Plasma *l*-[^{3}H]Norepinephrine, *d*-[^{14}C]Norepinephrine, and *d,l*-[^{3}H]Isoproterenol Kinetics in Essential Hypertension

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ABSTRACT We infused tracer-labeled *l*-[^{3}H]-norepinephrine, *d*-[^{14}C]norepinephrine, and *d,l*-[^{3}H]-isoproterenol simultaneously into patients with essential hypertension and into normotensive control subjects, in order to determine whether abnormalities in the disappearance kinetics of these substances characterized the hypertensive patients. The mean preinfusion venous plasma norepinephrine concentration was somewhat higher in the hypertensive group (260 vs. 194 pg/ml, $P = 0.06$), but the groups did not differ in the disappearance kinetics of *l*- or *d*-norepinephrine or of isoproterenol. Preinfusion plasma norepinephrine was significantly positively correlated with calculated spillover rates in both the hypertensive and normotensive groups, but not with norepinephrine clearances. The *d/l* ratio in plasma norepinephrine was the same as in the infusate during and after the infusion, even after pretreatment with the neuronal norepinephrine uptake blocker, desipramine. Because isoproterenol is not taken up by nerve endings, the ratio of [^{3}H]isoproterenol to *l*-[^{3}H]norepinephrine increased after the infusion ended. This increase was almost completely abolished by pretreatment with desipramine. These results indicate that (*a*) increased plasma norepinephrine levels seen in some patients with essential hypertension result from increased sympathetic neural activity and not from decreased clearance of norepinephrine, (*b*) changes in the isoproterenol/norepinephrine ratio after simultaneous infusion of both provide an index of neuronal norepinephrine uptake in man, and (*c*) neuronal norepinephrine uptake is not stereospecific.

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INTRODUCTION

The results of recent extensive literature reviews, as well as several large-scale comparative studies of patients with essential hypertension and of normotensive control subjects, have suggested that a subgroup of hypertensives have elevated resting, supine, venous plasma norepinephrine (NE)[1] levels, consistent with augmented sympathetic nervous system activity in some patients with essential hypertension (1–6).

The evidence, however, that plasma NE measures sympathetic neural activity in man is mainly indirect. Patients who have undergone sympathectomy for Raynaud's phenomenon show decreased venous plasma NE in the venous drainage from the sympathectomized limb (7). A variety of stimuli known to increase sympathetic neural activity in animals, e.g., exercise and environmental stress, also increase plasma NE in man (8, 9). Diabetic patients with autonomic neuropathy and patients with idiopathic orthostatic hypotension show decreased levels of NE either at rest or in response to standing (10, 11). Infusions of amphetamine or tyramine, which release NE from sympathetic nerve endings in vitro, produce increases in venous plasma NE in man associated with the pressor response—presumably a measure of the increment in NE at the neuroeffector junction (12). Finally, when sympathetic nerve activity has been recorded directly in

[1] *Abbreviations used in this paper:* COMT, catechol-*O*-methyltransferase; I, isoproterenol; LCED, liquid chromatography with electrochemical detection; MAO, monoamine oxidase; NE, norepinephrine; NMDA, *N*-methyldopamine; U_1 and U_2, $Uptake_1$ and $Uptake_2$.

man, peripheral plasma NE has correlated fairly well (13).

These findings indicate that venous plasma NE does bear some relationship, albeit indirect, to synaptic cleft NE. Even so, it is well known that venous plasma NE is the product of several complex, interacting processes, some of which are incompletely understood, intervening between sympathetically mediated NE release from presynaptic sites and norepinephrine measured in venous blood. Fig. 1 shows some of these processes, and serves as a reference for our discussion of the rationale for the study reported here.

Most endogenously released NE disappears from the synaptic cleft by reuptake ($Uptake_1$; U_1) into the presynaptic axon (14). U_1 is an active process that is not stereoselective (15–16), but inside the nerve ending, NE is catabolized by monoamine oxidase (MAO) or incorporated into storage vesicles, both processes being stereospecific.

NE also may be removed from extraaxonal sites by a nonneuronal, nonstereospecific uptake process ($Uptake_2$; U_2), e.g., into smooth muscle cells (14–15). Once inside these cells, NE may be catabolized by catechol-*O*-methyltransferase (COMT), which is not stereospecific.

Finally, a proportion of NE in the synaptic cleft diffuses through the blood vessel wall or out the adventitia and appears in the general circulation. Circulating NE is predominantly conjugated (17), taken up by platelets (18), and excreted by the kidney (19), as well as metabolized in the liver and other organs. The fact that plasma NE increases from artery to vein (20) suggests that there is a net release of NE into the general circulation along the vascular tree.

Whereas endogenously released NE is removed from the synaptic cleft mainly by U_1, U_2 and *O*-methylation appear to be more important removal mechanisms for exogenously administered NE (14).

Exogenously administered NE also can be removed by U_1, since pretreatment with the specific U_1 blocker desipramine prolongs the disappearance of NE from the bloodstream in man (21). It must be emphasized that details of this schema derive mainly from in vitro or in vivo studies in animals, not in people.

In an attempt to measure separately the contributions of these factors in determining plasma NE in patients with essential hypertension, Esler and associates (22, 23) measured the plasma disappearance of tracer amounts of infused radioactivity labeled NE. They reported that labeled NE disappeared with an

