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Research Article

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Studies on the Renal Excretion of Norepinephrine *

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Summary. Studies were carried out in anesthetized dogs to investigate the renal clearance of norepinephrine (NE) and to determine the origin of this amine in the urine. Infused radioactive NE was cleared from plasma at a rate averaging 63.8% of the glomerular filtration rate. NE was shown to be freely filterable, and evidence has been presented which suggests but does not prove that the amine is partially reabsorbed from the glomerular filtrate; metabolism of NE in the tubular fluid by catechol-o-methyl transferase has not been excluded. The clearance of this catecholamine was not affected by changes in urine pH or flow. Total chronic denervation of one kidney was shown to have no effect on the rate of excretion of endogenous NE. Therefore, the NE that is excreted in the urine would appear to be solely derived from the catecholamines in circulating blood.

Introduction

Measurement of the rate of norepinephrine excretion has been employed extensively as an index of generalized sympathetic activity by a number of investigators (1-6). Despite widespread usage of the measurement in this context, there is little detailed information available regarding the mechanism of excretion of this neurotransmitter hormone in mammalian species. Observations on the fate of NE injected into the circulating blood have indicated that only a small fraction of the material appears unaltered in the urine (6-8), and an even smaller fraction of NE secreted from nerve terminals escapes metabolism and appears unchanged in the urine (8). In addition, although the kidney apparently can clear this amine from the circulating blood, the major source of urinary NE is not known: there is no information about the relative contributions of NE derived from the blood and of material secreted from sympathetic nerves in the substance of the kidney.

Previous studies on the excretion of weak acids and bases have indicated the importance of the hydrogen ion concentration of tubular urine in regulating the rate of excretion of these substances (9). Since NE is a diffusible phenolic amine, it seemed likely that its excretion rate also might be determined by this hydrogen ion effect. Defining the influence of urinary pH on the excretion of this amine is important because changes in urinary acidity could invalidate the use of NE excretion rates as a quantitative method of assessing sympathetic activity. Some earlier investigations on the excretion of catechol- and indoleamines have been performed in the chicken; these have indicated a renal tubular secretion of both NE (10) and 5-hydroxytryptamine (11) in this species. The normal rate of NE excretion (6) and its concentration in normal plasma in either man or the dog (12-14) would appear to make the operation of net tubular secretion unlikely in these species. To determine whether net tubular secretion occurs, we measured the renal clearance of trace amounts of radioactive NE in the dog; we also studied the effect of changing urinary pH on this clearance. In addition, we investigated the excretion of endogenous NE after chronic unilateral denervation of the kidney to determine whether neurotransmitter released locally from renal nerves contributes significantly to the material present in the urine.

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Methods

In the clearance experiments 13 female dogs, weighing 14.0 to 22.8 kg, were studied, 6 control and 7 premedicated 14 hours before with the monoamine oxidase inhibitor, 1-phenyl-2-hydrazinopropane hydrochloride (Catron),¹ in a dose of 5.0 mg per kg. The dogs were anesthetized with pentobarbital; 30 mg per kg was administered intravenously initially, followed by doses sufficient to maintain light anesthesia. Glomerular filtration rate was measured from the clearance of administered creatinine in 12 animals and of inulin in 1 animal. Renal plasma flow was estimated from the clearance of para-aminohippuric acid (PAH) in 6 animals (Table I, no. 5, and Table II, no. 3 to 7). *dl*-Norepinephrine-7-³H (NE-³H) was added to these solutions and delivered at a rate of 0.38 μ c per minute (5.0 μ g per minute). This amount of NE produced no change in arterial blood pressure or heart rate. After a period of 40 to 60 minutes, when initial experiments had shown that the plasma levels of NE-³H were relatively constant, renal clearance measurements were carried out. The urine was collected through an indwelling catheter in the urinary bladder, and washouts were performed with distilled water. Arterial blood was obtained through a femoral arterial catheter.

