Assessment of Human Sympathetic Nervous System Activity from Measurements of Norepinephrine Turnover

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KEY WORDS • norepinephrine kinetics • catecholamines • sympathetic nervous system • hypertension • heart failure • cirrhosis

Since the sympathetic nervous system has such a central place in homeostasis in general, and in circulatory adaptation in particular, it is paradoxical that so little is known about the possible contribution of disturbed sympathetic nervous function to the development of human diseases. Clinical tests of sympathetic nervous system activity have, by and large, been routinely applied in one setting only: the detection of sympathetic nervous failure, from autonomic insufficiency syndromes, in patients with postural hypotension.

A list of "candidate" diseases for sympathetic nervous system dysfunction might include, for example, essential hypertension, cardiac failure, coronary artery spasm, cirrhosis, mitral valve prolapse, and Raynaud's syndrome. Until very recently, the picture of sympathetic nervous pathophysiology for conditions such as these was particularly sketchy, mainly because of the rudimentary nature of the tests of sympathetic nervous system function available to investigative clinical medicine. Pertinent questions have gone incompletely answered at best — questions such as: Is the sympathetic nervous system directly involved in the early pathogenesis of essential hypertension? Is increased renal sympathetic activity a common cause of sodium retention in edematous states? Is increased cardiac sympathetic nerve firing an important element in the development of cardiac arrhythmias in humans?

There have been rapid recent advances in the understanding of the physiology and pathophysiology of the sympathetic nervous system. Earlier pioneering contributions setting the stage for these advances should be mentioned. Soon after he had characterized the sympathetic nervous system transmitter as norepinephrine, von Euler introduced biochemical methods to the clinical study of the sympathetic nervous system by measuring the urinary excretion of norepinephrine in patients with essential hypertension, to gauge their level of sympathetic nervous activity. The development of a sensitive plasma assay for norepinephrine by Engelman et al. represented an important technical refinement of this biochemical approach. Doyle and Smirk introduced into clinical research the technique of pharmacological autonomic blockade for the study of the sympathetic nervous maintenance of cardiovascular function, to estimate in particular the "neurogenic component" in essential hypertension. Hagbarth and Vallbo devised clinical electrophysiological methods for studying nerve firing in subcutaneous sympathetic nerves distributed to skeletal muscle and skin, a technique later imaginatively applied by Wallin and coworkers.

Although these methods, and subsequent refinements of them, have proven valuable in the study of sympathetic nervous function in humans, each has its limitations. For example, with methods for estimating the "neural component in hypertension," based on the hemodynamic response to pharmacological autonomic blockade, measured responses are influenced by both the level of sympathetic nerve firing and secondary hypertrophy in the heart and arteries through a "cardiovascular amplifier effect." This undermines attempts to estimate sympathetic nervous activity in...
hypertensive patients from hemodynamic responses. With microneurographic electrophysiological methods for studying nerve firing rates, only the sympathetic nerves to skin and skeletal muscle can be studied; an important limitation in cardiovascular diseases is that the nerves to internal organs are not accessible to testing. The biochemical methods also have their deficiencies, but biochemical measurements of transmitter release might be more helpful in quantifying sympathetic nervous activity in internal organs. These biochemical methods will now be reviewed.

Biochemical Measures of Sympathetic Nervous System Activity

From the time of von Euler's demonstration that the sympathetic neurotransmitter was norepinephrine,\(^4\) the potential value of norepinephrine release rate measurements as an index of nerve firing was seen. Peart\(^17\) described norepinephrine overflow into the venous effluent of an organ (the cat spleen) on electrical stimulation of its nerve supply in 1949, followed soon after by the demonstration of Brown and Gillespie\(^18\) that the washout of norepinephrine was proportional to the rate of sympathetic nerve firing. By 1954 von Euler et al.\(^5\) had used norepinephrine measurements in urine as a measure of sympathetic nervous activity in a large-scale study of human hypertension.

From these early beginnings, clinical methods have been devised aiming to estimate sympathetic nervous system activity from measures of tissue norepinephrine concentration and turnover,\(^19,20\) norepinephrine release,\(^21\) the concentration of norepinephrine in the synaptic space,\(^22\) spillover of released norepinephrine to plasma,\(^23-27\) compartmental modeling of norepinephrine,\(^28-30\) total body turnover of norepinephrine (based on urinary excretion of norepinephrine and its metabolites),\(^31,32\) and biochemical markers of adrenergic receptor stimulation (Figure 1).\(^33\) Evaluation of the validity and utility of these techniques, as well as the findings obtained with them in clinical studies, will form the substance of this review.

These clinical methods for estimating sympathetic nervous system activity have their counterpart in experimental techniques in common use for studying sympathetic nervous function in animals. Measures of tissue norepinephrine synthesis (using radiolabeled norepinephrine precursors such as tyrosine and dopamine), and tissue norepinephrine turnover (based on the fall in unlabeled norepinephrine tissue concentration after synthesis block or decline in tissue norepinephrine specific activity after prior radiolabeling of norepinephrine stores) are widely used to estimate levels of sympathetic nerve firing in experimental animals.\(^34-36\) The particular strength of these methods in animal studies, usually lacking in the clinical setting, resides in the ability to estimate pool sizes from direct sampling of tissues. The interpretation of these biochemical measures of norepinephrine turnover in animals, however, is subject to the same sort of limitations that apply to the clinical methods: Tissue norepinephrine content and turnover cannot readily be equated, tissue norepinephrine turnover is not equivalent to norepinephrine release, and the extent of reuptake and reutilization of released norepinephrine is uncertain.\(^16,20,31\)

Estimation of Overall Sympathetic Nervous System Activity

For many clinical physiologists of the autonomic nervous system, the development of a reliable method for measuring overall sympathetic nervous system activity (integrated nerve firing rate) has been something of a Holy Grail. In the absence of such a method, for some years clinical researchers have had to make do with measurements of the urinary excretion or plasma concentration of norepinephrine, the principal transmitter of the sympathetic nervous system, as (rather imprecise) indices of total sympathetic nervous system activity.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Clinical methods have been devised to study sympathetic nervous system function using measurements of the tissue concentration of norepinephrine (NE; 1), the rate of release of NE from sympathetic nerve varicosities (2), the intrasynaptic concentration of NE (3), neuronal NE uptake (4), the rate of spillover of released NE to plasma (5), the plasma concentration of NE (6), and the urinary excretion of NE and its metabolites (7).
activity. More recently, several techniques have been developed, each based on measures of norepinephrine synthesis or release, which constitute an improvement on the previously used static measurements of norepinephrine concentration in urine or plasma. The relationship that usually exists between sympathetic nerve firing and norepinephrine synthesis and release rates\(^{31,32}\) provides the experimental justification for this use of norepinephrine turnover measurements as an index of sympathetic nervous activity.