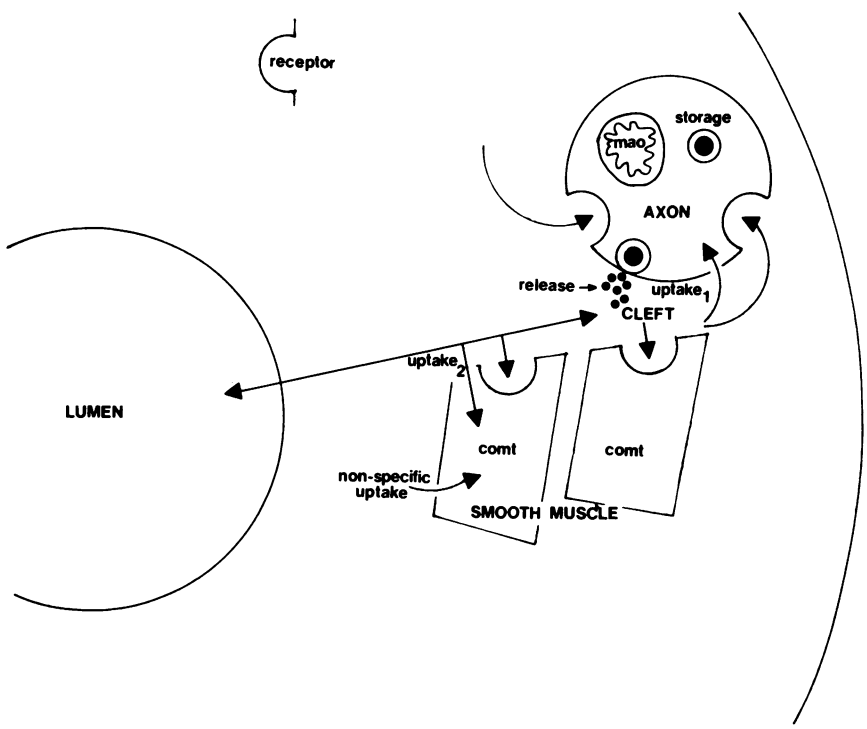

FIGURE 1 Diagrammatic cross-section of a blood vessel, showing several factors influencing the relationship between NE release from sympathetic nerve endings and circulating NE.

early, rapid half-life ($t_{1/2}^a$) of ~2 min and later with a slower half-life ($t_{1/2}^b$) of >30 min; that desipramine prolonged $t_{1/2}^a$; and that a subgroup of hypertensive patients showed prolongation of $t_{1/2}^a$. They concluded that in some patients with essential hypertension, faulty U_1 could account for elevated circulating levels of NE and the patients' high blood pressure. Paradoxically, however, in these studies U_1 blockade with desipramine did not appear to increase plasma NE. These investigators also reported that apparent spillover rates of NE into the bloodstream correlated well with resting plasma NE but NE clearance rates did not, indicating that differences across individuals in resting plasma NE depend more on differences in sympathetic tone than on differences in NE removal mechanisms (22).

The evidence that the technique used to measure U_1 actually did so is circumstantial. We have evaluated, in patients with essential hypertension and in normotensive controls, two somewhat similar techniques that we thought might be more specific.

The techniques we used exploited two generally accepted points: The first is that MAO activity and vesicular uptake are stereospecific, whereas COMT, U_2, other removal processes, and probably renal excretion are not. The second is that isoproterenol (I) is not taken up by U_1 (24), but undergoes COMT degradation and renal excretion in a manner similar to NE. If one were simultaneously to administer tritiated *l*-NE and *d*-[^{14}C]-NE, any differences in the disappearance kinetics of these substances—most simply reflected in the *d*/*l* ratio—would provide an index of the contribution of stereospecific processes. Similarly, any differences in the disappearance kinetics of I and *l*-NE—most simply reflected in the I/NE ratio—could be attributed to U_1.

On the other hand, if neither the *d*/*l* ratio nor the I/NE ratio changed in man during the plasma disappearance of simultaneously infused tracer amounts of *l*-[^{3}H]NE, *d*-[^{14}C]NE, and [^{3}H]I, one would conclude that the disappearance of exogenously administered NE was not so much due to U_1 as to U_2, *O*-methylation, renal excretion, or other removal processes.

To test whether changes in either the *d*/*l* or I/NE ratios provide an index of U_1 in man, we measured these ratios in normotensive individuals before and after treatment with desipramine.

In summary, we infused labeled stereoisomers of NE and labeled I simultaneously in patients with essential hypertension and in normotensive controls, and compared the plasma disappearance of these substances in these groups, in order to determine whether high NE levels seen in some hypertensive result from excessive sympathetic neural activity or from prolonged plasma disappearance of NE. We used the ratio of [^{3}H]I to *l*-[^{3}H]NE after the infusion as an index of neuronal uptake of NE.

METHODS

Subjects. 22 patients with essential hypertension participated in this study. Clinical data about them are shown in Table I. All the patients had systolic blood pressures averaging >140 mmHg or diastolic pressures averaging >90 mmHg during at least five outpatient visits. The patients either had never received antihypertensive medication or had not taken any for at least 2 wk. Secondary forms of hypertension were excluded by blood and urine tests (complete blood count, serum electrolytes, liver function tests, blood urea nitrogen, creatinine, glucose, uric acid, urinalysis, urine culture), medical history and physical examination, and, when appropriate, intravenous pyelography, renal scanning, abdominal computerized transaxial tomography, renal venous sampling, and arteriography. Patients were excluded and immediately treated if their diastolic pressures exceeded 120 mmHg.

13 subjects served as age-matched normotensive controls. None had shown systolic blood pressures >140 mmHg or diastolic pressures >90 mmHg. Most of them had no family history of hypertension. Medical history, physical examination, and routine laboratory blood and urine testing excluded any concurrent illness at the time of study. None of the controls were hospital or laboratory personnel. Most had been recruited through the National Institutes of Health (NIH) Normal Volunteer program.

All but two of the patients and all the controls were studied as outpatients. All subjects consented in writing to participate in this protocol, which was approved by the National Heart, Lung, and Blood Institute Institutional Review Board. In one hypertensive and one control subject, technical problems forced a premature end to the study, so that only base-line NE values from these subjects were included in the data analysis. These subjects were in addition to the 32 who underwent the entire infusion protocol.

Protocol. Most of the subjects were studied in the morning, after a light breakfast. With the subject supine, intravenous plastic catheters or butterfly needles were inserted in each forearm and isotonic saline infused slowly. After at least 15 more minutes, base-line blood pressure was mea-

TABLE I
Summary Clinical Data for the Hypertensive and Normotensive Groups

	Hypertensive	Normotensive
n	22	13
Age (*yr*)	42±11	43±14
Mean arterial pressure (*mmHg*)	108±8*	83±9
Pulse rate (*beats per minute*)	75±8‡	65±6
Weight (*kg*)	76±17	70±11
Creatinine (*mg/dl*)	1.1±0.2	1.0±0.1

All values expressed±1 SD.
* Significant hypertensive-normotensive difference, $P < 0.001$.
‡ Significant hypertensive-normotensive difference, $P < 0.01$.

sured in the arm that would be used for blood sampling. Just after this, two 10-ml blood samples were drawn from the sampling catheter, without use of a tourniquet, through a stopcock and directly into chilled, evacuated, heparinized tubes. About 10–15 seconds were required to fill each collection tube.

