0021-972X/95/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 80, No. 10
Printed in U.S.A.

Regional Release and Removal of Catecholamines and Extraneuronal Metabolism to Metanephrines

GRAEME EISENHOFER, BENGT RUNDQUIST, ANDERS ÅNEMAN, PETER FRIBERG, NADER DAKAK, IRWIN J. KOPIN, MARIE-CÉCILE JACOBS, AND JACQUES W. M. LENDERS

Clinical Neuroscience Branch, National Institute of Neurological Disorders and Stroke (G.E., I.J.K.), and Cardiology Branch, National Heart Lung and Blood Institute (N.A.D.), National Institutes of Health, Bethesda, Maryland 20892; the Departments of Cardiology (B.R.), Physiology (A.A.), and Clinical Physiology (P.F.), University of Göteborg, Göteborg, Sweden; and the Department of Internal Medicine, St. Radboud University Hospital (M.-C.J., J.W.M.L.), Nijmegen, The Netherlands

ABSTRACT

Norepinephrine (NE) and epinephrine (E) are metabolized extraneuronally by catechol-*O*-methyl-transferase to the metanephrines (MNs), normetanephrine (NMN) and metanephrine (MN). Subjects in this study received infusions of tritium-labeled NE and E. Concentrations of MNs and catecholamines were measured in plasma flowing into and out of the heart, forearm, lungs, kidneys, mesenteric organs (gastrointestinal tract, spleen, and pancreas), liver, and adrenals to examine the regional production of MNs from circulating and locally released catecholamines. NE spillover from mesenteric organs and kidneys accounted for 64% of the spillover from all tissues. There was detectable spillover of E from most extraadrenal tissues, but 91% was from the adrenals. The production of MNs from locally released and circulating catecholamines varied widely among tissues. The liver made the largest contribution to removal of circulating NE (57%) and E (32%) and the largest contribution to the production of NMN (54%) and MN (37%) from metabolism of circulating catecholamines. In all other tissues more NMN was produced from locally released than from circulating NE. Thus, the metabolism of circulating NE was responsible for only 19% of the total production of NMN. An even smaller portion (6%) of plasma MN was derived from metabolism of circulating E. Most plasma MN (91%) was produced within the adrenals, which also provided the largest single source (23%) of NMN. The regional variation in extraneuronal production of MNs indicates considerable heterogeneity in how circulating and locally released catecholamines are handled by different tissues. The substantial contribution of the adrenals to the production of MNs explains the extraordinary sensitivity of these metabolites for the diagnosis of pheochromocytoma. (*J Clin Endocrinol Metab* **80:** 3009–3017, 1995)

ESTIMATION of norepinephrine (NE) spillover into the circulation, using the dilution of iv infused [^{3}H]NE with endogenous NE in plasma (*i.e.* the specific activity of plasma [^{3}H]NE) provides a useful index of NE release from sympathetic nerves (1). Most released NE, however, is efficiently removed by neuronal and extraneuronal uptake, so that only a small portion escapes into the circulation (2). Additional measurements of dihydroxyphenylglycol, the intraneuronal deaminated metabolite of NE, enable estimation of neuronal NE reuptake (3–5). The metanephrines (MNs), normetanephrine (NMN) and metanephrine (MN) are extraneuronal *O*-methylated metabolites of NE and epinephrine (E); thus, measurements of their plasma concentrations enable examination of the extraneuronal uptake and metabolism of catecholamines (6, 7).

The above use of catecholamine metabolites to examine the neuronal and extraneuronal disposition of catecholamines depends on measurements of specific activities of metabolites and their precursor amines in plasma during the infusion of ^{3}H-labeled catecholamines. In particular, differences in specific activities of ^{3}H-labeled metabolites entering and leaving an organ are dependent on the relative amounts of metabolite derived from two sources: 1) metabolism of high specific activity catecholamine removed from in-flowing plasma, and 2) metabolism of locally released unlabeled catecholamine.

This study applied the above considerations to examine the sources of plasma MNs and assess whether the extraneuronal removal and metabolism of catecholamines vary among different tissues. Concentrations of MNs and catecholamines were measured in plasma flowing into and out of the heart, forearm, lungs, kidneys, adrenals, mesenteric organs, and liver. Samples of plasma draining mesenteric organs were obtained from the portal vein and, thus, represent plasma draining the gastrointestinal tract, spleen, and pancreas. Results in the intact innervated heart were compared with those in patients with heart transplants. Denervation of cardiac sympathetic nerves in these patients would be expected to produce little influence of locally released NE on the specific activity of [^{3}H]NMN leaving the heart.

Subjects and Methods

Subjects

Subjects were studied as part of ongoing protocols at 3 different institutions: the NIH, the University of Göteborg, and St. Radboud University Hospital. Regional blood sampling was carried out in 77 subjects studied in a cardiac catheterization laboratory, 8 patients studied during surgical anesthesia, and 10 subjects studied in a patient

Received February 7, 1995. Revision received April 18, 1995. Accepted April 24, 1995.

Address all correspondence and requests for reprints to: Dr. Graeme Eisenhofer, Building 10, Room 5N214, National Institutes of Health, 10 Center Drive, MSC 1424, Bethesda, Maryland 20892-1424.

observation room. All procedures were approved by the appropriate review committees, and all subjects gave informed written consent to participate in studies.

Subjects examined in a cardiac catheterization laboratory included 7 normal volunteers (6 males and 1 female; 29–39 yr old), 20 patients with angina pectoris with and without coronary artery disease (18 males and 2 females; 48–71 yr old), 39 patients with cardiac failure secondary to coronary artery disease or idiopathic dilated cardiomyopathy (30 males and 9 females; 33–75 yr old), 8 patients who had received heart transplants 1 yr previously (7 males and 1 female; 28–64 yr old), 2 male patients with renal artery stenosis (55 and 62 yr old), and 1 male patient during inferior vena caval blood sampling for localization of a suspected pheochromocytoma (38 yr old). The latter patient was subsequently determined not to harbor a tumor. Patients with heart failure were receiving a variety of medications (typically some combination of calcium channel blockers, digoxin, angiotensin-converting enzyme inhibitors, β-blockers, nitrates, and diuretics) that were discontinued for 12 h before the study. Patients with heart transplants were receiving cyclosporine, azathioprine, and prednisone. All other subjects remained unmedicated for at least 48 h before the studies.

Patients studied during surgical anesthesia included five males and three females (47–77 yr old). The presence of a gastric adenocarcinoma provided the reason for surgery in seven patients, whereas a pancreatic neoplasm was the reason for surgery in the other patient. No signs of hepatic or distant metastases were found in any patient. Anesthesia was induced with sodium thiopental (3–5 mg/kg) and vecuronium bromide (1.5 mg/kg). Patients were intubated and mechanically ventilated with 30% oxygen and 70% nitrous oxide. Anesthesia was maintained with enflurane (0.5–0.7 minimum alveolar anesthetic concentration), fentanyl (2.5–3.0 g/kg), and midazolam (1 mg/kg) as needed.

Subjects studied in a patient observation room included five normal volunteers and five patients with essential hypertension (six males and four females; 28–47 yr old). All subjects were studied in the supine position, did not receive any medication for 2 weeks before the study, and had abstained from nicotine, alcohol, and caffeinated beverages for at least 12 h before the study.

Infusions of tritium-labeled catecholamines

All subjects, except the suspected pheochromocytoma patient, received infusions of [^{3}H]NE (levo-2,5,6-[^{3}H]NE; 40–60 Ci/mmol; New England Nuclear Corp., Boston, MA) either delivered alone or, in most (65 of 95) cases, in combination with [^{3}H]E (levo-*N*-*methyl*-[^{3}H]E; 65–75 Ci/mmol; also from New England Nuclear). The radiotracers were infused into a forearm vein at 1.0–1.5 μCi/min.

