional kinetics of NE in the forearm. A biexponential curve fitted best for forearm venous radiolabeled NE, both before and during inhibition of neuronal uptake, and also for the NE released in the forearm by tyramine. Both before and during blockade of neuronal removal of NE only two compartments were detectable.

This implies that two compartments are the minimum for appropriate compartmental description of regional NE kinetics. A priori we envisioned that a four-compartment model could be present because there is a very steep concentration gradient for NE between the place of release, the synaptic cleft, and the plasma pool, and because there is extraneuronal uptake and metabolism.\(^2\)\(^-\)\(^5\) The four compartments (see Figure 6A) are the synaptic cleft into which NE is released by exocytosis and from which NE is taken up again into the neuron by a carrier-mediated uptake process, the smooth muscle cells, where NE derived from the synaptic cleft and from the circulation is subject to extraneuronal uptake and metabolism by catechol-O-methyltransferase (COMT), the interstitial space from which NE can be cleared by nonspecific processes, and the plasma pool, the only accessible compartment. The neuron is not considered to be a compartment because there is no concentration-dependent exchange of NE between the neuron and the synapse. The release of NE is determined by the electrical activity entering the nerve that triggers exocytosis and by various presynaptic regulatory mechanisms.\(^3\)

In Figure 6A it is assumed that three of the four compartments interact directly. Based on available knowledge of sympathetic innervation,\(^3\)\(^0\)\(^3\)\(^1\) however, this is not the most likely situation. Estimates for the number of varicosities that are of the "junctional" type (i.e., that are forming neuromuscular junctions,
FIGURE 6. Schematic drawing of compartmental modeling of organ norepinephrine kinetics. Neuron is not considered to be a compartment because release (R) of norepinephrine is not dependent on concentration in neuron. Compartments considered are synaptic cleft (C), plasma compartment (P), interstitial compartment (I), and extraneuronal or smooth muscle cell compartment (EN). After neuronal (re-)uptake (V_t), norepinephrine is either restored or metabolized via deamination by monoamine oxidase (MAO). After extraneuronal uptake (V_e), norepinephrine is metabolized via methylation by catechol-O-methyltransferase (COMT). From interstitial compartment some norepinephrine is probably cleared by nonspecific processes (U_3). Arterially delivered norepinephrine is arterial concentration (A) times plasma flow (Q). Venous outflow is venous norepinephrine concentration (V) times Q. PA is the plasma appearance rate of norepinephrine into the one compartment of panel D. Fractional extraction rate (E) of infused radiolabeled norepinephrine times A represents the amount removed from the arterially delivered norepinephrine, and E times PA represents the amount of PA removed in the organ. Thus, U_{1,2,3} = E:PA + E:A:Q. Panels are discussed in text.

usually with small cleft widths) vary widely between 10% and more than 85%. Thus, NE may be subject to extraneuronal uptake both from the cleft and from the interstitial space. Once taken up into a smooth muscle cell, it is likely that NE will leave that cell only as a COMT metabolite. Because of the lack of return of NE from the smooth muscle cells, the extraneuronal uptake functions as a sink and the smooth muscle cells therefore do not serve as a compartment. After uptake into the neuron NE can be either stored in the vesicles or metabolized by monoamine oxidase (MAO), and because the storage pool of NE inside the neuron is very large, only a small amount of the NE that recently got taken up can be re-released, which subsequently will be again subject to neuronal uptake. Therefore, the sympathetic varicosities likely also function as a sink for (tracer-labeled) NE that is infused into the circulation (Figure 6B).

Taking into account the K_m values for neuronal (about 300 nM) and extraneuronal (about 3.4 μM) uptake, the neuronal and extraneuronal uptake processes can be considered to obey first-order kinetics. The synaptic cleft is anatomically a very small space, making the concentrations reached in the cleft up to a thousandfold higher than outside. These concentrations exist only very briefly due to the very active neuronal reuptake and diffusion into the interstitial space. Both before and during neuronal uptake blockade, we could detect only a biexponential decay in the forearm, compatible with a twocompartment model. It seems that a very rapid equilibrium is reached between NE that is released from the nerve varicosities and the interstitium, probably because the volume of distribution in the synaptic clefts, especially the junctional ones, must be very small compared with the interstitial space. Therefore, we suppose that the synaptic cleft compartment may be lumped together with the interstitial space compartment (Figure 6C). During blockade of neuronal uptake there is another reason why the synaptic cleft cannot function as a compartment. Because the released NE can only flow over into the extrasynaptic interstitial space, the synaptic cleft and the interstitial space, from a compartmental point of view, merge. It is therefore likely that during blockade of neuronal uptake, the two compartments we found adequately describe the model, and the total release rate of NE may be solvable by compartmental modeling. We want to emphasize that additional experiments with compartmental analysis are needed to further elucidate the validity of this notion.