After the base-line sampling, an intravenous infusion was begun in the other arm. The infusate contained 50 μCi tritiated *l*-NE, 10 μCi ^{14}C-labeled *d*-NE, and 50 μCi tritiated *d,l*-I in 65 ml 5% dextrose. The infusion, which was controlled by an IMED (San Diego, CA) pump set to run at 180 ml/h, lasted 20 min.

At 1, 3, 5, 10, and 15 min and at the end of the infusion, blood samples were drawn through the stopcock into collection tubes as described above. In the few instances where the infusion ended just before 20 min, a sample was drawn immediately and the stopwatch restarted. After the infusion, blood samples were drawn at 1, 2, 3, 5, 10, 20, and, in several cases, at 40 and 60 min.

Drugs and dosimetry. We used labeled substances with high specific activity, so that the concentrations of physiological active NE and I would be too small to affect endogenous NE release. Tritiated *l*-NE (5 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL) and prepared by the NIH Radiopharmacy for use in humans. The ~46 μCi of *l*-NE infused consisted of 1.6 μg, resulting in an *l*-NE infusion rate of ~78 ng/min. During the study, ring-labeled *l*-NE of even higher specific activity was obtained, but the amount of radioactivity infused was not changed. The *d*-[^{14}C]NE (33 mCi/mmol) also was obtained from Amersham Corp. and tested and prepared for human use in the same way. The 9.2 μCi of *d*-NE (47 μg) resulted in a *d*-NE infusion rate of 2.4 μg/min. Of the increment in total plasma NE observed in this study, ~97% was due to physiologically inactive *d*-NE. The tritiated I (11.2 Ci/mmol) was infused at a rate of ~43 ng/min.

Solutions of these substances were aliquoted and stored in a −80°C freezer. When assayed for catecholamine content by the liquid chromatographic-electrochemical technique described below, the NE was free of any contamination with I and vice versa, and at least 95% of the radioactivity in these substances was contained in the appropriate chromatographic peaks.

Radiation dosimetry was calculated on the basis of a biological half-life of 1 d (19). We calculated that the patients would be exposed to ~1 mrad/infusion.

To assess whether inclusion of I or *d*-NE influenced the disappearance of tritiated *l*-NE, for five hypertensives the infusate contained only the tritiated *l*-NE.

Sample collection and assay technique. The blood samples were placed on ice until centrifuged at 4°C within 2 h. The plasma was transferred to plastic sample tubes without additives and stored either in a −80°C freezer or in a tank containing liquid nitrogen until the time of assay. All the samples from a given infusion were assayed at the same time.

Plasma NE and I were separated by liquid chromatography and quantified with electrochemical detection (LCED). Since we had previously validated and reported this technique for measuring plasma catecholamines (25), the following description will deal mainly with those aspects used for the first time in this study.

Either 1 or 2 ml freshly thawed and recentrifuged plasma was added to a plastic sample tube containing ~10 mg acid-washed alumina. To this were added the internal standard—1 ng *N*-methyldopamine (NMDA), 2 ng unlabeled I, or both—and then 400 μl Tris-EDTA buffer at pH 8.6. The tube was shaken vigorously for 20 min and the alumina then washed twice with water. The catecholamines were eluted with 100 μl of 0.1 M perchloric acid or 100 μl of 0.2 M acetic acid. In most cases, 90 μl of eluate were injected into the LCED apparatus.

The chromatographic and detection equipment included a Waters M6000 pump, U6K injector, and Microbondapak C18 reverse phase column (Waters Associates, Milford, MA). The mobile phase consisted of sodium acetate, acetonitrile, EDTA, and heptane-sulfonic acid as previously described (25). The mobile phase was pumped at 1.0 ml/min and recycled. The electrochemical detector was set at 0.5 V at a sensitivity in most cases of 1.0 nA/V. The limit of detection of this technique is ~10 pg/ml plasma. A sample chromatogram of an injection of a mixture of NE, epinephrine, dopamine, NMDA, and I standards is shown in Fig. 2. Sample chromatograms of plasma-derived eluates before and during the infusion are shown in Fig. 3.

As shown in the figures, NE and I were easily separated and detected by LCED. The NE and I peaks for each eluate were collected from the postdetector outlet tube into glass scintillation vials. For NE, collection began 20 s before the expected appearance of the peak and ended 70 s after the peak, for a total of 90 s (1.5-ml mobile phase). I was collected

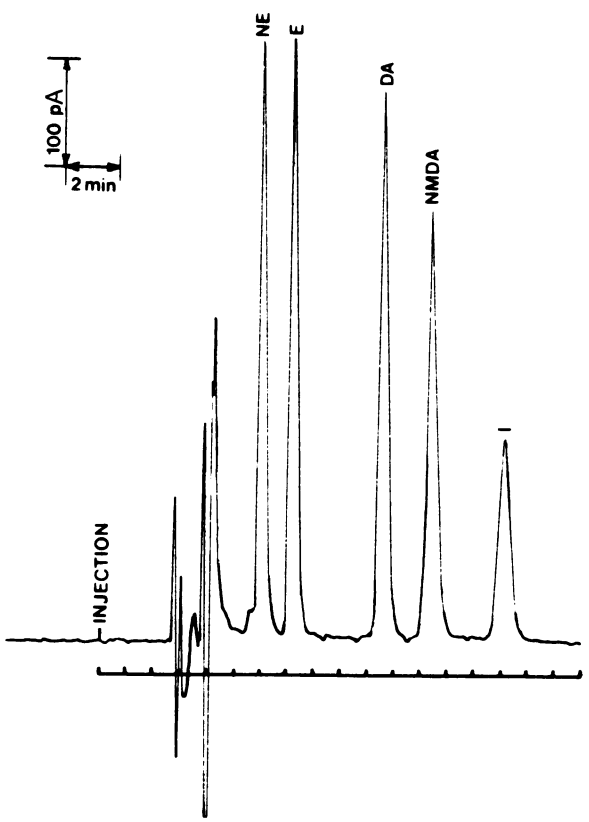

FIGURE 2 Chromatographic tracing after injection of 500 pg each of NE, epinephrine (*E*), dopamine (*DA*), NMDA, and I standards.