**Norepinephrine Production by the Sympathetic Nervous System**

The total urinary excretion of norepinephrine and all its metabolites can be used as a measure of norepinephrine synthesis rates.\(^{31,32}\) Although norepinephrine synthesis by sympathetic neurons leads to a variety of immediate outcomes (storage, intraneuronal metabolism, release followed by reuptake or extraneuronal metabolism),\(^{40}\) the ultimate fate of the synthesized norepinephrine is its excretion as unchanged amine or as metabolite in urine.\(^{31,32}\) The metabolism of norepinephrine in humans is very well characterized, largely through extensive earlier studies involving the administration of radiolabeled transmitter.\(^{41-43}\) These metabolites, representing the result of \(\sigma\)-methylation (normetanephrine), deamination (dihydroxyphenyl-ethylene glycol [DHPG], dihydroxymandelic acid [DOMA]), or combined \(\sigma\)-methylation and deamination (vanillylmandelic acid, 3-methoxy-4-hydroxy-phenylglycol), can all be measured in urine.

In establishing estimates of sympathetic nerve norepinephrine synthesis rates from the urinary excretion of norepinephrine and these metabolites, adjustment must be made for metabolites derived from epinephrine, for norepinephrine derived from the adrenal medulla, and for norepinephrine metabolites originating in the central nervous system.\(^{31,32}\) This raises some difficulties. The extent of passage of some norepinephrine metabolites to plasma from the central nervous system (particularly DHPG and DOMA) is uncertain. The fraction of norepinephrine that originates in the adrenal medulla may also be unclear under some circumstances; it is approximately 2% under resting conditions,\(^{44,46}\) but how this proportion changes with different forms of sympathoadrenomedullary activation is not known.

Another restriction placed on this method of estimating total sympathetic activity resides in the fact that rates of norepinephrine synthesis and norepinephrine release do not exactly coincide. Intraneuronal metabolism of an uncertain fraction of synthesized norepinephrine occurs before its release.\(^{40,47}\) Further, to some extent norepinephrine released by sympathetic nerves is repetitively recycled, through the medium of neuronal reuptake (such that synthesis measurements can underestimate release). This recycling, however, may be less than commonly thought, because norepinephrine entering the neuron through reuptake is preferentially metabolized.\(^{40,47}\)

Mean sympathetic nerve norepinephrine production rate in healthy humans is 3 to 4 \(\mu\)g/min.\(^{31,32,44}\) An increase in production rate with aging has been reported.\(^{44}\) Sympathetic nerve norepinephrine production is increased in patients with depressive illness and falls after administration of tricyclic antidepressants.\(^{32}\)

**Compartmental Modeling of Norepinephrine**

The disposition of many substances in the human body can be expressed by representing the body as a system of compartments. These compartments are mathematical constructs for the substance in question; no anatomical or functional entity is necessarily proscribed for a compartment. Many attempts have been made, in research spanning almost three decades,\(^{12,28-30}\) to use tracer methodology and the mathematical techniques of compartmental analysis to elucidate the kinetics of norepinephrine in humans. These studies have often been hampered by technical problems, such as the impracticality of assaying tissue norepinephrine levels in humans, and in earlier experiments, by the unavailability of radiolabeled \(\sigma\)-norepinephrine and the lack of a sufficiently sensitive and specific assay for plasma norepinephrine. The disposition of norepinephrine in fact is complex, defying precise compartmental analysis.\(^{28}\)

On a more general level, it might be questioned whether a whole-body approach to norepinephrine kinetic modeling could do justice to the topographical complexity of the sympathetic nervous system. Could a relatively simple two-compartment or three-compartment model of norepinephrine disposition, although conforming to the accepted mathematical precepts of compartmental analysis,\(^{46,49}\) be compatible with and illustrative of the known regional differentiation of sympathetic nervous system outflows\(^{50}\) and the pronounced regional heterogeneity of norepinephrine disposition (dependent on such factors as tissue differences in synaptic cleft width)?\(^{51}\)

Despite these difficulties, substantial contributions have followed from the application of techniques of this type. The early research of Gitlow et al.\(^3\) pointed to faulty neuronal uptake of norepinephrine in essential hypertension, a finding supported by several later studies.\(^52-54\) The study of De Quattro and Sjoerdsma,\(^55\) using labeled norepinephrine precursor and cardiac biopsy samples, was the first to measure tissue norepinephrine turnover in humans. In an interesting recent development, Linares et al.\(^56\) have devised a two-pool model of norepinephrine disposition that aims to quantify such physiologically important measurements as the rate of norepinephrine release into the extravascular space. The challenge that remains for proponents of compartmental analysis is to achieve a better synthesis of the mathematical compartmental models with the well-characterized physiological models of norepinephrine disposition. For example, it has not been possible to date to develop compartmental models that incorporate the neuronal norepinephrine pool.

**Norepinephrine Spillover to Plasma**

Norepinephrine in plasma is derived largely from transmitter released by sympathetic nerves, with only a small contribution from the adrenal medulla.\(^55,46,55\)
and apparently no input at all from the central nervous system.36-38 Faced with the lack of a completely acceptable compartmental model for determining whole-body norepinephrine kinetics, investigators turned to the plasma concentration of the transmitter as an index of overall sympathetic nervous system activity.7, 14, 59-62 Of several possible objections to this application of plasma norepinephrine values, one is the dependence of the plasma norepinephrine concentration on the rate at which norepinephrine is removed from the circulation, not solely on the rate of norepinephrine release.27, 29, 63, 64

To avoid this confounding influence of norepinephrine plasma clearance, the metabolic clearance rate methods have been developed, based on norepinephrine isotope dilution in plasma, to measure the rate of spillover of norepinephrine to plasma (plasma norepinephrine appearance rate). For infused tritiated norepinephrine (NE), the following relationship holds at steady state:26, 27:

\[
\text{Total NE spillover rate} = \frac{[\text{H}]\text{NE infusion rate}}{\text{plasma NE specific radioactivity}}
\] (1)

Rather than the rate of release of norepinephrine from sympathetic nerve varicosities, norepinephrine spillover rate gives the rate at which released norepinephrine enters plasma: In humans this rate appears to be approximately 20% of the total turnover of norepinephrine in sympathetic nerves.31 Since the overflow of released norepinephrine to plasma is influenced by the adequacy of neuronal norepinephrine reuptake,61, 63 the performance of tests of norepinephrine reuptake, in parallel with the metabolic clearance rate measurements, provides complementary information (answering such questions as: Is higher spillover due to increased norepinephrine release or deficient norepinephrine reuptake?). The normalcy or otherwise of neuronal norepinephrine uptake may be judged from a variety of clinical measures, all relying on the dependence of norepinephrine removal from plasma on neuronal uptake.27, 52, 53, 66-69

Several important assumptions underlie the use of isotope dilution methodology for determining norepinephrine spillover rates. One is that a well-mixed central plasma norepinephrine pool exists; the limitations of this idealization will be considered shortly. Another is that any rerelease of tracer, recycled through sympathetic nerves after uptake, is negligible in comparison with the rate of infusion of radioabeled norepinephrine. This does appear to be the case. In animal studies, when the dose of tritiated norepinephrine administered may be of the order of 100 that used in clinical studies, recycling of tracer by neuronal uptake and release is clearly evident.39 With the lower radio-isotope doses used in human studies, recycling of tracer is not readily apparent, since increasing endogenous norepinephrine release and plasma norepinephrine concentration (with dietary sodium restriction, diuretic therapy, or exercise, for example) and decreasing norepinephrine release (such as with clonidine or dietary calorie restriction) do not produce the matching changes in the plasma concentration of tritiated norepinephrine that would be expected if tritiated norepinephrine was rereleased in any quantity in parallel with the release of endogenous norepinephrine.27, 70, 71