Blood sampling and blood flow determinations

Blood samples (10 mL) were collected into prechilled syringes at least 15 min after the start of radiotracer infusions. For patients studied in catheterization laboratories, blood samples (10 mL) were taken simultaneously from a radial or femoral artery and from the coronary sinus (n = 62), the pulmonary artery (n = 36), or the right renal vein (n = 43). To examine MN production by the adrenals, blood samples were taken from an adrenal vein in four subjects and from the left and right renal veins in two subjects. In the latter subjects the catheter to the left kidney was advanced proximally to the vein draining the left adrenal so that the blood collected included effluent from both the left kidney and the left adrenal. Coronary sinus blood flow and cardiac output were determined by thermodilution, whereas renal blood flow was determined from the total body clearance and renal extraction of *p*-aminohippurate.

For patients studied during surgical anesthesia, blood samples were taken simultaneously from a radial artery, the portal vein, and the right hepatic vein. Samples from the portal vein were taken by venipuncture, whereas those from the hepatic vein were taken using a catheter advanced to the site from a femoral vein. Blood flow through the hepatic artery and portal vein were measured using ultrasound transit time flow probes connected to a HT207 dual channel flowmeter (Transonic Systems, Ithaca, NY).

Studies carried out in a patient observation room involved simultaneous sampling of blood from a brachial artery and a deep antecubital vein. Forearm blood flow was measured using venous occlusion strain gauge plethysmography with circulation to the hand excluded by inflation of a wrist cuff to 100 mm Hg above systolic pressure for the duration of each blood flow determination.

Blood samples were collected into ice-cold tubes containing an anticoagulant [heparin, ethylenediamine tetraacetate, or ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid] and stored on ice until studies were completed. Samples were then centrifuged at 4 C, and plasma was separated for storage at −80 C until assayed for plasma concentrations of catecholamines and MNs.

Assays

The MNs (NMN and MN) were extracted from 2 mL plasma using solid phase ion exchange columns and quantified by liquid chromatography with electrochemical detection (8). The catecholamines (NE and E) were extracted from 1-mL samples of plasma using alumina and quantified by liquid chromatography with electrochemical detection (9). Timed collections of eluants leaving the electrochemical cell, followed by liquid scintillation spectroscopy, enabled determination of plasma concentrations of ^{3}H-labeled catecholamines and MNs. Interassay coefficients of variation were 12.2% for NMN, 11.2% for MN, 6.5% for NE, and 11.4% for E. Intraassay coefficients of variation were 4.2% for NMN, 3.3% for MN, 1.9% for NE, and 3.0% for E.

Calculations

The fraction of catecholamine in in-flowing plasma removed during passage through an organ (fx_C) was estimated according to the equation $fx_C = ([^3H]C_I - [^3H]C_O)/[^3H]C_I$ (Eq I), where $[^3H]C_I$ and $[^3H]C_O$ are the respective in-flowing and out-flowing plasma concentrations of ^{3}H-labeled catecholamine (disintegrations per min/mL). Rates of removal (picomoles per min) of catecholamines by a specific organ or tissue were estimated from the product of fx_C with the in-flowing plasma concentration of catecholamine and the plasma flow through the organ.

The specific activity (SA) of an ^{3}H-labeled catecholamine precursor or O-methylated amine metabolite in plasma (*i.e.* the concentration of tritium bound to the amine divided by the concentration of the amine) was estimated according to the equation $SA = [^3H]A/A$ (Eq II), where $[^3H]A$ is the plasma concentration of the tritium bound to the amine (disintegrations per min/mL), and A is the plasma concentration of the amine (picomoles per mL).

For a tissue with negligible local release of catecholamine (*e.g.* the denervated transplanted heart or E in extraadrenal tissues), but which metabolizes circulating ^{3}H-labeled and endogenous catecholamines to MNs, the specific activity of the ^{3}H-labeled metabolite in out-flowing plasma (SA_O) is dependent on the higher specific activity of the [^{3}H]catecholamine precursor (SA_C) and the lower specific activity of the ^{3}H-labeled metabolite (SA_I) in in-flowing plasma. Under the above conditions, the proportion (p_O) of metabolite in out-flowing plasma derived from metabolism of in-flowing catecholamine can be estimated according to a previously described approach (10) using the equation $p_O = (SA_O - SA_I)/(SA_C - SA_I)$ (Eq III).

Under the same conditions, the fraction of metabolite removed during passage through the organ (fx_M) can then be estimated by correction of the out-flowing plasma concentration of endogenous metabolite for the contribution from in-flowing precursor according to the equation $fx_M = [M_I - (M_O - p_O \times M_O)]/M_I$ (Eq IV), where M_I and M_O are the respective concentrations (picomoles per mL) of MN (or NMN in a denervated organ) in in-flowing and out-flowing plasma, and p_O is estimated from Eq III. Rates of removal (picomoles per min) of MNs by specific tissues were estimated from the product of fx_M with the in-flowing plasma concentration of metabolite and the plasma flow through the organ.

The spillover of endogenous catecholamine or NMN into plasma from an organ (S) was then estimated according to the equation (11) $S = F \times [(A_O - A_I) + (fx \times A_I)]$ (Eq V), where F is the plasma flow through the organ (milliliters per min), A_I and A_O are the respective in-flowing and out-flowing plasma amine concentrations (picomoles per mL), and fx is the fractional extraction of the amine (from Eq I and IV). For estimation of NMN spillover, the fractional extraction of NMN was assumed to equal the fractional extraction of MN.

The proportion of extracted NE or E metabolized to NMN or MN (p_C)

was estimated according to the equation $p_C = [[^3H]M_O - ([^3H]M_I \times fx_M)]/([^3H]C_I \times fx_C)$ (Eq VI), where $[^3H]M_I$ and $[^3H]M_O$ are the respective concentrations of ^3H-labeled MN or NMN in in-flowing and out-flowing plasma, and the other terms are described in Eq I and IV above.

The spillover of NMN or MN derived from local metabolism of the NE or E extracted from in-flowing plasma by the organ or tissue (S_p) was then estimated using the equation $S_p = (p_C \times fx_C \times C_I) \times F$ (Eq VII); all terms are described in Eq I, IV, and VI above. The spillover of NMN derived from metabolism of locally released NE was then estimated by subtraction of S_p from the total spillover of NMN.

Plasma flows in the forearm were measured in units of milliliters per 100 mL tissue/min, so that forearm spillovers have units of picomoles per 100 mL/min. Previously documented data about the density of skeletal muscle (12) and the mass of skeletal muscle in the body as a function of gender and body weight (13) were used to transform rates of catecholamine and MN spillover and removal for the forearm into rates for skeletal muscle throughout the body (units of picomoles per min). The above transformation assumes that spillovers from the forearm reflect spillovers from skeletal muscle elsewhere in the body.

Rates of spillover of catecholamines and MNs from the adrenals were estimated using two methods. In the two patients from whom blood samples were obtained from the right renal vein and the common vein draining the left kidney and left adrenal gland, spillovers were estimated from the product of renal plasma flow and differences in catecholamine or MN concentrations at the two sampling sites. In the other patients from whom adrenal venous blood samples were obtained, adrenal spillovers of catecholamines were estimated according to Eq V above. Adrenal spillovers of MNs were estimated similarly, except that no correction was made for fractional extractions, which were assumed to be negligible in relation to the arterial-venous increase in concentrations. Adrenal blood flow was assumed to equal 30 mL/min, as derived from previously documented data (14, 15).