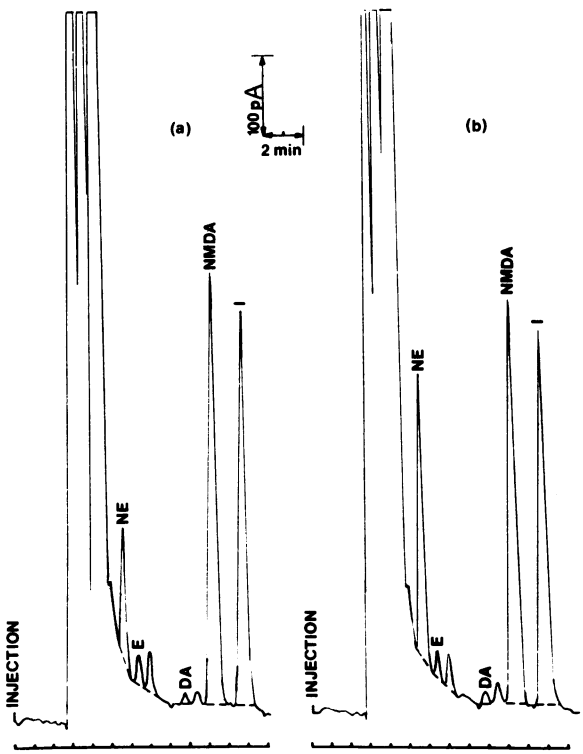

FIGURE 3 Chromatographic tracings after injection of plasma-derived eluates (*a*) before and (*b*) during infusion of *l*-[^{3}H]NE, *d*-[^{14}C]NE, and [^{3}H]I. Same abbreviations as in Fig. 2.

in two successive 1.5-ml aliquots beginning either 30 s before the expected appearance of the I peak or when the I internal standard peak began to rise (whichever came sooner) until 150 s after the I peak. The reason for this difference in collection times was that the I peak, being further from the solvent front, was wider than the NE peak. Whereas >95% of the radioactivity in NE occurred within the 90-s collection period, only 71% of the radioactivity in I occurred within the first 90 s of collection of the I peak. More than 95% of the radioactivity in I did appear, however, by the end of the 180-s collection. Accordingly, for I, the radioactivity for the two consecutive 1.5-ml aliquots was summed for each blood sample. In several cases, only one 1.5-ml aliquot was collected for I. In these cases, the quantity of radioactivity in I was back-calculated by dividing the counts per minute by 0.71, since this was the proportion of radioactivity in 3-ml aliquots that occurred in the first 1.5-ml aliquot in 12 individuals, and it also was the proportion of I standard occurring in the first 1.5-ml aliquot collected after the standard had undergone the alumina sample preparation step.

Plasma NE was calculated from the peak heights of NE and the internal standards and expressed in picograms per milliliter.

Without added unlabeled I, the plasma concentration of labeled I during the infusion was too small to produce a detectable peak in the obtained chromatograms, indicating a concentration <10 pg/ml.

When both I and NMDA were used as internal standards on the same specimen, the NE concentrations calculated from them were virtually identical. The I peak height decreased as the volume injected increased, even with the same number of picograms injected, so that standards had to be injected in identical volumes to obtain equivalent NMDA and I recoveries. The average recovery through the alumina preparation step was ~70% in samples from both the hypertensive and normotensive subjects.

Assay of ^{3}H and ^{14}C. The amount of *d*-[^{14}C]NE, tritiated *l*-NE, and tritiated I were measured by assay of the tritium and ^{14}C in the NE and I peaks. The samples were assayed by liquid scintillation spectrometry, counting disintegrations during 100 min, which was required because of the small amount of radioactivity per sample and the simultaneous counting of ^{14}C and tritium on one channel. The counts per minute were adjusted for background, crossover from the ^{14}C to the tritium channels (the crossover from tritium to ^{14}C being negligible), the volume of plasma assayed, and the recovery through the alumina extraction step. Background was determined from the results obtained from the blood sample drawn just before the infusion.

Spillover and clearance calculations. Apparent spillover and clearance rates of NE into and out of the circulation were calculated by applying previously described techniques (21, 22). Since 97% or more of the infused NE was the *d*-form, the steady-state endogenous NE concentration was calculated by subtracting the *d*-[^{14}C]NE concentration from the total in 15 subjects. This procedure demonstrated that, as expected, the steady-state endogenous NE concentration was unchanged from the preinfusion base-line level. Accordingly, apparent spillover rates were calculated in each subject from the *l*-[^{3}H]NE infusion rate, the amount of radioactivity in [^{3}H]NE at the steady state, and the preinfusion NE concentration. Because the infusion rate of the labeled substances was the same for each subject, calculated spillover and clearance rates varied inversely with the steady state level of labeled NE. Values of $t_{1/2}^a$ for the disappearance of NE were calculated from the slope of the line relating the logarithm of the plasma tritium content in NE to time over the first 5 min after the infusion ended. This technique was used because after ~20 min after the infusion levels of radioactivity were too low to yield reliable points for graphical analysis. Our procedure probably slightly overestimated $t_{1/2}^a$.

Data analysis. In each subject, for each sample, the ratio of *d*-[^{14}C]NE to tritiated *l*-NE was determined from the net levels of radioactivity in each; the ratio of I to *l*-NE was determined similarly. The I/NE and *d*/*l* ratios were expressed as percentages of the corresponding ratios in the infusate. The statistical significance of differences in trends between the hypertensive and normotensive groups was assessed by means of analyses of variance for repeated measures, Pearson correlation coefficients, and independent-means *t* tests (26).

Desipramine. In five normotensive and three hypertensive subjects, the infusion was repeated after at least a 2-wk interval. Three h before this second infusion, the subjects received a single oral dose of 125 mg desipramine.