There are some theoretical objections to the use of plasma isotope dilution techniques for the estimation of the metabolic clearance rate of substances such as norepinephrine for which there are multiple sites of production (release) and removal (uptake and metabolism) remote from the plasma pool.49 These general arguments for invalid application of metabolic clearance rate methodology, however, do not apply well in the case of norepinephrine, where the total overflow to plasma, measured by isotope dilution, happens to agree very well with the sum of regional norepinephrine inputs to the central pool, measured individually.55 Similarly, total plasma clearance (metabolic clearance rate) and summated regional clearances correspond closely.71

Adrenal Medullary Secretion of Catecholamines

Under resting conditions only a small percentage of the norepinephrine entering plasma derives from the adrenal medulla.45, 46 With certain stimuli, such as hypoglycemia, adrenal medullary secretion of norepinephrine increases.30, 62 Under conditions of medullary stimulation, the respective fractions of plasma norepinephrine originating from sympathetic nerves and adrenal medulla will be uncertain. A contrast should be drawn between the functional significance of norepinephrine spilling over into the circulation from sympathetic nerves and of epinephrine secreted into plasma by the adrenal medulla. The norepinephrine in plasma represents transmitter overflow rather than circulating hormone, and under most circumstances it is devoid of metabolic and cardiovascular effects; the plasma concentration typically must increase at least fivefold to reach threshold for such effects.25 Epinephrine secreted by the adrenal medulla is a potent circulating hormone, exerting powerful effects when the plasma concentration is doubled or trebled.62 The entry of epinephrine to plasma (secretion) is much more direct than is the mode of norepinephrine entry from sympathetic nerves (spillover); accordingly, fewer assumptions need be made for the clinical measurement of epinephrine secretion by isotope dilution.41, 62, 62

What Is the Preferred Sampling Site for Total Norepinephrine Spillover Measurements?

Insufficient attention has been paid to the issue of what constitutes the most appropriate plasma sampling site for norepinephrine spillover determinations.72-75 In earlier studies it was customary to sample peripheral venous blood, typically from an antecubital vein. The underlying assumption, that the infused norepinephrine is evenly mixed in plasma, is an oversimplification; regional differences in the plasma concentration of norepinephrine exist, dependent on local processes of norepinephrine release and removal.55, 59-77
tivity values differ throughout the circulation (Figure 2), so that norepinephrine spillover rate determinations (Equation 1) depend on the sampling site. Choice of the ideal sampling site requires consideration of the sites of release of norepinephrine to plasma, the site of infusion of the radiotracer, and the sites of extraction of both. Reduplication of the kinetics of unlabeled norepinephrine with the radiotracer infusion is complicated by the fact that the endogenous norepinephrine is released to plasma at two levels in series — first the lungs, then the systemic organs.\textsuperscript{53-74} Infusion of a radiotracer at a single site cannot replicate this arrangement.

No specific radioactivity value can be said to be representative of the central plasma pool as a whole. Regional distortion is greatest for regional venous blood, where recently infused tracer has been subjected to a cycle of extraction, but the endogenous norepinephrine freshly released from the systemic organs has not (see Figure 2). Accordingly, the specific radioactivity of venous plasma is lower and the calculated total norepinephrine spillover rate higher than for right ventricular or arterial plasma. In general, mixed venous and arterial sampling sites are preferable for the determination of norepinephrine spillover rates. Neither is ideal, and the spillover rate calculated from both is not identical (see Figure 2).

**Total Norepinephrine Spillover Rates**

The overall rate of spillover of norepinephrine to plasma in healthy subjects resting supine (arterial sampling) is of the order of 0.3 μg/min.\textsuperscript{55,72-74} This is an insufficient rate of entry of norepinephrine to plasma (based on comparisons with the effect of intravenously infused norepinephrine) to have direct cardiovascular or metabolic effects as a circulating hormone.\textsuperscript{25} Total norepinephrine spillover is lowered in the presence of reduced sympathetic nervous activity, whether from sympahtic nerve failure (idiopathic peripheral autonomic insufficiency), due to dosing with the sympathetic nervous system suppressant clonidine, or accompanying physical conditioning (Table 1). Increases in norepinephrine release are seen in some disease states, including cardiac failure, cirrhosis, depressive illness, and essential hypertension (see Table 1).

**Assay of Plasma Tritiated Norepinephrine**

A commonly used method has been to extract radioactivity from plasma with alumina,\textsuperscript{26,27,73} and regard extracted counts as almost exclusively representing tritiated norepinephrine. Earlier chemical characterization of alumina eluates (using thin-layer chromatography, organic extraction, and cation exchange methods) supported this practice, demonstrating negligible contribution by dihydroxy metabolites of norepinephrine (which are alumina-extractable) to the extracted plasma radioactivity.\textsuperscript{26} A recent report, using high performance liquid chromatography (HPLC) methods, challenges this conclusion, claiming that 30 to 50% of the alumina-extractable radioactivity represents the tritiated dihydroxy metabolite DHPG.\textsuperscript{101} We have been unable to replicate this finding (Figure 3). We have found, in plasma stored under our standard conditions\textsuperscript{26} for less than 1 month, that tritiated DHPG represents only 2 ± 3% of alumina-extracted radioactivity, thus constituting negligible interference. We did detect a degradation product of norepinephrine, formed with more prolonged storage of plasma, which, being extracted from plasma by alumina and having HPLC retention time very similar to that of DHPG, could easily be misidentified as radiolabeled DHPG. Decomposition of stock norepinephrine in storage, prior to its infusion, could lead to similar confusion (see Figure 3).

**Figure 2.** Nonuniformity of norepinephrine values in plasma. Mean plasma norepinephrine specific radioactivity (dpm/µg) at steady state, for different sampling sites, during constant-rate antecubital venous infusion of tritiated norepinephrine (0.35 μCi/min) in 50 patients with essential hypertension. Isotope dilution (signifying norepinephrine release to plasma) occurred across all organs except the gut and liver, but to differing degrees at different sites. Total norepinephrine spillover rate determinations are influenced by the sampling site chosen.
TABLE 1. Clinical Factors Influencing the Total Rate of Spillover of Norepinephrine to Plasma

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutrition</strong></td>
<td></td>
</tr>
<tr>
<td>Low salt intake</td>
<td>↑70, 79</td>
</tr>
<tr>
<td>Overeating</td>
<td>±80, 81</td>
</tr>
<tr>
<td>Caloric restriction</td>
<td>±80, 81</td>
</tr>
<tr>
<td><strong>Drugs</strong></td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>↓27, 82</td>
</tr>
<tr>
<td>Desipramine</td>
<td>↓96</td>
</tr>
<tr>
<td>β-adrenergic blockers</td>
<td>0 27, 62, 72, 83</td>
</tr>
<tr>
<td>Diuretics</td>
<td>↑27</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>↑84</td>
</tr>
<tr>
<td>Glucose clamp</td>
<td>↑85</td>
</tr>
<tr>
<td><strong>Disease</strong></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>↑86-88</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>↑27, 53, 78</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>↑63, 89</td>
</tr>
<tr>
<td>Depressive illness</td>
<td>↑90</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>↑24, 27</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>±27</td>
</tr>
<tr>
<td>Autonomic insufficiency</td>
<td></td>
</tr>
<tr>
<td>Idiopathic peripheral</td>
<td>↓91, 92</td>
</tr>
<tr>
<td>Sympathectomy</td>
<td>↓95</td>
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<tr>
<td>Shy-Drager syndrome</td>
<td>0 92</td>
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<tr>
<td>Diabetic</td>
<td></td>
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<tr>
<td>Surgery</td>
<td>↑95</td>
</tr>
<tr>
<td>Physical conditioning</td>
<td>↓96</td>
</tr>
<tr>
<td>Upright posture</td>
<td>↑64, 89</td>
</tr>
<tr>
<td>Aging</td>
<td>↑30, 97-100</td>
</tr>
</tbody>
</table>

Total norepinephrine spillover rate is increased (↑), unchanged (0), or lowered (↓).