The dogs were rendered acidotic by administering 6 g of ammonium chloride 18 hours before the study and infusing sodium sulfate in hypotonic saline intravenously during the initial clearance periods. Sodium bicarbonate was substituted for the sodium sulfate infusion in amounts sufficient to produce a maximally alkaline urine. The blood pH remained between 7.3 and 7.6 in all clearance experiments. Mannitol (20% solution) was also added to the infusion in 3 animals at the end of a series of clearance measurements in order to produce an osmotic diuresis.

Total chronic denervation of the left kidney was performed to determine the effect of this maneuver on NE excretion. This was accomplished in 6 anesthetized animals by approaching the left kidney through a midline incision, mobilizing the kidney, removing the capsule, and dissecting the nerves at the hilum. Divided renal function studies were performed in these animals 3 to 5 weeks later as well as in 4 normal dogs. These animals were anesthetized with pentobarbital, and after cannulation of the ureters with polyethylene catheters via a lower abdominal incision, bilateral urine collections were made during infusion of creatinine and PAH. Clearance measurements of creatinine and PAH and endogenous NE excretion rates were carried out during periods of 30 to 60 minutes in 3 control, and then in 2 successive periods during which tyramine was infused intravenously at a rate of 10 and then 20 μ g per kg per minute. At the termination of the study the animal was sacrificed, and both kidneys were excised and frozen immediately for later assay of NE content.

Blood and urine samples were collected on ice and

stored briefly in this condition until assayed for NE. Plasma was separated by centrifugation ($25,000 \times g$) at 0° C. The NE in samples of plasma and urine was adsorbed on aluminum oxide (15) and eluted in 0.2 N acetic acid. In the NE-³H determinations, nonradioactive NE was added to all specimens to permit calculation of exact recoveries in each determination; these averaged 70.0%. NE-³H was measured by lyophilizing a portion of the acetic acid eluate, redissolving it in 0.2 ml distilled water, adding 10 ml of counting solution (16), and measuring the radioactivity in a liquid scintillation spectrometer. Total urinary radioactivity was measured by adding 0.1-ml portions of urine directly to the counting solution. Quenching was monitored in all experiments by adding internal standards. Sufficient counts were accumulated in all measurements to maintain the counting error below 2%. In 2 experiments a portion of this eluate from the urine specimens was also extracted with ethyl acetate (17) to determine the amount of extractable radioactivity representing neutral and acidic catechol compounds. This fraction was found to contain consistently less than 2.5% of the total activity, averaging 0.97%. To determine the portion of plasma NE-³H that was diffusible, we compared the amount of NE-³H in whole plasma with that in an ultrafiltrate of plasma prepared by centrifuging plasma contained in a collodian membrane sac for 30 minutes at 0° C ($35,000 \times g$).

Excretion of endogenous NE from the control and denervated kidneys was determined by adsorbing samples of the urine on aluminum oxide, eluting them with acetic acid, and oxidizing the catecholamines in the eluates to the trihydroxyindole with ferricyanide (18). Fluorescence measurements were carried out in a spectrophotofluorometer. Readings were made at 395/500 and 430/530 $m\mu$ (uncorrected wavelengths of activation and fluorescence, respectively) in order to differentiate NE from epinephrine. Recoveries in this method are identical with those in the assay of NE-³H, and the values were not corrected for this recovery. Tissue NE analyses were performed similarly, with a trichloroacetic acid extract. Tyramine excretion was measured in 4 control dogs and in 5 dogs pretreated with Catron. Samples of urine were analyzed by an extraction method (19). Creatinine, inulin, and PAH were measured colorimetrically in trichloroacetic acid extracts of plasma and urine (20-22). Standard methods of statistical analysis were used in these studies (23).

Results

Renal clearance of NE. The renal clearance of NE-³H was found to be less than the clearance of creatinine in all experiments. A representative experiment (Figure 1) demonstrates results typical of those obtained in these studies. The clearance of NE-³H ranged from 29.5 to 42.5 ml per minute compared to the clearance of creatinine, which ranged from 64.2 to 68.6 ml per minute.

¹ Catron was kindly supplied by Lakeside Laboratories, Cleveland, Ohio.