RESULTS

Total NE. The hypertensive subjects had a higher mean preinfusion NE level than the normotensive subjects (260 vs. 194 pg/ml, $t = 1.99$, $P = 0.06$). The increment in total NE during the infusion, virtually entirely due to *d*-[^{14}C]NE, was similar in the hyper-

TABLE II

Levels of Radioactivity in l-[^3H]NE, d-[^{14}C]NE, and [^3H]I and Total Plasma NE during and after Simultaneous Infusion of the Three Substances in Patients with Essential Hypertension (EH) and in Normotensive Controls (NT)

	l-[^{3}H]NE		*d*-[^{14}C]NE		[^{3}H]I		Total plasma NE	
Time	EH	NT	EH	NT	EH	NT	EH	NT
min	*net cpm/ml*		*net cpm/ml*		*net comp/ml*		*pg/ml*	
1	37±44	19±33	10±13	6±9	30±33	17±24	328±145	228±86
3	170±91	172±119	52±28	47±32	153±77	143±120	533±150	454±184
5	202±83	186±83	59±26	56±30	189±77	171±80	594±209	480±173
10	248±106	261±116	73±29	71±31	241±81	253±113	664±223	540±174
15	253±93	258±103	73±27	70±32	258±72	273±87	637±209	522±170
20	249±93	239±92	73±27	66±26	260±73	269±83	646±226	508±189
21	189±58	217±87	56±17	58±24	221±52	249±93	526±156	458±139
22	126±32	150±53	36±10	40±16	160±42	196±63	473±168	393±135
23	89±26	103±34	24±8	28±9	124±34	151±42	378±172	366±130
25	58±21	62±22	16±6	17±4	99±31	104±24	337±158	276±81
30	32±13	31±13	8±3	9±3	58±24	61±17	301±138	230±75
40	19±12	21±14	6±2	5±3	31±17	36±17	285±141	227±72

All values expressed±1 SD.

tensive and normotensive groups, so that at all time points during and after the infusion, the mean total NE level was higher in the hypertensive subjects (Table II). In the five hypertensive subjects who received only tritiated *l*-NE, total plasma NE at 20 min after the start of the infusion was the same as before the infusion (194 vs. 216 pg/ml). Pretreatment with desipramine did not influence base-line plasma NE (182 pg/ml without desipramine, 193 pg/ml with desipramine among the eight subjects tested).

l-[^3H]NE. As indicated in Fig. 4, tritiated *l*-NE accumulated rapidly in plasma during the infusion and disappeared rapidly after the infusion ended. During the first 5 min after the infusion, *l*-[^{3}H]NE disappeared with a $t_{1/2}$ of ~2.5 min, after which the disappearance rate declined and a second component with a much slower disappearance rate predominated. By 60 min after the infusion, the radioactivity in *l*-[^{3}H]NE could not be distinguished from background, despite the 100-min period of scintillation counting.

Table II and Fig. 4 demonstrate that the hypertensive and normotensive groups showed virtually identical plasma *l*-[^{3}H]NE levels at all points during and after the infusion. Furthermore, the mean $t_{1/2}$[a] for the disappearance of *l*-[^{3}H]NE during the first 5 min after the infusion also was similar in the hypertensive and normotensive groups (Table III). The plasma level of *l*-[^{3}H]NE at 20 min after the start of the infusion was similar in the patients receiving only *l*-[^{3}H]NE in the infusate as in those receiving *l*-[^{3}H]NE, *d*-[^{14}C]NE, and tritiated I simultaneously.

d,l-[^3H]. The plasma disappearance rate of [^{3}H]I was slower than that of *l*-[^{3}H]NE. This difference was most apparent during the first several minutes after the infusion ended. The $t_{1/2}$[a] for tritiated I was ~3.5 min. As with *l*-[^{3}H]NE, the overall mean disappearance from plasma of [^{3}H]I was similar in the hypertensive and normotensive groups.

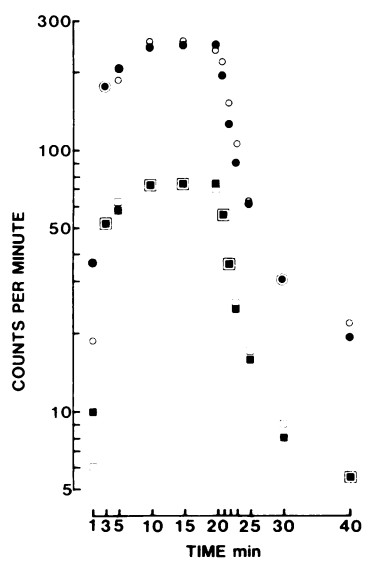

FIGURE 4 Plasma mean levels of radioactivity in *l*-[^{3}H]NE and *d*-[^{14}C]NE during and after the infusion in the hypertensive (*EH*) and normotensive (*NT*) groups. ●, EH *l*-[^{3}H]NE; ○, NT, *l*-[^{3}H]NE; ■, EH *d*-[^{14}C]NE, □, NT, *d*-[^{14}C]NE.