What Is the Validity of Biochemical Tests of Total Sympathetic Nervous System Activity?

Global tests of sympathetic nervous activity, such as plasma norepinephrine concentration and spillover measurements, have one intrinsic deficiency: the sympathetic nervous system is not organized as an all-or-nothing system.15, 50 The sympathetic nervous outflow to all organs is not uniform, and local, organ-specific increases or decreases in sympathetic activity can occur with different reflexes and in different disease states.63, 102-105 A biochemical index of overall sympathetic activity, in merely representing the algebraic sum of these changes, conveys rather imprecise physiological information. For more penetrating analysis of sympathetic nervous function in humans, and in particular of sympathetic nervous pathophysiology in disease states, techniques allowing study of regional, organ-specific sympathetic activity are needed.

Estimation of Regional Sympathetic Nervous System Activity

Biochemical methods have been developed aiming to estimate organ-specific sympathetic nervous system activity from measurements of the regional release of norepinephrine21 and the spillover of released norepinephrine to plasma (see Figure 1).108

Regional Norepinephrine Release

Cousineau et al.21 have developed, in the dog, a multiple tracer technique for studying norepinephrine release from the cardiac sympathetic nerves. The method involves the simultaneous rapid injection of radiolabeled forms of albumin (confined to the plasma space), sucrose (free access to the extravascular space), and norepinephrine into the left coronary ar-
tery, with subsequent sampling from the coronary sinus. In principle the technique should be applicable to organs other than the heart. The method, which also has been applied in humans,\textsuperscript{107} has considerable power, carrying the potential for measuring organ-specific norepinephrine release and reuptake.

One major difficulty with the technique, not generally appreciated, concerns the specific radioactivity level of available radiolabeled norepinephrine. A dose of 25 to 50 \( \mu \text{Ci} \) of tritiated norepinephrine is needed, injected as a bolus into the left coronary artery.\textsuperscript{21, 107} With available supplies of the preferred isotope, \( \text{[7-}^{3}\text{H}]\)norepinephrine (specific radioactivity 20–30 C/mmol), this dose is equivalent to approximately 0.15 to 0.3 \( \mu \text{g} \) of unlabeled norepinephrine. With coronary artery plasma flow in humans of 40 to 100 ml/min,\textsuperscript{55, 107} this bolus dose of norepinephrine would be expected to produce a peak plasma concentration of the order of 5 to 20 ng/ml, contrasting with an arterial plasma norepinephrine concentration in resting humans of approximately 0.25 ng/ml.\textsuperscript{75–77} Since this would constitute a biologically active dose, and not a tracer dose,\textsuperscript{25} there would seem to be valid concerns that the tracer prerequisites of the method cannot be met with existing bolus methodology and the radiolabeled norepinephrine currently available. The use of a constant-infusion technique, allowing reduced rates of administration of the tritiated norepinephrine, might overcome this difficulty.

**Regional Norepinephrine Spillover to Plasma**

The rate at which released norepinephrine spills into the venous drainage of individual organs usually is proportional to their rate of sympathetic nerve firing. This point has been demonstrated, with electrical stimulation of the nerves of supply, for a range of organs, including the heart,\textsuperscript{37, 45} kidneys,\textsuperscript{38} lungs,\textsuperscript{37} and skeletal muscle.\textsuperscript{39} This relationship provides the experimental justification for using measures of regional norepinephrine overflow to plasma as a clinical index of regional sympathetic nervous activity.

**Net** spillover of norepinephrine from an organ to plasma can be calculated, by the Fick principle, from the venoarterial difference in plasma norepinephrine concentration across the organ and the plasma flow.\textsuperscript{108, 109} Since norepinephrine flux is bidirectional, however, with uptake and release by all organs except the brain,\textsuperscript{55, 56, 103} net spillover calculations represent an underestimate for regional norepinephrine outward flux. Adjustment needs to be made for norepinephrine uptake.\textsuperscript{76, 106, 110} Perhaps best determined using a constant-rate infusion of radiolabeled norepinephrine (NE),\textsuperscript{108, 110} At steady state\textsuperscript{110ut}: \[ \text{Organ NE spillover} = [(C_v - C_a) + C_a(NEE)] \times \text{PF} \] (2)

where \( C_v \) is plasma norepinephrine concentration in draining vein, \( C_a \) is arterial plasma norepinephrine concentration, \( \text{NEE} \) is fractional extraction of tritiated norepinephrine, and PF is organ plasma flow.

It should be emphasized that norepinephrine spillover is measured, and from this measure, norepinephrine release (and sympathetic activity) is inferred. A variety of factors other than sympathetic nerve firing rates could influence the rate at which released norepinephrine spills into plasma (Table 2). Of these, the possible influence of blood flow on norepinephrine washout is of particular concern. The increase in nerve firing underlying sympathetic-mediated vasoconstriction, for example, could go undetected, based on norepinephrine spillover measurements, if norepinephrine washout fell in proportion to the reduction in blood flow. Conversely, high flow rates, with regional vasodilation, might favor increased norepinephrine washout. Early reports did, in fact, suggest a marked flow dependence of norepinephrine spillover.\textsuperscript{112, 113} Several recent studies do not support this.\textsuperscript{38, 39}

We have studied the flow dependence of regional norepinephrine kinetics in the kidney of awake, uninephrectomized dogs. Trained dogs were prepared at a preliminary operation at least 2 weeks before the experiment, as previously described.\textsuperscript{115} With the dog under general anesthesia, one kidney was removed, a perivascular Silastic balloon cuff was placed around the renal artery to the remaining kidney, a Doppler flowmeter was placed on the renal artery distal to this cuff, and catheters were placed in the aorta and renal vein.\textsuperscript{115}