Statistical methods

Results are expressed as means with variance for paired data (*i.e.* in-flowing and out-flowing plasma concentrations of MNs and fractional extractions of NE and E) shown using SEs of the difference and for other data using SEM. Plasma concentrations of catecholamines and MNs are not normally distributed. Therefore, levels of statistical significance were determined using nonparametric methods; Wilcoxon's signed rank sum test was used for comparisons of paired data, and the Mann-Whitney test was used for comparisons of other data. Unless stated, statistical significance was defined as $P < 0.05$.

Results

Regional differences in plasma MNs

There was a 12 ± 2% arterial-venous increase ($P < 0.001$) in plasma concentrations of NMN across the heart (Table 1). No other organ, apart from the adrenals, showed evidence of local production of NMN, as indicated by increases in NMN concentrations from in-flowing to out-flowing plasma. In contrast, concentrations of MN in in-flowing plasma were consistently higher ($P < 0.01$) than those in out-flowing plasma for every tissue, except the adrenals. The kidneys and liver showed the largest arterial-venous decreases ($P < 0.005$) in plasma MN. These organs also showed decreases ($P < 0.01$) in in-flowing to out-flowing plasma concentrations of NMN, indicating local extraction of both NMN and MN. Local production of MNs was most evident for the adrenals, which showed a 10-fold arterial-venous increase in plasma NMN and an 87-fold increase in plasma MN.

TABLE 1. Concentrations of metanephrines in plasma flowing into and out of various organs and tissues

	No.	Inflowing	Outflowing	SED
Heart				
NMN	57	0.293	0.329	±0.005[a]
MN	62	0.256	0.210	±0.004[b]
Forearm				
NMN	10	0.260	0.299	±0.024
MN	10	0.279	0.175	±0.012[b]
Lungs				
NMN	34	0.317	0.314	±0.007
MN	36	0.260	0.244	±0.005[b]
Kidneys				
NMN	40	0.281	0.256	±0.008[b]
MN	41	0.246	0.154	±0.007[b]
Mesenteric organs				
NMN	7	0.399	0.380	±0.024
MN	8	0.283	0.195	±0.027[b]
Liver				
NMN	7	0.384	0.212	±0.047[b]
MN	7	0.243	0.090	±0.034[b]
Adrenals				
NMN	4	0.378	3.951	±2.122
MN	4	0.274	23.857	±16.362

Results represent mean plasma concentrations (picomoles per mL) with variance between inflowing and outflowing concentrations shown by the SE of the difference (SED).

[a] Significantly higher concentration in outflowing than inflowing plasma.

[b] Significantly lower concentration in outflowing than inflowing plasma.

Handling of MNs by the transplanted and innervated heart

In contrast to the innervated hearts of normal volunteers and patients with angina, in which there were small, but fairly consistent, increases ($P < 0.002$) in NMN from arterial to coronary venous plasma, patients with heart transplants showed consistent arterial-coronary venous decreases ($P < 0.02$) in plasma NMN (Fig. 1, *top panel*). Plasma concentrations of MN showed similar arterial-coronary venous decreases ($P < 0.02$) in both innervated control subjects and denervated heart transplant patients (Fig. 1, *bottom panel*). This and lack of a difference in arterial and coronary venous concentrations of $[^3H]$MN resulted in a highly consistent 20.2 ± 2.2% increase ($P < 0.001$) in the specific activity of $[^3H]$MN from arterial to coronary venous plasma.

In contrast to the increase in specific activity of $[^3H]$MN across the heart, control subjects showed a small, but consistent, decrease ($P < 0.001$) in the specific activity of $[^3H]$NMN from arterial to coronary venous plasma. On the other hand, heart transplant patients showed an increase ($P < 0.03$) in the specific activity of $[^3H]$NMN from arterial to coronary venous plasma (Fig. 2, *lower panel*). Control subjects showed a large decrease ($P < 0.001$) in the specific activity of $[^3H]$NE from arterial to coronary venous plasma, whereas patients with heart transplants showed a much smaller arterial-venous decrease ($P < 0.05$) in the specific activity of $[^3H]$NE across the heart (Fig. 2, *upper panel*).

The fractional extraction of $[^3H]$NE was decreased by 81% in patients with heart transplants compared to that in innervated controls (0.70 ± 0.02 *vs.* 0.13 ± 0.2). Correction of out-flowing plasma concentrations of MNs for the contribution from metabolism of in-flowing catecholamines (see Eq IV in *Materials and Methods*) indicated no difference between fractional cardiac extractions of plasma NMN (0.20 ± 0.05) and MN (0.21 ± 0.01) in patients with heart transplants.

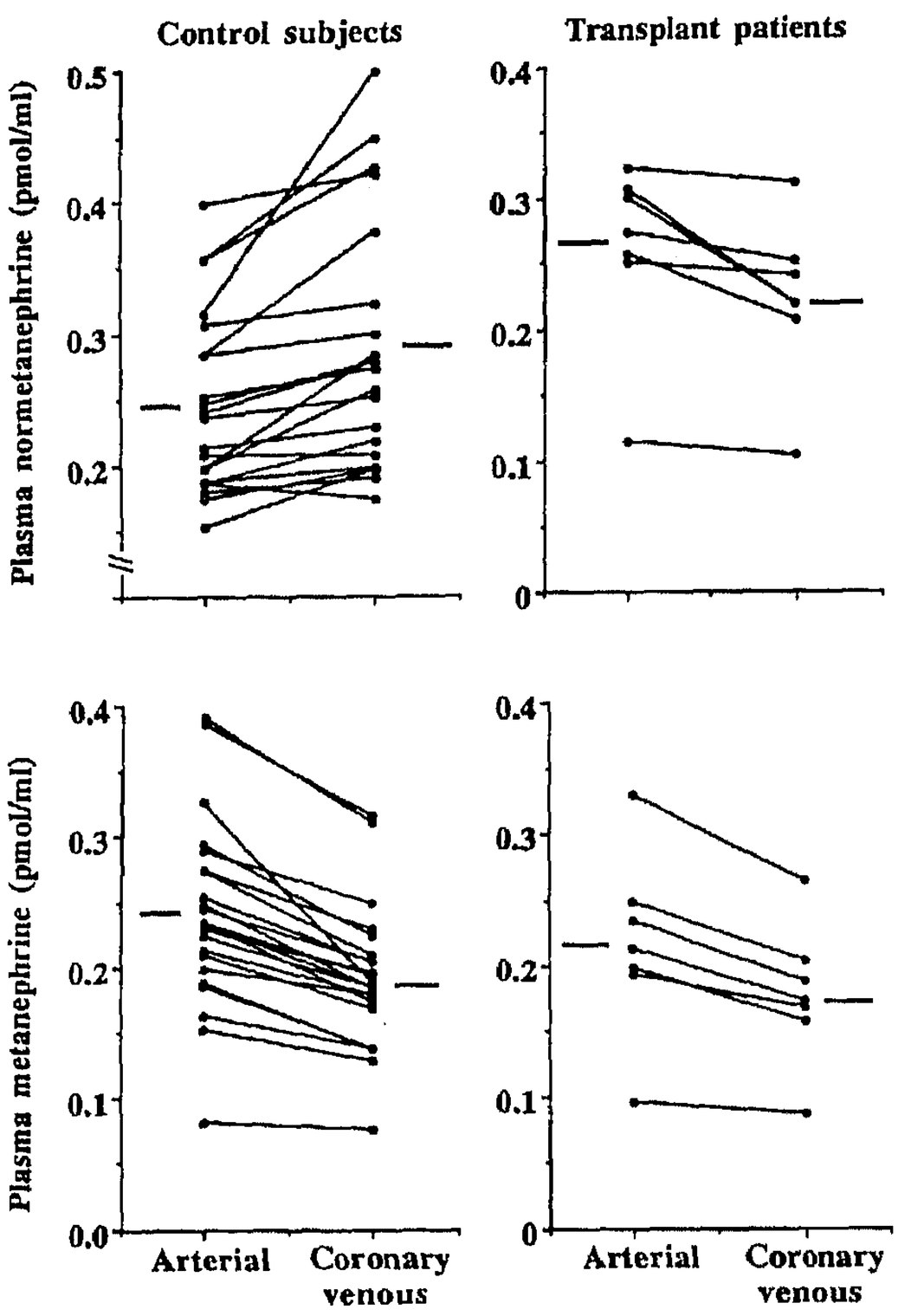

FIG. 1. Plasma concentrations of NMN (*upper panel*) and MN (*lower panel*) in arterial and coronary venous plasma in control subjects (*left*) and patients who had received heart transplants 1 yr earlier (*right*). Data represent individual paired values for arterial and coronary venous concentrations, with means shown by *horizontal bars*.