TABLE III
Individual Patient Norepinephrine Kinetic Data

Patient	Base-line NE	Spillover rate	Clearance	I/NE % slope	$t_{1/2}$*	NE/U_l
	pg/ml	*mcg/min*	*liters/min*		*min*	
Hypertensive						
1	304	0.71	2.35	0.15	2.74	2,027
3	291	0.88	3.03	0.09	2.15	3,233
4	338	0.95	2.80	0.05	2.15	6,760*
6	267	1.01	3.79	0.23	1.51	1,161
7	301	1.02	3.38	0.03	3.34	10,033*
8	471	2.38	5.05	0.06	3.01	7,850*
10	207	1.03	5.00	0.21	2.51	986
13	204	0.74	3.64	0.08	2.74	2,550
19	274	1.11	4.04	0.16	2.15	1,713
20	226	1.35	5.99	0.14	2.51	1,614
23	407	3.26	8.00	0.04	6.02	10,175*
24	109	0.43	4.03	0.11	2.32	991
25	177	1.26	7.13	—	2.74	—
26	140	0.78	5.56	—	2.74	—
27	379	0.95	2.50	—	2.01	—
28	310	1.05	3.40	—	2.01	—
29	75	0.18	2.41	—	1.77	—
30	247	0.67	2.78	0.13	1.58	1,900
31	90	0.24	2.70	0.28	1.67	321
32	215	1.43	6.65	0.22	2.01	977
33	356	1.90	5.34	0.11	2.18	3,236
Mean	257	1.11	4.27	0.13	2.47	3470
±1 SD	±105	±0.70	±1.67	±0.07	±0.94	±3305
Normotensive						
2	199	0.50	2.50	0.07	2.15	2,843
5	257	0.69	2.69	0.07	2.01	3,671
9	132	0.86	6.52	0.07	3.76	1,886
11	138	0.60	4.33	0.06	2.32	2,300
12	177	1.18	6.67	0.09	3.01	1,967
14	386	1.49	3.86	0.31	2.15	1,245
15	131	0.43	3.30	0.22	2.74	595
16	221	0.71	3.22	0.15	2.51	1,473
17	274	0.99	3.60	0.37	1.51	741
18	179	0.68	3.79	0.04	3.01	4,475
21	131	0.92	7.03	0.06	3.76	2,183
22	203	1.03	5.05	0.10	2.15	2,030
Mean	202	0.84	4.38	0.13	2.59	2,117
±1 SD	±76	±0.30	±1.58	±0.11	±0.69	±1,128

I/NE % slope = slope of line relating the ratio of counts per minute per milliliter tritiated isoproterenol to counts per minute per milliliter tritiated L-NE expressed as a percentage of the ratio in the infusate, across the time interval from 0 to 5 min after the infusion ended; $t_{1/2}$* = first half-life of plasma disappearance of NE determined by net counts per minute per milliliter tritiated l-NE across the time interval from 0 to 5 min after the infusion ended. Patient No. indicates sequence in which subjects actually were studied. Five hypertensive subjects received only tritiated l-NE, accounting for the unreported data.

* Individual value > 2 SD from the normotensive mean.

I/NE ratio. In marked contrast to the lack of change in the *d/l* ratio during or after the infusion, the I/NE ratio increased slowly during and rapidly after the infusion in both the hypertensive and normotensive groups (Fig. 5). Pretreatment with desipramine virtually abolished this change in ratios (Fig. 5).

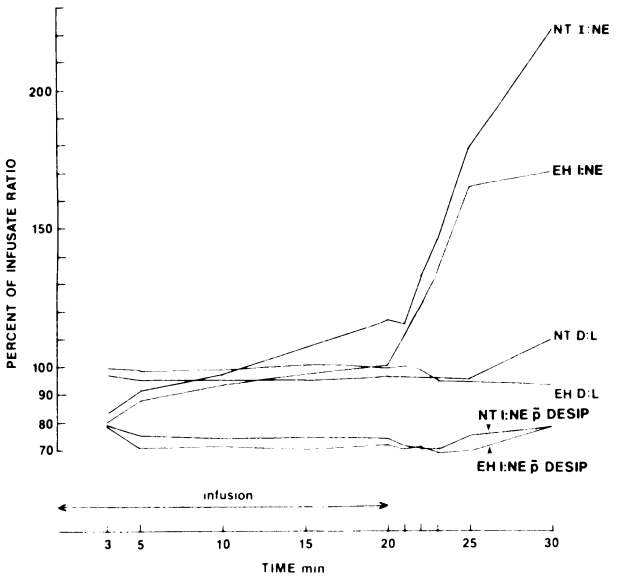

FIGURE 5 Ratio of I to *l*-[^{3}H]NE (*I/NE*) and of *d*-NE to *l*-NE (*d/l*) expressed as percentages of the ratios in the infusate in the hypertensive and normotensive groups. I/NE mean values for five normotensive and 3 hypertensive subjects pretreated with desipramine (*p̄DESIP*) are also shown.

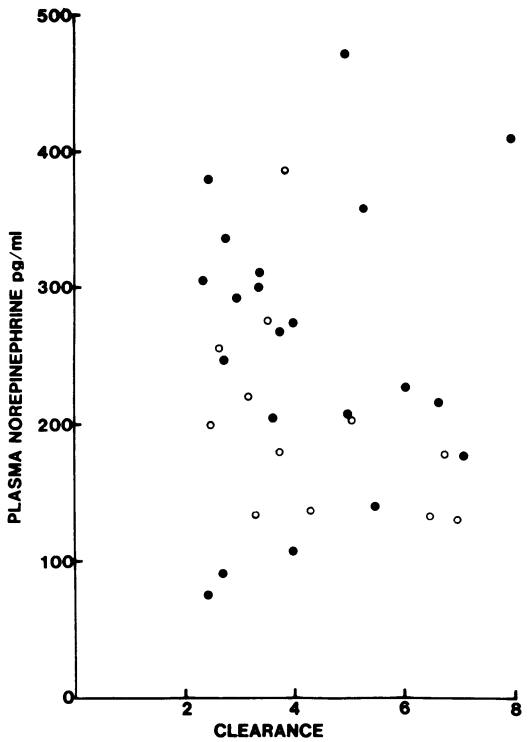

FIGURE 6 Preinfusion plasma NE as a function of NE clearance (in liters per minute). ●, individual hypertensives, ○, individual normotensives.

The degree of increase in the I/NE ratio after the infusion ended did not differ significantly between the hypertensive and normotensive groups, as assessed by the interaction effect in an analysis of variance for diagnostic category and time.

Table III shows individual values for the slope of the regression line relating to time the I/NE ratio (expressed as a proportion of that in the infusate) during the first 5 min after the infusion ended. U_I activity estimated using this technique was significantly negatively correlated with the $t_{1/2}{}^a$ of *l*-[^{3}H]NE ($r = -0.57$ in the hypertensive and -0.63 in the normotensive groups, $P < 0.01$). Table III also shows individual values for the ratio between preinfusion plasma NE and U_I activity (NE/U_I) by the I/NE ratio technique. As indicated, four hypertensive subjects (25% of the studied group) had a NE/U_I ratio > 2 SD from the normotensive mean. No clinical characteristics (in particular, serum creatinine, body weight, and severity of hypertension) differentiated these patients from the other hypertensives.