Measurements of renal norepinephrine kinetics were made with the trained dog lying on a padded table in a quiet laboratory, after pretreatment with enalapril, 0.5 mg/kg i.v. Following measurements at rest, renal blood flow was lowered, in the six dogs studied, by inflating the renal artery cuff. Mean flow reductions of 12%, 29%, and 51% were achieved with the three levels of cuff inflation, each of which was maintained for 30 minutes. Measurements were made over the last 5 to 10 minutes of each period. Renal blood flow measurements were derived from \( p \)-aminohippurate clearance at rest and from the Doppler flow shift with progressive cuff inflation.\textsuperscript{115} Renal plasma norepinephrine kinetics measurements were calculated, as for humans,\textsuperscript{71, 103} from the venoarterial difference in plasma norepinephrine concentration across the kidney, the fractional extraction of tritiated norepinephrine in transit through the kidney (during constant-rate infusion of tritiated norepinephrine, 0.35 \( \mu \text{Ci} / \text{min} \), and the renal plasma flow.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
\textbf{Possible Determinants of the Rate of Spillover of Norepinephrine from an Organ to Plasma} & \\
\hline
Sympathetic nerve firing rate\textsuperscript{18, 23, 37–39, 65} & \\
Sympathetic nerve density & \\
Organ mass & \\
Synaptic cleft width\textsuperscript{51} & \\
Capacity for \( \alpha \)-methylation\textsuperscript{111} & \\
Capacity for \( \beta \)-methylation\textsuperscript{21} & \\
Capillary permeability to norepinephrine\textsuperscript{21} & \\
Blood flow\textsuperscript{21, 37, 39, 112–114} & \\
\hline
\end{tabular}
\caption{Possible Determinants of the Rate of Spillover of Norepinephrine from an Organ to Plasma}
\end{table}

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FIGURE 4. Effect of changes in renal blood flow (achieved with an inflatable renal artery cuff) on renal norepinephrine kinetics in six awake, chronically instrumented, uninephrectomized dogs. An approximately proportional fall in norepinephrine clearance was evident during progressive reduction in renal blood flow. In contrast, norepinephrine spillover was unchanged with blood flow reductions of less than 30% but fell substantially when renal blood flow was halved. Means ± SE are listed. No reflex sympathetic nervous system activation was apparent during inflation of the renal artery cuff, since total norepinephrine spillover to plasma (not shown) was unchanged. $D_1$, $D_2$, and $D_3$ signify the three settings of the renal artery cuff, producing mean flow reductions of 12, 29, and 51%.

No reflex sympathetic nervous system activation was apparent during inflation of the renal artery cuff, since total norepinephrine spillover to plasma was unchanged. With progressive reduction in renal blood flow, norepinephrine plasma clearance by the kidney fell in proportion to the fall in flow. In contrast, norepinephrine spillover was unaltered by a flow reduction of less than 30%, but reduced norepinephrine washout was evident with flow reduction of approximately 50% (Figure 4). Our results indicate a flow dependence of norepinephrine clearance for flow reduction of any extent and for norepinephrine spillover at high degrees of flow reduction only. We have not studied the effects of increasing flow in this model.

A second deficiency of organ-specific norepinephrine release measurements, in addition to this flow dependence of norepinephrine washout, is the failure to indicate from which sympathetic nerves within an organ the released norepinephrine is derived, and in what proportions. Does it come from the sympathetic nerves to cardiovascular components of the organ (principally small arteries, arterioles, and venules) or from the innervation of noncardiovascular cells and tissues? The dense sympathetic innervation typical of blood vessels, plus the presence of (characteristically) broad neuroeffector synaptic clefts, suggests that for many organs the norepinephrine released into venous blood is predominantly vascular in origin and that in plasma it most closely reflects cardiovascular sympathetic nervous function. However, this may not always be the case. Direct sympathetic innervation has been demonstrated for noncardiovascular tissues and cells as diverse as liver hepatocytes, renal tubules, gastrointestinal and genitourinary smooth muscle, exocrine glands, and adipose tissue adipocytes. In certain instances norepinephrine release may be an expression of thermoregulatory or metabolic sympathetic nervous functions than of neural cardiovascular control.

Norepinephrine Spillover from the Heart
Measurement of the rate of overflow into plasma of norepinephrine released by the sympathetic nerves of the heart has been used to estimate cardiac sympathetic
NOREPINEPHRINE RELEASE IN HUMANS/Esteller et al.

Typically, this process involves measuring the coronary sinus-arterial plasma norepinephrine concentration difference, the fractional extraction of radiolabeled norepinephrine across the heart, and the coronary sinus blood flow (measured by thermodilution).71,74

Cardiac norepinephrine spillover in healthy subjects at rest accounts for only 2 to 3% of total body norepinephrine release to plasma.1103 Although the heart has a dense sympathetic innervation, density of innervation presumably is outranked by other determinants of norepinephrine spillover, by factors such as organ mass and blood flow and mean width of the synaptic cleft (narrow in the heart; see Table 2).122-123 A relatively modest increase in cardiac norepinephrine spillover (approximately a doubling) was seen with right ventricular pacing to ischemia in patients with coronary artery disease (Figure 5) and with mild laboratory-induced mental stress (mental arithmetic involving serial subtraction of 7 or 13 from a large number for 10 minutes) in untreated subjects with essential hypertension. The increase in cardiac norepinephrine spillover was very much greater in healthy subjects at the end of a 10-minute period of supine exercise on a bicycle ergometer (exercise level 50% of maximum work capacity); a 30-fold increase in norepinephrine overflow from the heart was noted (see Figure 5).

Increased release of norepinephrine from the heart to plasma, presumptive evidence of increased cardiac sympathetic activity, has been reported in some disease states, including cardiac failure,63,89,123 essential hypertension,74 and cirrhosis (see Figure 5). The possible pathophysiological consequences of this have been incompletely explored to date.

Norepinephrine Spillover from the Kidneys

Renal norepinephrine spillover measurements may be derived clinically from the venoarterial plasma norepinephrine concentration difference across the kidneys (with sampling from the right renal vein to avoid the contamination by adrenal medullary drainage that occurs on the left), the fractional extraction of radiolabeled norepinephrine occurring in transit across the kidney, and the renal plasma flow determined by thermodilution or p-aminohippurate clearance and extraction.71,106 Net norepinephrine spillover measurements are possible if norepinephrine removal by the kidney is not determined.108

Overflow of norepinephrine from the kidneys accounts for approximately 25% of the total spillover of norepinephrine to plasma in healthy subjects at rest.55,103 This norepinephrine is derived from sympathetic nerve firing, and not from other hypothetical sources, such as from circulating norepinephrine conjugates124 deconjugated in the kidney, since the measured norepinephrine spillover is markedly reduced by the sympathetic nervous suppressant clonidine.103 Renal sympathetic nervous activity and norepinephrine spillover increase in sodium-depleted states,123 a sympathetic activation that appears to preferentially

Figure 5. Mean spillover of norepinephrine from the heart to plasma was increased at rest in patients with essential hypertension, cirrhosis, and cardiac failure. Modest increases in cardiac norepinephrine spillover were seen with right ventricular pacing to ischemia in patients with coronary artery disease and with mental arithmetic in hypertensive patients. Supine bicycle ergometry (50% maximum work capacity) produced a 30-fold increase in norepinephrine overflow from the heart.
involve the kidneys. A striking increase in renal norepinephrine spillover is seen in patients with cirrhosis. Increased norepinephrine overflow from the kidneys has also been reported in cardiac failure and essential hypertension.

**Norepinephrine Spillover into the Hepatomesenteric Circulation**

Hepatomesenteric norepinephrine spillover rate measurements represent a composite of gut, spleen, pancreas, and liver norepinephrine release to plasma and may be derived clinically from the systemic artery–hepatic vein plasma norepinephrine concentration difference, the hepatomesenteric extraction of triitated norepinephrine, and the hepatic plasma flow (calculated typically from the steady state clearance and hepatic extraction of indocyanine green). Net norepinephrine spillover measurements are less helpful for estimating regional sympathetic activity in this circulatory bed than for the heart and kidney, since hepatic vein norepinephrine plasma concentration values are typically much lower than arterial values, due to high rates of combined norepinephrine extraction by the gut and liver.