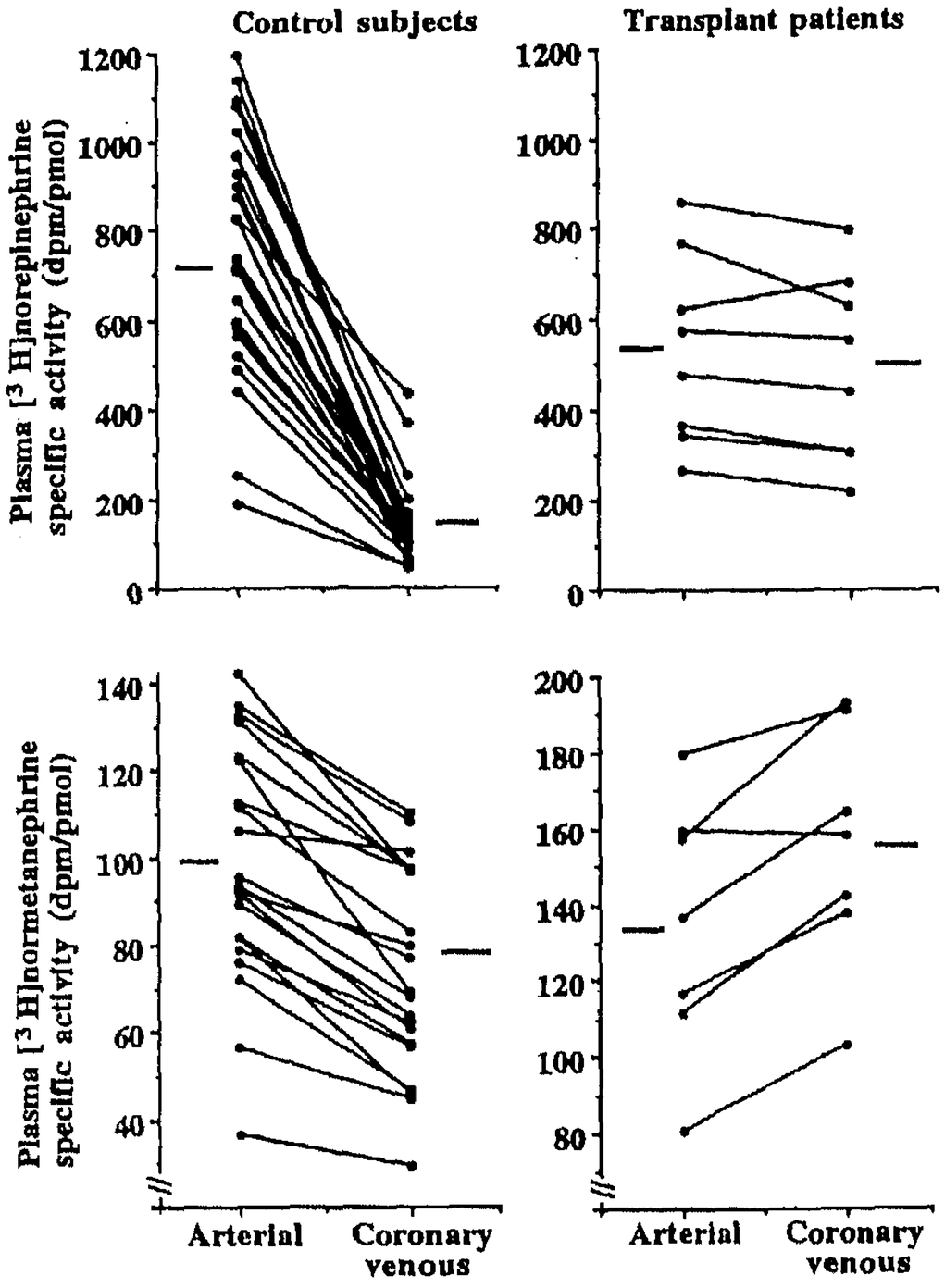

FIG. 2. Specific activities of [^{3}H]NE (*upper panel*) and [^{3}H]NMN (*lower panel*) in arterial and coronary venous plasma in control subjects (*left*) and patients who had received heart transplants 1 yr earlier (*right*). Data represent individual paired values for arterial and coronary venous specific activities, with means shown by *horizontal bars*.

Regional differences in specific activities of plasma [^{3}H]MNs

Changes in specific activities of [^{3}H]NMN from in-flowing to out-flowing plasma showed considerable variation among different tissues (Fig. 3). Like the heart, mesenteric organs showed an arterial-venous decrease ($P < 0.02$) in the specific activity of [^{3}H]NMN, whereas increases ($P < 0.02$) in [^{3}H]NMN specific activity were observed across the kidneys and liver. There were no differences in specific activities of [^{3}H]NMN in the in-flowing and out-flowing plasma of the forearm and lungs.

In contrast to the variable changes in specific activities of [^{3}H]NMN from in-flowing to out-flowing plasma of different tissues, specific activities of [^{3}H]MN showed consistent increases ($P < 0.02$) from in-flowing to out-flowing plasma for the heart, forearm, lungs, mesenteric organs, liver, and kidneys (Fig. 3). The magnitude of the increase in specific activity of [^{3}H]MN was highly variable from organ to organ. The liver showed the largest increase, with a specific activity of [^{3}H]MN in out-flowing plasma that was 97.3 ± 19.8% higher than that in in-flowing plasma. The lungs showed the smallest increase, with a specific activity of [^{3}H]MN in out-flowing plasma that was 9.0 ± 2.8% higher than that in in-flowing plasma. The adrenals, in contrast to all other tissues, showed a large arterial-venous fall in the specific activity of plasma [^{3}H]MN (data not shown).

Regional extractions of catecholamines

Differences in magnitudes of increases in specific activities of [^{3}H]MN from in-flowing to out-flowing plasma among the different organs and tissues (Fig. 3) showed a pattern similar to the differences in regional fractional extractions of [^{3}H]E (Fig. 4). The lungs, with the lowest fractional extraction of [^{3}H]E, also had the smallest increase in specific activity of [^{3}H]MN from in-flowing to out-flowing plasma. The liver, with the highest fractional extraction of [^{3}H]E, also had the largest increase in [^{3}H]MN specific activity from in-flowing to out-flowing plasma. In contrast, there was no relationship between fractional extractions of [^{3}H]NE and differences in specific activities of [^{3}H]NMN in in-flowing and out-flowing plasma.

Fractional extractions of [^{3}H]NE showed variable differences from those of [^{3}H]E, depending on the tissue (Fig. 4). The fractional extraction of [^{3}H]E was 35 ± 2% lower than that of [^{3}H]NE in the heart, 10 ± 2% higher than that of [^{3}H]NE in the forearm, 10 ± 4% higher than that of [^{3}H]NE in the kidneys, not different in mesenteric organs, and 4 ± 1% lower than that of [^{3}H]NE in the liver.