Apparent spillover and clearance rates. The congruity of plasma *l*-[^{3}H]NE and of plasma *d*-[^{14}C]NE disappearance curves in the hypertensive and normotensive groups meant that their overall NE clearances were identical (Table III). Preinfusion plasma NE was unrelated to clearance in both groups (Fig. 6).

In contrast, apparent spillover rates correlated significantly with preinfusion plasma NE in the hypertensive ($r = 0.72$, $P < 0.01$) and normotensive ($r = 0.69$, $P < 0.01$) groups (Fig. 7).

Although the hypertensive mean spillover rate exceeded the normotensive mean spillover rate, the difference was not statistically significant (Table III). Hypertensive subjects with preinfusion NE levels greater than the median hypertensive value (250 pg/ml) had a higher mean spillover rate than hypertensive subjects with preinfusion NE less than the median value (1384 vs. 815 ng/min, $t = 2.00$, $P = 0.06$) and also a higher mean spillover rate than the normotensive subjects (839 ng/min, $t = 2.20$, $P < 0.05$).

Desipramine. As mentioned above, desipramine pretreatment did not alter preinfusion plasma NE levels but did profoundly influence plasma NE kinetics. After desipramine pretreatment, the infusion produced an extremely rapid accumulation of *l*-[^{3}H]NE and *d*-[^{14}C]NE in plasma, with plateau levels averaging about double the levels seen without desipramine pretreatment (Fig. 8). Desipramine prolonged the disappearance rates of both *d*- and *l*-NE. The rapid initial decrease in plasma levels was slowed to an extent consistent with the elevated steady-state levels, i.e., about twofold, since the later, slower disappearance rate was

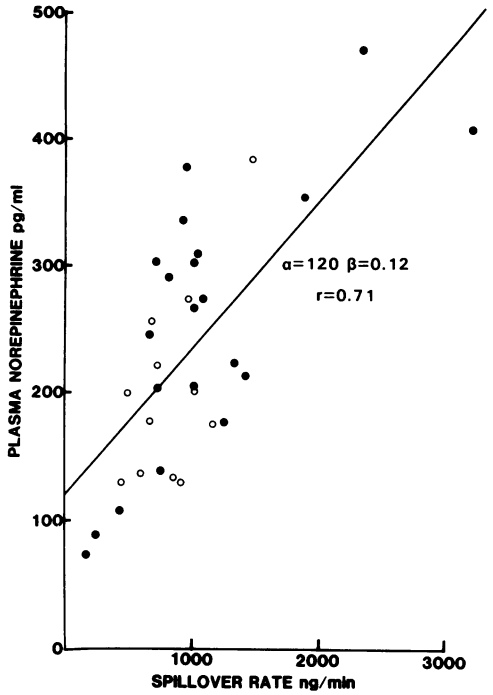

FIGURE 7 Preinfusion plasma NE as a function of NE spillover rate.

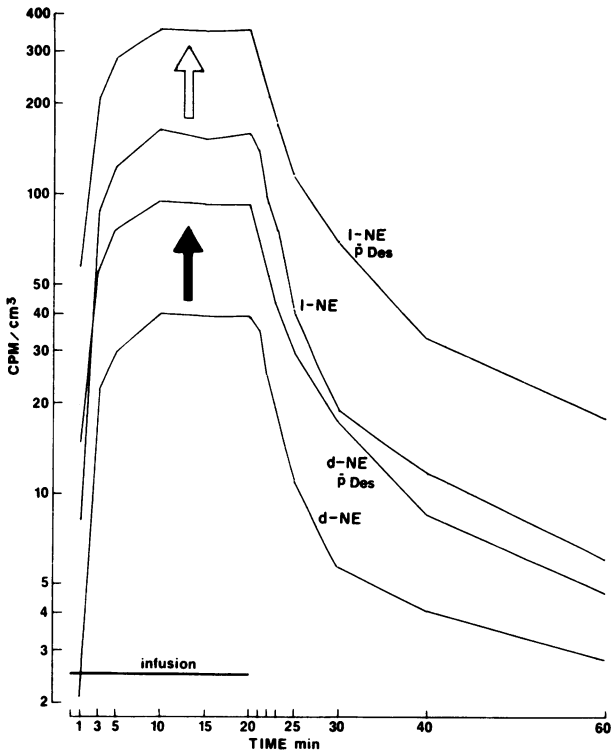

FIGURE 8 Effect of desipramine (*p̄ Des*) on plasma *l*-[^{3}H]NE and *d*-[^{14}C]NE levels during and after the infusion in five normotensive subjects.

unchanged by the drug. Desipramine had no effect on the *d/l* ratio but, as mentioned above, virtually completely abolished the increase in the I/NE ratio during and after the infusion. None of these effects of desipramine differentiated the hypertensive and normotensive groups.

DISCUSSION

Several studies involving large numbers of individuals have suggested that a minority of patients with essential hypertension have elevated levels of plasma NE. The relationship between sympathetic neural activity and circulating NE levels, however, is complex. This study was designed to examine factors that influence plasma NE levels, in an attempt to explain why those levels are high in some patients with hypertension.

Concentrations of circulating, free NE depend not only on sympathetically mediated neural secretion, but also on metabolic degradation, uptake and binding processes, the site of sampling, and renal excretion. In this study, we infused tracer-labeled NE to determine whether hypertensives show decreased plasma clearance of NE. To assess the contribution of stereospecific processes, we compared the disappearance kinetics of *d*- and *l*-NE, most simply reflected in the *d/l* ratio. To examine differences that might depend on neural NE uptake, we compared the disappearance of infused I and NE, most simply reflected in the I/NE ratio, because I is not taken up by sympathetic nerve endings, but NE is inactivated to an important degree by neuronal uptake.