The application of radiotracer methodology for estimating release of norepinephrine into the hepatomesenteric circulation, particularly with norepinephrine extensively labeled in the ring position ([2,5,6-3H]norepinephrine), encounters technical problems. A net rise in plasma norepinephrine specific radioactivity is noted across the hepatomesenteric circulation in hypertensive subjects, unlike the isotope dilution (signifying norepinephrine release) seen in all other organs (see Figure 2). This must represent an “isotope effect,” with extraction of endogenous norepinephrine by the gut and liver being underestimated by the radio-labeled norepinephrine. The combination of such an isotope effect, plus low intrinsic rates of norepinephrine release, could cause a rise in plasma norepinephrine specific radioactivity. In all other organs an isotope effect presumably applies but is not apparent, since plasma norepinephrine specific radioactivity values invariably fall in transit. Higher rates of norepinephrine release than for the gut and liver and single extraction of norepinephrine (rather than the dual radiotracer extraction of the hepatomesenteric circulation) presumably account for this effect. This isotope effect should be less of a problem with single-labeled norepinephrine, [7-3H]norepinephrine. Hepatomesenteric norepinephrine release appears to be markedly increased in patients with untreated cirrhosis, an abnormality reversed in most instances by clonidine treatment, 100–300 μg i.v. (Figure 6). This increase in hepatomesenteric norepinephrine spillover in cirrhosis is particularly noteworthy, since portosystemic shunting of blood in this condition would lead to underestimation of spillover. Evidence is available to suggest that a regional increase in sympathetic activity in the hepatomesenteric circulation (involving intrahepatic postsinusoidal vascular resistance?) contributes to portal hypertension in cirrhosis. Within the limitations of the methodology, hepatomesenteric norepinephrine spillover appears to be normal in cardiac failure and essential hypertension.

**Norepinephrine Spillover from Other Organs**

Dispersed organ systems, such as skeletal muscle and skin, present two problems to the application of the method. The first is that one site is chosen for venous sampling, based on the necessary assumption that this site is representative of skin or skeletal muscle venous drainage in general. The second problem is that the plasma flow cannot be measured directly; it must be calculated from the cardiac output and arterial hematocrit (measured concurrently) and from published estimates of the fraction of the cardiac output received by the organ system in question. This fraction is approximately 18% of the cardiac output for skeletal muscle and 6% for skin.

It has been surmised that as much as 60% of the norepinephrine entering plasma derives from skeletal muscle sympathetic nerves. This seems most improbable, based on simple consideration of the kinetics of norepinephrine exchange, given that at rest skeletal muscle receives no more than 20% of the cardiac output and that the plasma concentration of norepinephrine in veins draining skeletal muscle is only marginally higher than the arterial plasma concentration. A perhaps more realistic estimate is that, of the total norepinephrine spillover, approximately 20 to 25% represents skeletal muscle sympathetic spillover; 5 to 6% represents norepinephrine overflow from skin in a neutral thermal environment.

The extent to which sympathetic nerves of the lungs contribute to the norepinephrine plasma pool is disputed. The lungs typically are the site of net pulmonary extraction of norepinephrine, but in the course of radiolabeled norepinephrine infusions in humans, a fall in plasma norepinephrine specific radioactivity during passage through the lungs has been noted (see Figure 2), demonstrating dilution of the radiotracer by released norepinephrine. The blood vessels of the human lungs receive a sympathetic innervation. It has been estimated that approximately one third of the norepinephrine entering plasma is derived from the sympathetic nerves of the lungs. This estimate is disputed, and the issue remains unsettled.

A blood-brain barrier to norepinephrine is thought to exist, blocking the passage of norepinephrine between the circulation and brain. The recent report of net spillover of norepinephrine into the cerebral circulation, although needing confirmation, raises the possibility that this barrier to norepinephrine flux is unidirectional only, blocking transfer from blood to brain, but allowing norepinephrine to pass in the reverse direction, from the central nervous system into the circulation.

**Removal of Norepinephrine from the Circulation**

Norepinephrine is rapidly removed from the circulation in humans. Norepinephrine plasma clearance,
measured by isotope dilution methods (with arterial sampling) is typically 1 to 3 L/min in healthy people. Removal of norepinephrine from plasma involves the combined processes of neuronal uptake into sympathetic nerves, extraneuronal uptake by a variety of other tissues including vascular endothelium, and metabolic conversion by o-methylation, oxidative deamination, and sulfoconjugation.

**Total Norepinephrine Plasma Clearance**

The overall rate of removal of norepinephrine from plasma is dependent on both the cardiac output and the activity of these cellular mechanisms of norepinephrine removal, particularly neuronal norepinephrine uptake. If the cardiac output is lowered, such as through α-adrenergic blockade, or in cardiac failure, norepinephrine plasma clearance is reduced (Table 3). If neuronal uptake of norepinephrine is diminished, as a result of disease of the peripheral sympathetic nerves (idiopathic peripheral autonomic insufficiency) or through the administration of norepinephrine uptake-blocking drugs, such as tricyclic antidepressants, regional norepinephrine extraction by individual organs and total norepinephrine plasma clearance falls. The rate of removal of norepinephrine from plasma is altered by a variety of clinical factors (see Table 3), most of which are associated with either defective neuronal norepinephrine uptake or change in cardiac output.

Such changes in norepinephrine plasma clearance bear on the validity of plasma norepinephrine measurements as a clinical index of overall sympathetic nervous system activity. As can be seen in the following list, alterations in norepinephrine clearance disturb the relationship expected between the rate of entry of the neurotransmitter to plasma and the plasma concentration values.

1. Plasma norepinephrine concentration greater than expected from norepinephrine release (removal of norepinephrine from plasma slowed):
   - Cardiac failure, α-adrenergic blockers, upright posture, tricyclic antidepressants, idiopathic peripheral autonomic insufficiency, lumbosacral sympathectomy, aging (in healthy subjects and in patients with essential hypertension)

2. Plasma norepinephrine concentration less than expected from norepinephrine release (norepinephrine plasma clearance increased):
   - Renal failure, high intake of dietary calories

3. Norepinephrine plasma concentration and release in agreement (norepinephrine plasma clearance normal):
   - Diseases: essential hypertension, cirrhosis, hypothyroidism, hyperthyroidism, central autonomic failure (Shy-Drager syndrome), diabetes, drugs: diuretics, clonidine, insulin, monoamine oxidase inhibition, hydrocortisone, other influences: physical conditioning, low salt diet, surgical procedure

Reduced clearance leads to higher plasma norepinephrine concentration values than expected for a given level of sympathetic nervous activity and norepinephrine release. A case in point concerns the effect of head-up tilting on plasma norepinephrine kinetics.