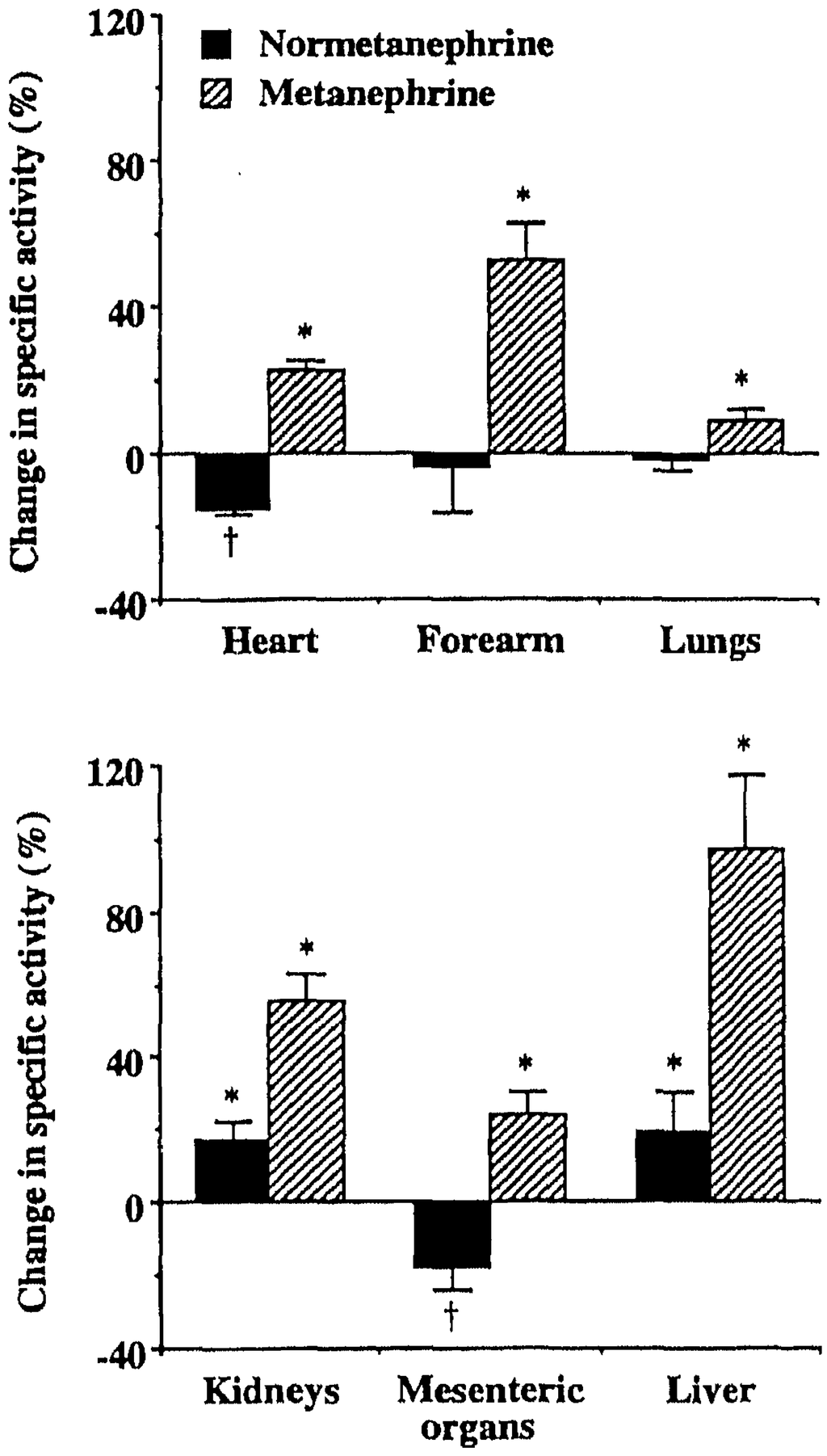

FIG. 3. Percent differences in specific activities of [^{3}H]NMN and [^{3}H]MN from in-flowing to out-flowing plasma across the heart, forearm, and lungs (*upper panel*) and across the kidneys, mesenteric organs, and liver (*lower panel*). Values represent the mean ± SEM. *, An increase ($P < 0.05$) in specific activity from in-flowing to out-flowing plasma. †, A decrease ($P < 0.05$) in specific activity from in-flowing to out-flowing plasma.

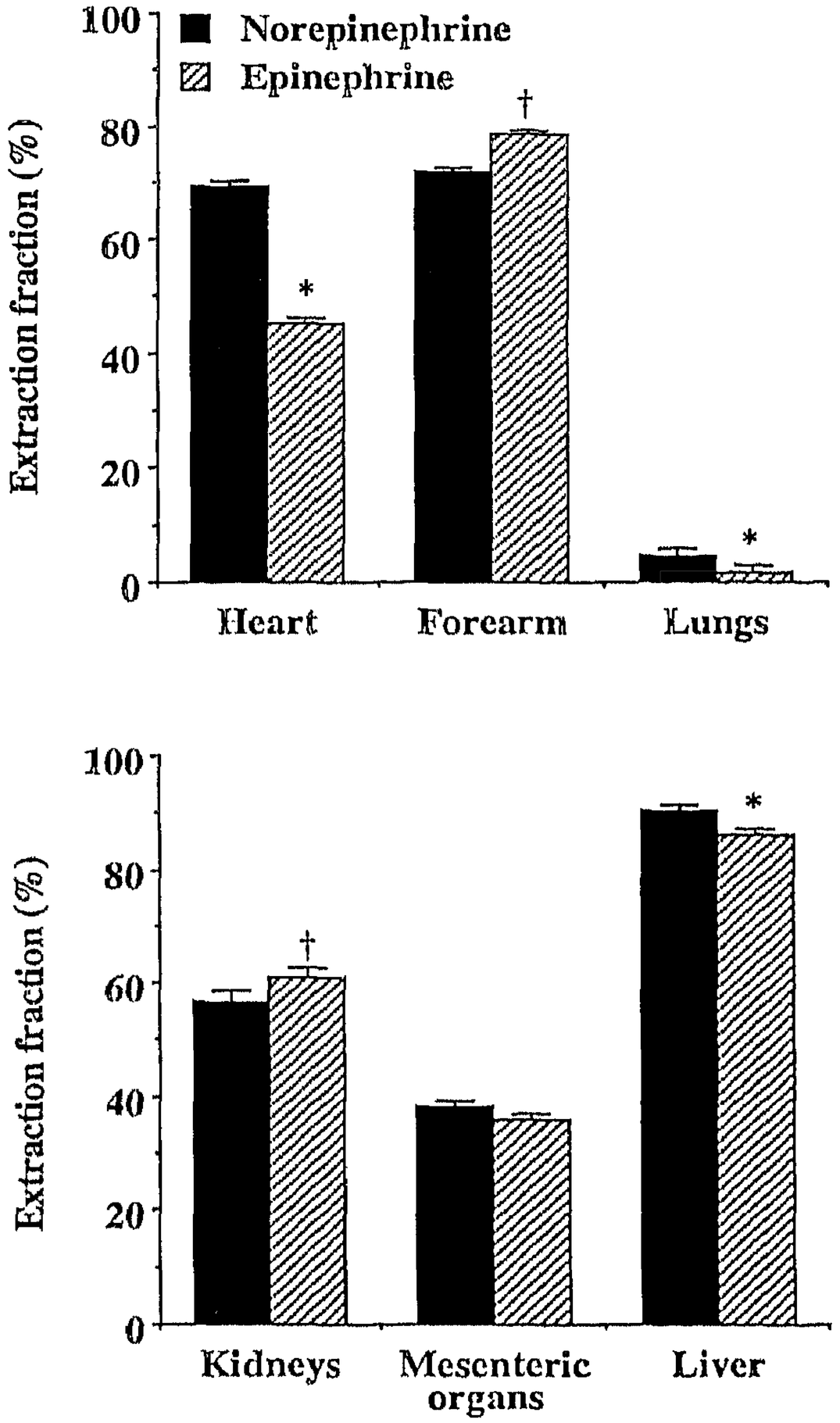

FIG. 4. Fractional extractions of NE and E by the heart, forearm, and lungs (*upper panel*) and by the kidneys, mesenteric organs, and liver (*lower panel*). Values represent the mean ± SEM. *, Lower ($P < 0.05$) fractional extractions of E than NE. †, Higher ($P < 0.05$) fractional extractions of E than NE.