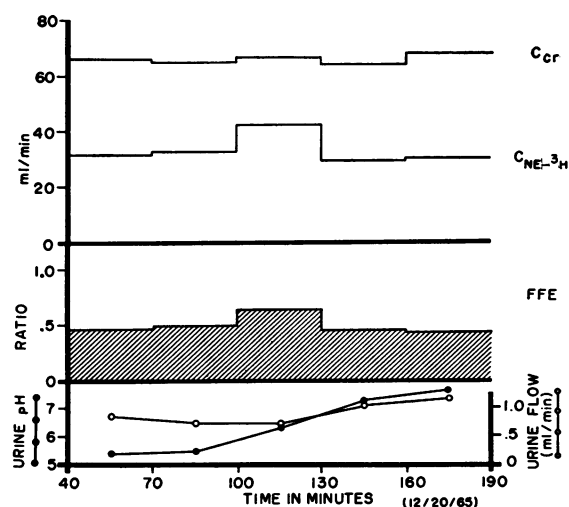


FIG. 1. RENAL CLEARANCE OF NOREPINEPHRINE (C_{NE-3H}) IN A TYPICAL EXPERIMENT IN A CONTROL DOG. Clearance of creatinine (C_{Cr}) and of $NE-3H$ are depicted together with the filtered fraction of $NE-3H$ that is excreted (FFE). Urine flow and pH are plotted below.

The amount of filtered NE actually appearing in the urine, the filtered fraction excreted (FFE), varied between 44.1 and 63.7%. Neither the clearance of $NE-3H$ nor the FFE was affected by a change in urinary pH from 5.36 to 7.62, nor by a minimal increase of urine flow from 0.85 to 1.2 ml per minute. In these studies a state of relative constancy appeared to be present in regard to the excretion of $NE-3H$ and of total radioactivity in the urine. One experiment has been reproduced to demonstrate this observation (Figure 2). The total radioactivity excreted was 160 $m\mu c$ per minute compared to the rate of introduction of $NE-3H$, which was 380 $m\mu c$ per minute. A small fraction of the total radioactivity was represented by unmetabolized $NE-3H$, and this fraction, 9%, remained essentially constant throughout the experiment.

The results of clearance experiments in 6 control dogs (Table I) demonstrated that the production of an alkaline urine did not diminish the clearance of $NE-3H$, which averaged 38.1 ml per minute in an acidic urine and 38.6 ml per minute in an alkaline urine, or significantly reduce the FFE, which averaged 67.2% in an acidic urine and 65.5% in an alkaline urine. An increase of urine flow was observed in the course of all experiments, but this was small with flow rising from

TABLE I
Summary of clearance values in control dogs*

Dog no.	Acid urine (pH 5.0-5.8)				Alkaline urine (pH 7.0-7.8)			
	Urine vol	C_{NE}	GFR	FFE	Urine vol	C_{NE}	GFR	FFE
	ml/min	ml/min	ml/min	%	ml/min	ml/min	ml/min	%
1	1.0	31.9	43.5	63.5	2.0	28.2	46.6	60.3
2	1.0	51.9	64.3	82.7	1.3	47.3	61.4	77.4
3	1.1	53.1	68.0	79.3	2.1	66.5	79.0	84.1
4	3.1	23.6	63.6	44.0	3.9	24.5	45.3	52.7
5	0.8	32.0	65.9	48.8	1.1	29.9	66.4	45.0
6	0.3	36.1	42.6	84.6	0.7	35.4	49.3	73.5
Mean	1.2	38.1	57.9	67.2	1.9	38.6	58.0	65.5
SEM	0.4	4.9	4.8	7.3	0.5	6.5	5.5	7.2

* These figures represent the mean values for clearance periods in the pH range indicated. C_{NE} = clearance of $NE-3H$; GFR = glomerular filtration rate; FFE = filtered fraction excreted.

an average of 1.2 ml per minute during the excretion of an acidic urine to 1.9 ml per minute during the excretion of an alkaline urine. Although there was considerable variation in the FFE, this measure of NE excretion was always less than 100%, ranging from 44.0 to 84.6%. The $NE-3H$ present in plasma was found to be ultrafiltrable *in vitro* and, therefore, not bound irreversibly to plasma protein. In 3 experiments, in which the $NE-3H$ concentration in whole plasma ranged from 0.068 to 1.73 $m\mu c$ per ml, there was no evidence of a significant reduction of $NE-3H$ in the plasma filtrate; the ratio of concentration of $NE-3H$ in plasma to filtrate was 1.08 (0.79 to 1.29).