**Figure 6. Spillover of norepinephrine into the hepatomesenteric circulation was increased overall in patients with cirrhosis. Treatment with the sympathetic nervous system suppressant clonidine normalized norepinephrine overflow in most of these patients. Increased hepatomesenteric sympathetic nervous activity has been implicated in the pathogenesis of portal hypertension. Values are means ± SE. In subjects showing no isotope dilution across the hepatomesenteric circulation, or isotope enrichment, signifying reduced tracer extraction caused by an isotope effect, norepinephrine spillover was recorded as zero.**

**Table 3. Clinical Factors Influencing the Rate of Removal of Norepinephrine from Plasma**

<table>
<thead>
<tr>
<th>Decreased clearance</th>
<th>Increased clearance</th>
</tr>
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<tbody>
<tr>
<td><strong>Altered norepinephrine uptake?</strong></td>
<td></td>
</tr>
<tr>
<td>Tricyclic antidepressants</td>
<td></td>
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<tr>
<td>Idiopathic peripheral autonomic insufficiency</td>
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<tr>
<td><strong>Altered cardiac output?</strong></td>
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</tr>
<tr>
<td>Cardiac failure</td>
<td>Renal failure137</td>
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<tr>
<td>β-adrenergic blockers</td>
<td>Curhosis96</td>
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<tr>
<td>Upright posture</td>
<td>High intake of dietary calories80, 81</td>
</tr>
<tr>
<td>Low intake calories</td>
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<tr>
<td>Aging</td>
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Modified from Esler et al. with permission of the editors of the *Journal of the Autonomic Nervous System.*
The rise in plasma norepinephrine concentration with upright posture has been used extensively, and often uncritically, as a clinical index of reflex sympathetic nervous responsiveness. But norepinephrine plasma clearance falls with upright posture (see Figure 7) presumably due to a reduction in cardiac output and organ blood flows, contributing to the rise in plasma norepinephrine concentration. The rise in plasma norepinephrine concentration appears to be due at least as much to this fall in norepinephrine plasma clearance as to any increase in norepinephrine release (see Figure 7).

Regional Removal of Norepinephrine from the Circulation

One dimension of norepinephrine removal from plasma is the cellular mechanism involved (uptake, metabolism), the other is the organs participating. Circulating norepinephrine appears to be extracted by all organs except the brain; extraction has been demonstrated directly in humans for the limbs, skin, skeletal muscle, heart, kidneys, hepatomesenteric circulation, and lungs. The fractional extraction of norepinephrine in passage across individual organs (studied with radiolabeled norepinephrine) is highest for the hepatomesenteric circulation, 64% (Figure 8). Extraction by the heart, kidneys, and skeletal muscle is somewhat less, 35 to 55%. Skin extraction is less again, 25 to 30%. We find mean pulmonary norepinephrine extraction in both healthy subjects and patients with essential hypertension to be approximately 15%, using pulmonary artery sampling (see Figure 8). Earlier estimates of steady state pulmonary extraction (based on right atrial and right ventricular sampling) were considerably higher, a discrepancy attributed to incomplete mixing of the infused radiotracer in the right atrial and ventricular samples, leading to an overestimation of extraction. Norepinephrine extraction across the brain, with high internal jugular venous sampling, constitutes only a few percent (see Figure 8), a blood-brain barrier to norepinephrine extraction across the cerebrovascular circulation exists.

Fractional norepinephrine extraction by an organ, organ plasma flow, and regional norepinephrine (NE) plasma clearance are related in the following fashion:

$$	ext{Organ NE clearance} = \frac{\text{NE}_e \times \text{PF}}{\text{NE}_e}$$

where $\text{NE}_e$ is the fractional extraction of tritiated NE and PF is the organ plasma flow. Regional norepinephrine plasma clearance is highest across the lungs and hepatomesenteric circulation, with removal by the kidneys and skeletal muscle also contributing substantially to total plasma clearance (see Figure 8).

Patterns of Sympathetic Nervous Activation in Circulatory Disorders, Based on Measurements of Regional Norepinephrine Spillover

Increased overall rates of release of norepinephrine to plasma have been reported in a number of circulatory diseases, including cardiac failure, portal hypertension, and arterial hypertension (Figure 9). In certain instances, the pattern of sympathetic nervous activation, once elucidated, may give clues as to causal mechanisms involved. For example, is the regional sympathetic nervous activation present in patients with essential hypertension evocative of the defense reaction, so as to suggest the importance of mental stress as an etiological factor?

Regional Norepinephrine Spillover in Cardiac Failure and Cirrhosis

In cardiac failure, based on norepinephrine spillover measurements, the regional pattern of sympathetic nervous activation appears to be a very pronounced increase in cardiac, and a rather less pronounced increase in renal sympathetic activity, with no increase in pulmonary or hepatomesenteric sympathetic tone (see Figure 9). Electrophysiological measurements of firing rates in sympathetic nerves lying in subcutaneous nerves distributed to skeletal muscle indicate that sympathetic nervous discharge to muscle is also increased in cardiac failure. But the interpretation of these measurements of the rate of release of norepinephrine to plasma in heart failure, it must be remem-
NOREPINEPHRINE RELEASE IN HUMANS/Esler et al.

Figure 8. Removal of norepinephrine from the circulation by different organs in 50 untreated patients with essential hypertension. The fractional extraction of tritiated norepinephrine in single passage was greatest for the hepatomesenteric circulation (64%) and least for the brain (6%). Regional norepinephrine plasma clearance, determined by fractional extraction and organ plasma flow, was greatest for the hepatomesenteric circulation and the lungs, which together accounted for more than 60% of total plasma clearance. Means ± SE are shown.

Figure 9. Total (arterial sampling) and regional spillover of norepinephrine to plasma in 20 patients with cardiac failure, 50 patients with essential hypertension, and 35 patients with cirrhosis. The regional patterns of sympathetic nervous activation, based on measurements of norepinephrine overflow, differed in these three disorders with increased total sympathetic nervous system activity. Means ± SE are indicated. The mean values in the reference population of 28 healthy subjects are listed as 100%. Single (p < 0.05) and double (p < 0.01) asterisks indicate significant difference, by Mann-Whitney U test.139

Total norepinephrine spillover to plasma is increased in essential hypertension, but to a lesser degree than that noted in patients with cardiac failure or cirrhosis (see Figure 9). Approximately 50% of the increase in total norepinephrine spillover in our own series of 50 patients with untreated essential hypertension was explicable in terms of increased overflow of norepinephrine from the kidneys and heart.

Regional Norepinephrine Spillover in Essential Hypertension

Total norepinephrine spillover to plasma is increased in essential hypertension, but to a lesser degree than that noted in patients with cardiac failure or cirrhosis (see Figure 9). Approximately 50% of the increase in total norepinephrine spillover in our own series of 50 patients with untreated essential hypertension was explicable in terms of increased overflow of norepinephrine from the kidneys and heart.