Regional extractions of MN

Fractional extractions of MN by the various organs and tissues were lower than those of the catecholamines, but, in general, showed similar variation among organs as the precursor amines (Table 2). The highest fractional extraction of MN was in the liver; the lowest was in the lungs.

Regional removals and spillovers of catecholamines

Rates of NE spillover and removal showed wide variation among different tissues and organs (Table 3). As expected, the adrenals showed the largest spillover of E, amounting to 91% of the summed total (Table 4). The highest spillover rates of NE were observed for the kidneys and mesenteric organs, whereas the liver made the largest contribution to the removal of circulating NE and E. Although there were large differences between NE spillover and removal rates among individual tissues, the sum of NE spillover rates for all tissues was similar to the sum of removal rates.

Regional metabolism of catecholamines to MNs

Only small proportions (<6%) of the E or NE extracted from the plasma entering the various organs and tissues appeared in the plasma effluent as MN or NMN (Tables 3 and 4). Conversion of extracted catecholamines to MNs varied widely among tissues and organs; it was highest in the liver and lowest in the heart. Between 30–200% more MN appeared in out-flowing plasma from extracted E than NMN from extracted NE.

Much more NMN was derived from the metabolism of locally released than circulating NE for all tissues, apart from the liver (Table 3). The liver, producing 60% of its NMN from extracted NE, also made the largest single contribution (54%) to the NMN derived from metabolism of circulating NE.

TABLE 2. Regional fractional extractions and removal rates of metanephrines

	Fractional extraction (%):	Removal rates (pmol/min)	
	MN	NMN	MN
Heart	19.9 ± 1.2	5.8 ± 0.7	5.1 ± 0.5
Skeletal muscle	39.1 ± 1.9	40.1 ± 8.5	46.8 ± 8.5
Lungs	7.2 ± 1.5	71.2 ± 19.7	52.0 ± 12.4
Kidneys	40.4 ± 1.5	60.6 ± 4.4	50.4 ± 4.5
Mesenteric organs	30.5 ± 4.0	61.5 ± 13.9	44.5 ± 12.2
Liver	68.5 ± 4.2	184.2 ± 33.4	118.6 ± 23.7
Total		423	317

Results represent the mean ± SEM. Fractional extractions of MN were estimated from decreases in inflowing and outflowing plasma MN concentrations (see Table 1) after correction of the outflowing concentration for the contribution of local metabolism of extracted E (see Eq IV in *Materials and Methods*). Removal rates were estimated from the product of the fractional extraction of MN, the inflowing plasma concentration of MN or NMN, and the plasma flow through the organ.

However, only 19% of the NMN produced by all tissues was derived from metabolism of NE after entry into the circulation; most (81%) was derived from metabolism of locally released NE before entry into the circulation. Regional spillovers of NMN derived from locally released NE were typically a small fraction (3–13%) of NE spillovers; the exception to this was the adrenals, where NMN spillover was 33% that of NE spillover. The adrenals also made the single largest contribution (23%) to the spillover of NMN from all tissues.

The pattern of total and regional spillovers of MN derived from locally released and removed E (Table 4) showed some similarities to the pattern for NMN (Table 3), but also many differences. Similar to the situation for NMN, the adrenals made the largest single contribution to the combined spillovers of MN from all organs and tissues; however, the extent of this contribution (91%) was much larger than that for NMN (23%).

Some spillover of E was detected from most organs and tissues, although far less than from the adrenals. Thus, like NMN, some spillover of MN from these extraadrenal tissues was derived from metabolism of locally released E (Table 4). However, unlike the situation for NMN, all extraadrenal tissues produced more MN from E removed from the circulation than from locally released E. Similar to the situation for NE and NMN, the liver made the single largest contribution to removal of circulating E and produced the most MN from circulating E. However, metabolism of E after its release into the circulation made only a minor (6%) contribution to total MN production, and only a small amount (3%) of MN was produced locally from E released by extraadrenal tissues. Most MN (91%) was formed by local metabolism of E produced within the adrenals.

Discussion

In addition to providing an examination of regional release and removal of NE and E, this study shows how the catecholamines are metabolized to MNs after their local release within tissues or removal from the circulation. There are considerable differences in the production of MNs among various tissues. The adrenals in particular are an important source of MNs, providing an explanation for the extraordinary sensitivity of these metabolites for the diagnosis of pheochromocytoma.

The lower concentrations of NMN in hepatic and right renal venous plasma than in arterial plasma agree with previous findings (16). This and the extraction of MN by all extraadrenal tissues indicate that assessment of regional production of MNs, like catecholamines, requires consideration of their local removal. For catecholamines, this is achieved by analysis of arterial-venous decreases in the specific activity of iv infused ^{3}H-labeled catecholamines (11). The pronounced decrease in the specific activity of [^{3}H]NE from arterial to coronary venous plasma in control subjects and the negligible decrease in heart transplant patients, who have little cardiac NE release (17), illustrate how arterial-venous decreases in the specific activity of plasma [^{3}H]NE reflect local release of endogenous NE.

Differences in specific activities of [^{3}H]NMN are more complex to interpret than those in [^{3}H]NE. The decrease in specific activity of [^{3}H]NE from in-flowing to out-flowing plasma is dependent only on local release of unlabeled NE, whereas any change in that of [^{3}H]NMN is dependent on both metabolism of locally released NE and [^{3}H]NE removed from in-flowing plasma. Reversal of the normally observed arterial-coronary venous increase in plasma NMN to a decrease in heart transplant patients reflects the lack of cardiac NE release in these subjects; the resulting single source of NMN from in-flowing plasma NE together with the much higher specific activity of plasma [^{3}H]NE than of [^{3}H]NMN results in an increase in the specific activity of [^{3}H]NMN from arterial to coronary venous plasma. In the innervated heart, additional production of NMN from locally released unlabeled NE results in an overall arterial-venous decrease in [^{3}H]NMN specific activity.

Increases in specific activity of plasma [^{3}H]MN across all tissues, except the adrenals, reflect a similar situation to the transplanted heart, where specific activity differences depend on the extraction, but not the local release, of catecholamines. The variable regional differences in specific activity of [^{3}H]NMN reflect differences in the amounts of NMN derived from locally released or extracted NE. The liver, with the largest removal and smallest spillover of NE, also had the largest in-flowing to out-flowing increase in the specific activity of plasma [^{3}H]NMN.