The effect of increases of tubular flow on the excretion of $NE-3H$ was evaluated in 3 dogs in which an osmotic diuresis was produced by man-

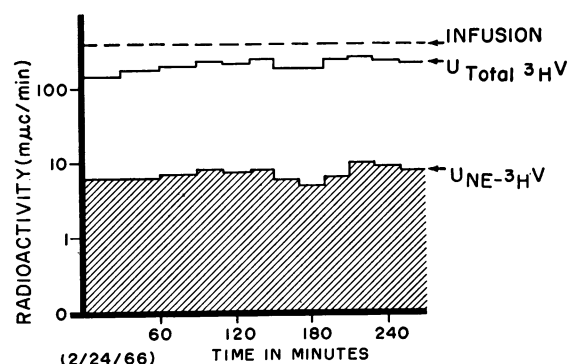


FIG. 2. COMPARISON OF THE RATE OF INFUSION OF $NE-3H$ WITH THE EXCRETION OF TOTAL RADIOACTIVITY ($U_{total\ 3HV}$) AND OF $NE-3H$ (U_{NE-3HV}).

TABLE II
Summary of clearance values in dogs treated with
monoamine oxidase inhibitor*

Dog no.	Acid urine (pH 5.0-5.8)				Alkaline urine (pH 7.0-7.8)			
	Urine vol	CNE	C _{Cr}	FFE	Urine vol	CNE	C _{Cr}	FFE
	ml/min	ml/min	ml/min	%	ml/min	ml/min	ml/min	%
1	0.6	29.1	52.3	56.0	0.5	23.3	59.3	59.3
2	1.4	23.1	38.7	59.8	1.7	15.0	40.1	40.1
3	3.1	37.9	68.8	56.4	3.7	35.0	57.3	61.7
4	0.9	28.9	47.8	57.3	1.2	35.4	55.9	63.3
5	1.3	41.0	76.8	53.8	1.4	26.6	63.3	42.0
6	1.4	32.9	49.0	67.5	1.4	29.6	55.0	54.1
7	1.6	40.1	65.3	62.7	2.1	25.4	67.2	38.6
Mean	1.5	33.3	57.0	59.1	1.7	27.5	56.9	51.3
SEM	0.3	2.6	4.9	1.8	0.5	2.6	3.1	4.7

* These values represent the mean values for clearance periods in the pH range indicated. C_{Cr} = clearance of creatinine.

nitro infusion. In these experiments the FFE averaged 54.3 (50.5 to 57.6) % before mannitol infusion and increased only slightly to 60.2 (57.3 to 64.7) % during mannitol when urine flows had increased from 1.56 (1.27 to 1.99) ml per minute to 7.60 (2.23 to 15.90) ml per minute. One of these experiments demonstrating the clearances of creatinine and NE-³H before and during man-

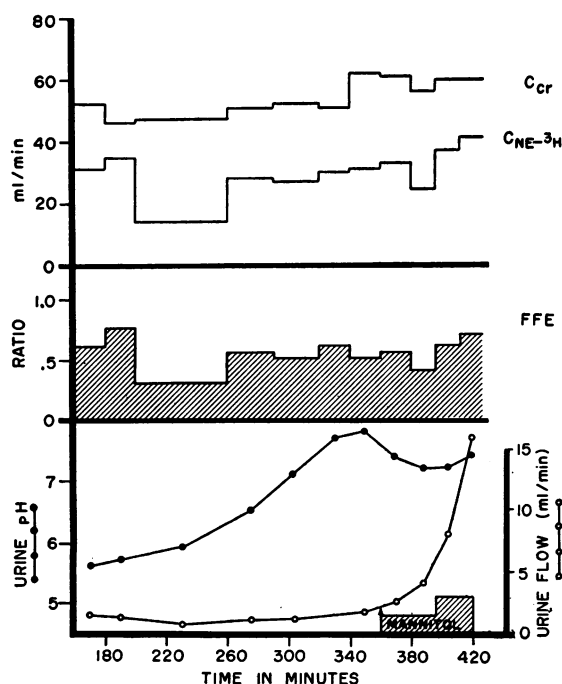


FIG. 3. RENAL CLEARANCE OF NE-³H BEFORE AND DURING AN OSMOTIC DIURESIS INDUCED BY MANNITOL. Symbols are as shown on the previous clearance study.