The increased renal norepinephrine spillover in patients provides presumptive evidence of increased renal sympathetic nervous activity, which is not readily accounted for by other factors possibly influencing norepinephrine spillover, such as altered renal blood flow, is complicated by an important consideration: Have low organ blood flows led to reduced washout of the released norepinephrine? This factor would apply in particular to organs with a disproportionate reduction in flow, such as the kidney and hepatomesenteric circulation, but not to the heart, for which blood flow is preserved in cardiac failure.51,121

In cirrhosis also, cardiac and renal norepinephrine spillover rates are increased. In addition, a striking increase in norepinephrine release into the hepatomesenteric circulation is apparent (see Figure 9). The stimulus for sympathetic nervous activation in cirrhosis is uncertain; circulatory underfilling and vasodilatation and arteriovenous shunting have been suggested. At present it is not clear whether this increased spillover of norepinephrine into the hepatomesenteric circulation derives from the sympathetic nerves of the gut, the liver, or both. Portal venous sampling for plasma norepinephrine measurements would be needed to decide this point. Available pharmacological evidence does suggest that increased intrahepatic sympathetic activity is present in cirrhosis, elevating the postsinusoidal vascular resistance and contributing to the portal hypertension commonly present.129
flow and norepinephrine washout (renal blood flow was not increased in the hypertensive patients) or defective neuronal norepinephrine uptake (renal extraction of radiolabeled norepinephrine was normal). Renal norepinephrine spillover is increased particularly in young patients, markedly so in some instances, to a level matching that seen in cardiac failure and cirrhosis (Figure 10). The findings complement rather less direct observations from several sources\textsuperscript{143-145} that have previously suggested that renal sympathetic nervous activity is increased in young adults with essential hypertension. Sympathetic nerves to the kidney terminate in blood vessels, the juxtaglomerular apparatus, and the renal tubules.\textsuperscript{2,146} To what extent the increased norepinephrine release originates from each of these three sites is not known. Participation of the sympathetic nerves to the juxtaglomerular apparatus is suggested by the relation observed between renal norepinephrine spillover rates in hypertensive patients and the rate of renal renin secretion and, as a consequence, between norepinephrine spillover and clinical renin status.\textsuperscript{103,143,147} It is unclear whether parallel increases exist in the firing rates of sympathetic nerves to the renal tubules, contributing to sodium reabsorption, and to the renal resistance vessels.

The increased release of norepinephrine from the heart to plasma noted in patients with essential hypertension is unlikely to be due to coexistent left ventricular hypertrophy, since cardiac norepinephrine content and turnover do not increase in parallel with the increase in ventricular mass in hypertensive hypertrophy of the heart.\textsuperscript{148,149} The finding of increased cardiac norepinephrine spillover supports earlier results based on hemodynamic and pharmacological techniques, which suggest that cardiac sympathetic activity is increased in essential hypertension, especially in younger patients, contributing to higher heart rates and cardiac output.\textsuperscript{1,13,143,150}

No increase in norepinephrine release from the hepatomesenteric circulation was evident in hypertensive patients (see Figure 9). Any conclusion that sympathetic nervous activity in the gut and liver is normal in essential hypertension, however, must be tentative, because of the technical problems that exist when the radiotracer method for measuring norepinephrine spillover is applied to this particular vascular bed. Sympathetic nervous activity appears to be normal in the lungs of hypertensive patients (see Figure 9). The same conclusion can be tentatively reached based on venoarterial plasma norepinephrine concentration gradients\textsuperscript{133} for the sympathetic nerves to skin and skeletal muscle. The hemodynamic studies of Brod et al.\textsuperscript{151} and the electrophysiological studies of Wallin and colleagues\textsuperscript{10,11} also suggest that sympathetic nervous activity in skeletal muscle is not increased in essential hypertension. How might this regional pattern of sympathetic nervous system activation in essential hypertension, apparently confined to the sympathetic outflows to the kidney and heart, have originated?

One possibility, which can neither be confirmed nor dismissed with certainty, is that faulty neuronal uptake of norepinephrine produced a selective increase in norepinephrine spillover, restricted to the heart and kidneys. Neuronal norepinephrine uptake does appear to be defective in some patients with essential hypertension.\textsuperscript{12,22-24} A norepinephrine uptake defect might selectively increase cardiac norepinephrine spillover, as in the heart many sympathetic synaptic junctions are narrow and facilitation of norepinephrine spillover by reduced uptake is greatest at tight synaptic junctions.\textsuperscript{51,117,122} But this mechanism would not readily explain selectively increased renal spillover, since the kidney has a preponderance of broad, vascular neuroeffector junctions.\textsuperscript{2,146} Further, norepinephrine uptake in the heart and kidneys, at least as reflected by cardiac and renal extraction of radiolabeled norepinephrine, seems to be normal in essential hypertension. Could the sympathetic nervous activation result from sodium depletion due to pressure natriuresis?\textsuperscript{215} Probably not, if one considers the regional pattern of sympathetic nervous stimulation resulting from low salt states. With sodium depletion from diuretic use or a low salt diet, increased sympathetic activity seems to occur in the renal, but not the cardiac, sympathetic nervous outflow,\textsuperscript{70,71} in contrast to the finding in hypertensive patients.

Folkow\textsuperscript{15} has recently reviewed one view of hypertension pathogenesis that gives priority to psychosomatic mechanisms, emphasizing the importance of neurohumoral responses in the defense reaction. Falkner et al.,\textsuperscript{153,154} in studies on blood pressure in

\begin{figure}[h]
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\caption{An influence of age on renal norepinephrine spillover was evident in hypertensive patients, but not healthy subjects. Increased renal sympathetic activity (based on measurements of norepinephrine overflow) was most apparent in patients aged less than 40 years. Single (*p < 0.05) and double (\textsuperscript{*}p < 0.01) asterisks indicate significant difference, by Mann Whitney U test.\textsuperscript{129}}
\end{figure}
adolescents, have consistently noted greater sympathetic nervous system responsiveness to experimental mental stress in those subjects with a family history of hypertension, whether their blood pressure is currently elevated or not. Light et al. 15, 16 report similar findings, noting greater cardiovascular responsiveness and sodium retention with mental challenge in young men genetically predisposed to essential hypertension. Hollenberg et al. 145 describe exaggerated renal vasoconstrictor responses with experimental mental stress in patients with essential hypertension. Could findings of increased cardiac and renal norepinephrine overflow represent a similar accentuation of the stress response in hypertensive patients? Increased renal and cardiac sympathetic nervous activity in the presence of normal skeletal muscle sympathetic activity represents the regional sympathetic nervous response expected with the defense reaction. 15, 102 Not consistent, however, is the finding of apparently normal hepatomesenteric sympathetic activity. In short, the precise cause of these changes in hypertensive patients is unclear.

The expectation that mapping of the regional pattern of sympathetic nervous system activity in patients with essential hypertension might indicate the primary cause of the hypertension remains unfulfilled. The increased renal and cardiac sympathetic nervous activity present in some patients with essential hypertension, particularly younger ones, does, however, provide a plausible mechanism for the development of their hypertension, whatever the fundamental cause. An unresolved issue is how increased sympathetic nervous system activity could be present in young patients with essential hypertension, contributing to the development of their hypertension, but, by and large, be absent in older patients. Although there is no ready answer to this question, a parallel might be drawn between essential hypertension and some experimental models of hypertension. In one-kidney, one-clip Goldblatt renovascular hypertension in the dog, for example, increased renin secretion, the trigger for hypertension development with renal artery stenosis, is present only in the early stages. At a later phase in the course of the hypertension the cardiovascular amplifier effect of arteriolar and cardiac hypertrophy predominates as the hypertensive mechanism. 16, 115 In essential hypertension there may be a similar dissociation of the mechanisms responsible for initiating and for maintaining the high blood pressure.

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_Hypertension_. 1988;11:3-20
doi: 10.1161/01.HYP.11.1.3

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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