In all tissues, except the adrenals, regional spillovers of NMN were determined using the fractional extractions of MN, which were assumed to equal those of NMN. Fractional extractions of MN were determined after correction of the amount of locally extracted E converted to MN; it was assumed that the amount of locally released E converted to MN was negligible. Both assumptions deserve comment. Similar total body clearances of NMN and MN and equal contributions of deamination to plasma clearances of both metabolites (7) support the assumption of equivalent fractional extractions of MN and NMN. Further support is provided here by the similar fractional extractions of MN and NMN in transplanted hearts. The small, but detectable, spillover of E from extraadrenal tissues is in agreement with previous find-

TABLE 3. Regional spillover and removal rates of NE and spillovers of NMN derived from NE released locally or removed from plasma

	NE		NMN spillover		
	Spillover	Removal	Released NE	Removed NE	Total
Heart	230 ± 31	142 ± 15	9.3 ± 0.9	1.1 ± 0.2	10.4 ± 1.0
Skeletal muscle	435 ± 78	312 ± 62	52.4 ± 16.3	4.0 ± 1.4	56.4 ± 16.6
Lungs	253 ± 114	328 ± 109	53.6 ± 25.6	14.5 ± 8.6	68.1 ± 30.7
Kidneys	976 ± 77	629 ± 45	33.5 ± 4.3	11.4 ± 1.2	44.9 ± 4.5
Mesenteric organs	1571 ± 392	347 ± 74	48.4 ± 12.6	4.1 ± 0.8	52.5 ± 10.3
Liver	214 ± 63	2374 ± 589	27.1 ± 11.7	40.8 ± 11.4	67.9 ± 8.0
Adrenals	274 ± 51	20 ± 1	91.3 ± 35.9	<20	91.3 ± 35.9
Total	3953	4152	316	76	392

Values represent the mean ± SEM rates (picomoles per min). Spillover rates of NE and total spillover rates of NMN were estimated according to Eq V in *Materials and Methods*. Removal rates of NE were estimated from the product of its fractional extraction, inflowing plasma concentration, and the plasma flow through the tissue. The spillover of NMN derived from local removal of plasma NE was estimated according to Eq VII in *Materials and Methods*. Subtraction of the latter estimate from the total spillover of NMN provided an estimate of the spillover of NMN derived from locally released NE. For the adrenals, estimates were derived without correction for the negligible influence of local extraction.

TABLE 4. Regional spillover and removal rates of E and spillovers of MN derived from E released locally or removed from plasma

	E		MN spillover		
	Spillover	Removal	Released E	Removed E	Total
Heart	7 ± 1	24 ± 3	0.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
Skeletal muscle	2 ± 1	133 ± 51	0.5 ± 0.3	2.8 ± 1.2	3.3 ± 1.3
Lungs	<1	46 ± 39	<0.5	7.7 ± 1.8	7.7 ± 1.8
Kidneys	23 ± 5	166 ± 22	1.7 ± 0.4	4.1 ± 0.4	5.8 ± 0.5
Mesenteric organs	33 ± 19	79 ± 20	3.4 ± 1.9	3.5 ± 0.7	6.9 ± 1.7
Liver	31 ± 18	216 ± 50	10.1 ± 5.6	10.9 ± 2.2	21.0 ± 3.7
Adrenals	979 ± 175	4 ± 1	448.8 ± 193	<4	448.8 ± 193
Total	1075	668	465	29	494

Values represent the mean ± SEM rates (picomoles per min). Spillover and removal rates of E and spillover rates of MN derived from local removal of plasma E were estimated as described in Table 3 for NE and NMN. The spillover of MN derived from locally released E was estimated from the product of the E spillover with the ratio of NMN spillover to NE spillover and the ratio of extracted E converted to MN to extracted NE converted to NMN. Total spillovers of MN were then estimated from the sum of MN spillovers derived from locally released and removed E.

ings (2, 18) and suggests that some MN may be formed from locally released E. However, MN production from locally released E was less than that from extracted E, and the latter source influenced regional differences in plasma MN by only 2–10%. Therefore, the second assumption introduces negligible error into estimates of MN extraction (<3% underestimation) for most tissues. In the liver, fractional extractions of MN may have been underestimated by up to 8%.

Larger regional production of MNs from extracted E than NE is in agreement with findings in rats (7, 19) and humans (20), in which infusions of E and NE produced larger increases in plasma MN than NMN. This is consistent with more efficient removal of E than NE by extraneuronal uptake and less efficient removal by neuronal uptake (2, 21, 22). NE is also a better substrate than E for monoamine oxidase (23). All of these differences contribute to greater availability of E than of NE for catechol-*O*-methyltransferase.

Larger fractions of NMN derived from locally released than circulating NE are consistent with findings in rats (19) and humans (20) that more NMN is produced from NE before than after its release into plasma. In humans, 16% of plasma NMN is derived from NE after its release into plasma (20), a result close to the 19% contribution indicated here by the sum of regional NMN spillovers. In the liver, however, 60% of NMN is derived from plasma NE, reflecting the importance of this organ for the removal of circulating catecholamines.

Although most NMN derived from circulating NE is formed in the liver, the most important single source of NMN is the adrenals. There is some previous evidence for an adrenal source of NMN; catechol-*O*-methyltransferase is present in adrenal tissue (24), plasma NMN concentrations are lower in adrenalectomized patients than in control subjects (20), and plasma NMN concentrations are higher in the common vein draining the left kidney and the left adrenal gland than the vein draining the right kidney (16). The present study extends these findings by showing 10-fold higher NMN concentrations in adrenal venous than arterial plasma and a 23% contribution of the adrenals to circulating NMN.

In humans, only 10% of the MN in plasma is derived from circulating E, suggesting that the other 90% is formed from *O*-methylation of E in the adrenals (20). This conclusion is supported here by the 87-fold increase in plasma concentrations of MN from arterial to adrenal venous plasma, which translates into a 91% contribution of the adrenals to the spillover of MN from all tissues.

The finding that the adrenals are a major source of circulating MNs has important implications for the use of these metabolites in the diagnosis of pheochromocytoma. The superiority of 24-h urinary excretion rates of MNs over other catecholamine metabolites for the diagnosis of pheochromocytoma (25, 26) is explained not only by preferential metabolism of circulating catecholamines by extraneuronal path-

ways (10), but also by the primary source of MNs from chromaffin tissue.

Other findings suggest that measurements of MNs in plasma provide a useful test for the presence of a pheochromocytoma (8, 27, 28). Our own observations establish that measurements of plasma MNs offer a superior method for diagnosis of the tumor than measurements of plasma catecholamines (29). Even when quiescent and not releasing catecholamines, pheochromocytomas appear to actively metabolize catecholamines to the *O*-methylated derivatives, regardless of their adrenal or extraadrenal location.

MNs are produced extraneuronally, whereas catecholamines are removed by neuronal and extraneuronal uptake (30). Differences in the relative importance of neuronal and extraneuronal uptake explain the small and highly variable amounts of NMN or MN produced by extraadrenal tissues relative to the amounts of NE or E removed from plasma. The heart, which removes NE largely by neuronal uptake (6), also produces the smallest fraction (0.77%) of NMN from extracted NE. In this study, the innervated heart removed 70% of the [^{3}H]NE entering the coronary circulation, whereas in cardiac transplant patients, only 13% was removed, presumably by extraneuronal uptake. If it is assumed that the ratio of extraneuronal uptake to coronary venous outflow of [^{3}H]NE is the same in transplanted and innervated hearts, then only 4.5% (13/87 of 30%) of in-flowing NE and 6.4% (4.5% of 70%) of extracted NE is removed by extraneuronal uptake in innervated hearts. Thus, 12% (0.77% of 6.4%) of the NE removed by extraneuronal uptake appears in coronary venous plasma as NMN. Assuming a similar proportion for locally released NE indicates, from the total cardiac spillover of NMN (10.4 pmol/min), that extraneuronal uptake of NE is 87 pmol/min (10.4/0.12). Cardiac NE spillover is about 3 times this rate, and neuronal uptake is 10-fold that of NE spillover (5). Thus, extraneuronal uptake removes a small portion (<4%) of the NE released by cardiac sympathetic nerves. This is less than the 10% estimated for the whole body (6), consistent with the greater importance of neuronal over extraneuronal uptake for removal of catecholamines in the heart than in other tissues (21).