Derivation of the Norepinephrine Plasma Appearance Rate Parameter

At this point discussion of the organ spillover of NE and the proposed plasma appearance rate parameter, both derived from the isotope-dilution method, is indicated. Because of the inherent assumptions of homogeneous mixing of the tracer in the pool into which the tracer is infused and from which it is sampled, the explicit association of all sources, sinks, and
measurements with the one (sampled) pool, and the equivalence of all sources and sinks, this method basically presumes a one-compartment model. In the present study we have clearly shown that at least two compartments are discernible in the forearm. In the whole body also at least two compartments are detectable. Because a multiple compartment model for regional NE kinetics may not be easily solvable for tissue NE release and may need additional assumptions (Reference 38 and personal communication from Dr. J. Jacquez, March 1989), the isotope-dilution method may be the best method for obtaining an estimate of regional NE release. From the definition of organ spillover (see “Analysis” section) and our own measurements, it is clear that spillover must be directly related to regional flow and that it does not correct for inward flux of NE that originates from the organ, makes it into the blood (the sampled pool), and subsequently is subject to reuptake from the blood and metabolism (by COMT or MAO or stored in the vesicles of sympathetic neurons), without ever leaving the organ.

The flow dependency of the spillover can easily be explained as follows. In steady state there is a concentration gradient between the sites of release in the tissue and the blood that depends on diffusion or transport equilibrium between the NE that is being secreted in the tissues and the NE that is in the blood. When regional blood flow is low the transit time of the NE that is in the blood is longer and therefore more NE can diffuse (back) into the tissue and can be removed by neuronal and extraneuronal processes. Conversely, when the blood flow is high the transit time of the blood is short and, as there is a high concentration at the sites of release in the tissue favoring diffusion into the blood, both more of the NE originating from the tissue and entering the organ with the blood will be washed out of the organ.

The proportion of released NE that enters plasma would be dependent on the concentration gradient. During the addition of nonlabeled NE, which increased the calculated arterial NE concentration to around 8 nM, to the tritiated NE infusion a decreased concentration gradient between the tissue and the plasma and consequently also a decreased extraction of NE from the circulation would be expected. In fact the fractional extraction of tritiated NE remained equal (0.78 ± 0.06 versus 0.79 ± 0.05), indicating that in this concentration range the removal of NE from the circulation is little dependent on the concentration gradient, even though in humans the intrasynaptic NE concentration has been estimated to be around 3 nM. The reason for this may be that this estimate is an averaged concentration for all varicosities while probably only 1 in every 100 varicosities actively secretes NE at a time and therefore the concentration around actively secreting varicosities may be a hundredfold higher, around 300 nM. Such a concentration gradient would favor diffusion into the plasma, virtually independent from the plasma concentration. In contrast, all the varicosities are likely to have a continuously active neuronal uptake process, serving as a sink for any NE that enters the tissue from plasma or from local release.

We therefore propose that, when using the isotope-dilution approach, the fact should be included that the NE that is being released and makes it into the blood is also subject to the same extraction process from the plasma as the NE that entered the organ via the circulation. The mass balance equation for such a model (Figure 6 D) in steady state would be: input of NE into the sampled plasma pool = output of NE from the sampled pool, or

\[ A-Q + PA = VQ + U_{1+2+3} \]

(See Figure 6.) \( U_{1+2+3} \) equals fractional extraction of A-Q plus fractional extraction of plasma appearance rate. The plasma appearance rate is the total amount of NE that enters into the sampled, plasma compartment. The locally extracted and metabolized part of the plasma appearance rate of NE equals fractional extraction of plasma appearance rate. The fractional extraction of A-Q is the extracted and metabolized part of the arterial input of NE. From this and the definition of spillover it follows that

\[ \text{spillover} = \frac{\text{PA}}{1 - E} \]

It has to be emphasized that plasma appearance rate, being derived from the isotope-dilution method, still does not represent total organ release of NE because it can not account for locally released NE that recirculates (neuronal reuptake) without ever reaching the plasma compartment.

Support for the notion that the spillover can change without an actual change in sympathetic activity was found in the present study. Local blockade of neuronal uptake by intra-arterial infusion of desipramine doubled the spillover of NE from the forearm, whereas a diminished release of NE would be expected because of higher NE concentration in the synapse that stimulates inhibitory presynaptic α-adrenergic receptors. Also eventual systemic effects of desipramine would be expected to reduce sympathetic activity reflexively by inhibition of neuronal uptake in the central nervous system. There were, however, no clear signs of systemic effects during the desipramine infusion. Although systolic blood pressure readings increased, this was more likely due to the local changes in flow and not the result of systemic effects because heart rate, diastolic blood pressure, and arterial NE concentration remained unchanged. It could also be argued that desipramine has α-adrenergic receptor antagonistic properties opposing the NE effect on presynaptic facilitating adrenergic receptors. More likely, the increase in NE spillover was simply due to the diminished elimination by neuronal uptake because we found that the intrinsic clearance capacity for NE in the forearm decreased by 65%. We also found that during local nitroprusside-induced vasodilatation in the forearm, the spillover rate of NE increased, even though local clearance increased and in the absence...
of clear systemic effects or changes in the intrinsic clearance capacity. Because nitroprusside is devoid of direct effects on the release or uptake of NE, an increased washout of circulating and locally released NE is likely to have accounted for this increase in spillover. In our present study the values of plasma appearance rate during the desipramine and nitroprusside infusions indeed did not show significant changes and thus appeared to be less sensitive for local changes in flow and clearance.