Preinfusion resting NE levels tended to be higher on average in the patients with essential hypertension than in the age-matched normotensive controls, yet no aspect of the kinetics of infused *l*-[^{3}H]NE, *d*-[^{14}C]NE, or [^{3}H]I significantly differentiated the groups. Plasma NE levels were significantly positively correlated with apparent spillover rates but not at all with NE clearances. Relatively hypernoradrenergic hypertensive subjects tended to have higher spillover rates than normonoradrenergic hypertensive subjects. These results indicate that the increased resting NE levels seen in some patients with essential hypertension do not result from prolonged plasma disappearance of NE so much as from increased NE release.

Because no change in the *d/l* ratio occurred in hypertensive or normotensive subjects or after the infusion, the disappearance of exogenously administered NE from plasma is probably not influenced to any important extent by stereospecific processes such as MAO oxidation or incorporation of NE into storage

vesicles, which have been reported to be stereospecific (15, 16). The lack of effect of desipramine, a specific blocker of neuronal NE uptake (U_1), on d/l ratios suggests that in man, U_1 is not stereoselective.

The clearcut, progressive, and large increases in the I/NE ratio after termination of the infusion in all normotensive and most hypertensive subjects indicate that neural NE uptake does play a role in the plasma disappearance of exogenously administered NE. The validity of changes in the I/NE ratio as an index of neuronal NE uptake in man was tested by repeating the infusion in five healthy and three hypertensive subjects after treatment with desipramine. U_1 blockade virtually abolished the increase in the I/NE ratio in the first 5 min after the infusion, confirming that a substantial proportion of injected NE is taken up by nerve endings in man.

A few other groups of investigators have compared plasma NE disappearance in hypertensive and normotensive groups (22, 23, 27, 28) after an infusion to steady state, with discrepant results. Esler and coworkers found that the first half-life of NE disappearance is prolonged in a minority of patients with essential hypertension (22), which they attributed to decreased U_1 (29). Our results complement theirs, because U_1 activity using the I/NE ratio was significantly positively correlated with U_1 activity using the $t_{1/2}{}^a$ for the plasma disappearance of l-[^{3}H]NE, and a few hypertensive subjects showed decreased U_1 activity by both techniques. Also consistent with the findings of Esler et al. (22), hypertensive subjects with relatively high resting plasma NE levels also tended to have decreased U_1 activity. Four hypertensives (25% of the tested group) had a NE/U_1 ratio > 2 SD from the normotensive mean. No clinical characteristics distinguished these patients from other hypertensive subjects.

The proportion of the rapid disappearance of l-[^{3}H]NE due to U_1 can be estimated from the data presented in Table III as follows: First-order disappearance kinetics for I (I) and NE (N) means that $I = I_0e^{-k_1t}$ and $N = N_0e^{-k_2t}$, where k is the proportion of radioactivity lost in time t. At time t, the ratio of tritium in I to that in NE is $I/N = I_0/N_0e^{(k_2-k_1)t}$, and so the I/NE ratio as a proportion of that in the infusate is $(I/N)/(I_0/N_0) = \exp[(K_2 - K_1)t]$. Since for low values of a, $e^a = 1 + a$, the values for the slopes for I/NE reported in Table III were roughly equal to $k_2 - k_1$. From Table III, $k_2 = 0.693/2.48 = 0.28$, and $k_2 - k_1 = 0.13$. The proportion of the early, rapid disappearance of NE due to U_1, therefore, is 0.13/0.28, a bit under 1/2. These calculations demonstrate that the two techniques for measuring U_1 activity were not independent, accounting for the correlation between their values. Furthermore, the slope of the I/NE ratio more accurately measures U_1 than does the $t_{1/2}{}^a$ of l-[^{3}H]NE, since the latter is determined by other factors (U_2, metabolism, etc.) besides U_1.

Our interpretation of the findings of the present study differs somewhat from the model used by Esler et al. (22). According to their conception, faulty neuronal reuptake of norepinephrine causes higher NE spillover rates into plasma in hypernoradrenergic hypertensives. This hypothesis can account neither for the lack of effect of extensive U_1 blockade with desipramine on plasma NE nor for the fact that directly measured sympathetic activity correlates well with venous plasma NE (13). Our working hypothesis is that in some hypertensive subjects, there is both decreased U_1 activity and increased NE release from sympathetic nerve endings, and that these may be due to the same basic neuronal defect, the net result being increased stimulation of postsynaptic adrenoceptors. We agree with Esler et al. (22) that it is possible that decreased U_1 activity accentuates circulating NE responses to stress.

Neither Esler et al. (22) nor we noted significantly increased apparent spillover rates in the groups of hypertensive subjects as a whole. We did find evidence for increased spillover rates in the hypertensive subjects with preinfusion NE levels greater than the hypertensive median value, although with substantial scatter in the data. The calculation of apparent spillover rates includes errors due to variability in the specific activities of the infused NE, assay of tritium where the amount of radioactivity is small, inexact timing of sample collection, inconstancy of the infusion rate, and accuracy of the NE assay. It is therefore possible that differences in preinfusion NE concentrations between the hypertensive and normotensive groups may have been obscured by errors inherent in the calculation of spillover rates.

Apparent spillover rates and clearances, being pharmacokinetic rather than physiologic entities, need not be related to specific processes influencing circulating NE. For instance, calculated NE clearances may or may not correlate with U_1, and apparent spillover rates, which decrease after desipramine (because the specific activity of labeled NE increases), may or may not be associated with a decreased concentration of NE in the synaptic cleft.

Because antecubital venous samples provided the basis for the spillover and clearance calculations, effects of pulmonary and arm extraction of NE probably led to overestimation of the spillover and clearance rates due to lower steady-state levels of the labeled NE.

The results of the present study lead to the following conclusions: (*a*) Most patients with essential hypertension have normal venous plasma NE levels. (*b*) No abnormality in the disappearance kinetics of either l- or d-NE occurs in essential hypertension, even in the mi-

nority of patients with relatively high resting plasma NE levels. (*c*) Apparent NE spillover rates correlate well with resting plasma NE, but NE clearances do not. (*d*) U_1 activity can be measured in man by means of the I/NE ratio. (*e*) U_1 is not stereospecific in man but does contribute importantly to the disappearance from plasma of exogenously administered NE. And (*f*) a minority of patients with essential hypertension show coexistent decreased U_1 activity and enhanced NE release, resulting in increased circulating levels of NE.

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