Although this study provides a comprehensive examination of regional spillover and removal rates of MNs and catecholamines, it should be recognized that the data were obtained from a variety of patient groups studied under different conditions. Thus, some caution must be exercised in extrapolating the present comparisons among tissues to normal regional variations in sympathetic out-flow and catecholamine metabolism. The present data, however, do indicate wide regional variability in the extraneuronal production of MNs from catecholamines released locally or removed from the circulation. The substantial contribution of the adrenals to production of MNs explains the extraordinary sensitivity of these metabolites for the diagnosis of pheochromocytoma.

Acknowledgments

The authors are grateful for the expert technical assistance of Douglas Hooper and Anneli Ambring, and thank Dr. David S. Goldstein for helpful discussion during preparation of the manuscript.

References

1. **Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G.** 1990 Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev. 70:963–985.
2. **Eisenhofer G, Smolich JJ, Esler MD.** 1992 Disposition of endogenous adrenaline compared to noradrenaline released by cardiac sympathetic nerves in the anaesthetized dog. Naunyn Schmiedebergs Arch Pharmacol. 345:160–171.
3. **Eisenhofer G, Goldstein DS, Kopin IJ.** 1989 Plasma dihydroxyphenylglycol for estimation of noradrenaline neuronal reuptake *in vivo*. Clin Sci. 76:171–182.
4. **Halbrügge T, Wölfel R, Graefe K-H.** 1989 Plasma 3,4-dihydroxyphenylglycol as a tool to assess the role of neuronal uptake in the anaesthetized rabbit. Naunyn Schmiedebergs Arch Pharmacol. 340: 726–732.
5. **Eisenhofer G, Esler MD, Meredith IT, et al.** 1992 Sympathetic nervous function in the human heart as assessed by cardiac spillovers of dihydroxyphenylglycol and norepinephrine. Circulation. 85:1775–1785.
6. **Eisenhofer G.** 1994 Plasma normetanephrine for examination of extraneuronal uptake and metabolism of noradrenaline in rats. Naunyn Schmiedebergs Arch Pharmacol. 349:259–269.
7. **Eisenhofer G, Finberg JPM.** 1994 Different metabolism of norepinephrine and epinephrine by catechol-O-methyltransferase and monoamine oxidase in rats. J Pharmacol Exp Ther. 268:1242–1251.
8. **Lenders JWM, Eisenhofer G, Armando I, Keiser HR, Goldstein DS, Kopin IJ.** 1993 Determination of plasma metanephrines by liquid chromatography with electrochemical detection. Clin Chem. 39:97–103.
9. **Holmes C, Eisenhofer G, Goldstein DS.** 1994 Improved assay for plasma dihydroxyphenyl-acetic acid and other catechols using high-performance liquid chromatography with electrochemical detection. J Chromatogr. 653:131–138.
10. **Eisenhofer G, Pecorella W, Pacak K, Hooper D, Kopin IJ, Goldstein DS.** 1994 The neuronal and extraneuronal origins of plasma 3-methoxy-4-hydroxyphenylglycol in rats. J Auton Nerv Sys. 50:93–107.
11. **Esler M, Jennings G, Korner P, Blombery P, Sacharias N, Leonard P.** 1984 Measurement of total and organ-specific norepinephrine kinetics in humans. Am J Physiol. 247:E21–E28.
12. **Méndez J, Keys A.** 1960 Density and composition of mammalian muscle. Metabolism. 9:184–185.
13. **Clarys JP, Martin AD, Drinkwater DT.** 1984 Gross tissue weights in the human body by cadaver dissection. Hum Biol. 56:459–473.
14. **Nishijima MK, Breslow MJ, Raff H, Traystman RJ.** 1989 Regional adrenal blood flow during hypoxia in anesthetized, ventilated dogs. Am J Physiol. 256:H94–H100.
15. **Williams LR, Leggett RW.** 1989 Reference values for resting blood flow to organs of man. Clin Phys Physiol Meas. 10:187–217.
16. **Kobayashi K, DeQuattro V, Bornheimer J, Kolloch R, Miano L.** 1980 Plasma normetanephrine: a biochemical marker of sympathetic nerve function in man. Life Sci. 26:1821–1826.
17. **Rundquist B, Eisenhofer G, Dakak NA, Elam M, Waagstein F, Finberg P.** 1994 Cardiac noradrenergic function one year following cardiac transplantation. Blood Pressure. 2:252–261.
18. **Esler M, Kaye D, Thompson J, et al.** 1995 Effects of aging on epinephrine secretion and regional release of epinephrine from the human heart. J Clin Endocrinol Metab. 80:435–442.
19. **Lenders JWM, Kvetnansky R, Pacak K, Goldstein DS, Kopin IJ, Eisenhofer G.** 1993 Extraneuronal metabolism of endogenous and exogenous norepinephrine and epinephrine in rats. J Pharmacol Exp Ther. 266:288–293.
20. **Eisenhofer G, Friberg P, Pacak K, et al.** 1995 Plasma metanephrines: do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clin Sci. 88:533–542.
21. **Eisenhofer G, Esler MD, Cox HS, et al.** 1990 Differences in the neuronal removal of circulating epinephrine and norepinephrine. J Clin Endocrinol Metab. 70:1710–1720.
22. **Paiva MQ, Guimaraes S.** 1978 A comparative study of the uptake and metabolism of noradrenaline and adrenaline by the isolated saphenous vein of the dog. Naunyn Schmiedebergs Arch Pharmacol. 303:221–228.

23. **Grohmann M.** 1987 The activity of neuronal and extraneuronal catecholamine-metabolizing enzymes of the perfused rat heart. Naunyn Schmiedebergs Arch Pharmacol. 336:139–147.
24. **Kaplan GP.** 1980 Immunohistochemical localization of catechol-O-methyltransferase in the mammalian central nervous system and in peripheral tissues. PhD Thesis. St. Louis: Washington University; 13–14.
25. **Manu P, Runge LA.** 1984 Biochemical screening for pheochromocytoma: superiority of urinary metanephrines measurements. Am J Epidemiol. 120:788–790.
26. **Rosano TG, Swift TA, Hayes LW.** 1991 Advances in catecholamine and metabolite measurements for diagnosis of pheochromocytoma. Clin Chem. 37:1854–1867.
27. **Mornex R, Peyrin L, Pagliari R, Cottet-Emard JM.** 1991 Measurement of plasma methoxyamines for the diagnosis of pheochromocytoma. Horm Res. 36:220–226.
28. **Foti A, Adachi M, DeQuattro V.** 1982 The relationship of free to conjugated normetanephrine in plasma and spinal fluid of hypertensive patients. J Clin Endocrinol Metab. 55:81–86.
29. **Lenders JWM, Keiser HR, Goldstein, et al.** 1995 Plasma metanephrines in the diagnosis of pheochromocytoma. Ann Intern Med. 123:101–109.
30. **Graefe K-H, Henseling M.** 1983 Neuronal and extraneuronal uptake and metabolism of catecholamines. Gen Pharmacol. 14: 27–33.

Call for Abstracts
Diabetic Foot Research

Investigators involved in diabetic foot research are encouraged to submit abstracts by January 5, 1996, for the 56th Annual Meeting of Scientific Sessions of the American Diabetes Association, June 8–11, 1996. Original data may be accepted for exhibition in abstract, poster, and/or oral presentations.

Abstract applications and meeting information may be obtained by contacting: Jill Thompson, American Diabetes Association, 1660 Duke Street, Alexandria, VA 22314. Phone: 800-232-3472 ext. 212; Fax: 703-683-1839.