**Kinetics of Circulating and Locally Released Norepinephrine**

The kinetics of diffusion of NE to and from the sympathetic synapses and the blood vessel lumen appeared to be equal in the forearm. When NE was released by tyramine the time constants for appearance into and disappearance from the blood were practically identical to the time constants for radio-labeled, circulating NE during blockade of neuronal uptake by desipramine. This is based on the findings in the six subjects who received both the [$^3$H]NE infusion during blockade of neuronal uptake by desipramine and the tyramine infusion before desipramine. A comparison of these half times seems to be justified for several reasons. The determinants of the kinetic parameters are the elimination rate constants, the apparent volumes of distribution, and the transfer rate constants between the compartments. The elimination from the synaptic cleft by neuronal uptake was probably zero both during the infusion of tyramine and the infusion of radiolabeled NE combined with desipramine. Tyramine acts via uptake by the neuronal uptake carrier and subsequently displaces NE from the storage vesicles. The dose of tyramine used resulted in a calculated plasma tyramine concentration around 20 µM, which is likely to have competitively blocked the neuronal uptake of NE. During the desipramine infusion tyramine did not have any significant effect on the venous plasma NE concentration nor on the blood flow, indicating that indeed there was complete blockade of neuronal uptake. During blockade of neuronal uptake higher NE concentrations were reached in the tissue, as indicated by the vasoconstriction that was observed. It is unknown whether this may have induced changes in the apparent volumes of distribution of the compartments. However, it is unlikely that this would be different for the blockade induced by tyramine or desipramine. The elimination by extraneuronal mechanisms was probably not different during the two infusions because tyramine and tracer doses of NE are not known to have inhibitory effects on these mechanisms. The main determinant of the NE elimination rate constant in the plasma compartment, the blood flow, was practically identical during the infusion of tyramine before desipramine, and the tracer NE infusion with desipramine. There are no reasons to assume that tyramine or tracer doses of NE change the transfer rate constants between the compartments.

It was not unexpected that there does not seem to be a difference in the transfer of NE from the synaptic cleft to the blood vessel lumen and vice versa. Kopin et al. found in pithed, vagotomized, α₂-adrenergic receptor-blocked, adrenal-demululated rats that changes in blood pressure–plasma NE curves during electrical stimulation of sympathetic outflow or NE infusion, with and without blockade by desipramine, were equal in magnitude and reciprocal. Therefore, diffusion barriers or active transport of catecholamines by the endothelium as found in isolated arteries may not be relevant for compartmental modeling in vivo. It is not known whether a diffusion barrier, as found in the myocardium of dogs, exists in the heart of humans, but it may not be significant because more than 80% of circulating NE can be removed in a single pass.

During infusion of desipramine the venous plasma NE gradually increased due to overflow of released NE from sympathetic synapses that escaped reuptake into the neurons. The resulting appearance of released NE in venous plasma fitted best to a monoexponential curve with a relatively long half-time that was almost equal to the second-phase half time of the tyramine-induced NE release. The difference with the tyramine-induced NE release may be related to the different mode of action. Tyramine competes with NE for the neuronal uptake carrier and displaces NE from the storage vesicles. Desipramine seems to act by binding to a site on the sympathetic neuron close to the catecholamine uptake carrier and subsequently inhibits the uptake carrier. Therefore, the second phase of the tyramine-induced appearance of NE in venous plasma may be related to the uptake inhibiting effect, whereas the first phase may be more related to the releasing effect of tyramine. This is supported by the half time of the second phase during tyramine, which was practically equal to the half time of the desipramine effect.

The behavior of circulating radiolabeled and unlabeled NE was practically equal as indicated by the close relation between the extraction of radiolabeled NE and the low dose of unlabeled NE in the forearm that we observed in six subjects at various levels of forearm blood flow. Due to inclusion of the hand blood flow during the experiments, shunting of blood in the hand circulation may have had a decreasing effect on the fractional extraction rates of both the labeled and the unlabeled NE. This would lead to underestimations of the spillover and the plasma appearance rate but the relative changes and proportions would remain unaffected.

In conclusion, the kinetics of NE in the forearm can be described by a two-compartmental model. The organ spillover of NE is markedly influenced by regional changes in flow and clearance. It is proposed that, when using the isotope-dilution method, the plasma appearance rate parameter may give a better estimate of total organ NE that enters the blood than.
the spillover parameter. We found no evidence for differences in diffusion of NE to and from sympathetic synapses and the blood vessel lumen.

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