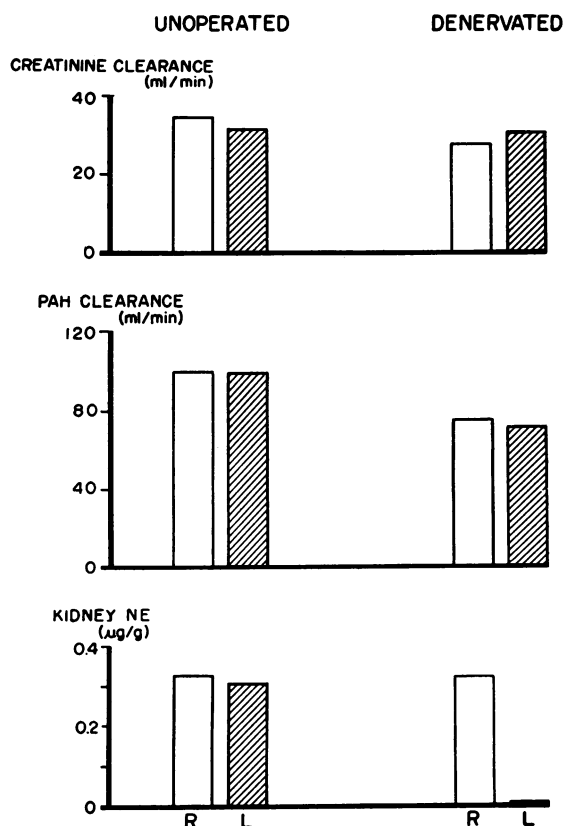


FIG. 4. RENAL HEMODYNAMIC DATA AND KIDNEY NE CONTENT IN THE RIGHT (R) AND LEFT (L) KIDNEYS OF CONTROL AND LEFT RENAL DENERVATED DOGS. PAH = para-aminohippuric acid.

nitro infusion is reproduced (Figure 3). Urine flows were increased from 1.75 to 15.9 ml per minute by mannitol, and no consistent change in clearance of NE-³H or FFE was found.

Similar clearance experiments were performed in 7 dogs in which monoamine oxidase had been inhibited by pretreatment with Catron (Table II). The clearances of NE-³H and of the FFE were not increased in these monoamine oxidase inhibited dogs. In acidic urine, the average clearances of NE-³H and the FFE, 33.3 ml per minute and 59.1%, respectively, were not significantly different from values observed in control animals given in Table I. In alkaline urine, the average clearances of NE-³H and the FFE, 27.5 ml per minute and 51.3%, respectively, did not differ significantly from values observed in acidic urine. The effectiveness of monoamine oxidase inhibition, in the dose of inhibitor used in these studies, was demonstrated by a striking increase of tyramine

TABLE III
Excretion of norepinephrine from the right (R) and left (L) kidneys of dogs with left renal denervation and of unoperated dogs

Experimental dog	Control*		Tyramine			
	R	L	R	L	R	L
	<i>μg/hour</i>		<i>10 μg/kg/min</i>		<i>20 μg/kg/min</i>	
Denervated dogs						
1	0.05	0.00	0.08	0.09	0.24	0.25
2	0.35	0.44	0.80	0.69	0.37	0.95
3	0.10	0.11	0.14	0.18	0.41	0.40
4	0.27	0.22	0.39	0.40	0.60	0.64
5	0.14	0.18	0.25	0.24	0.74	0.85
6	0.11	0.08	0.32	0.42	0.81	0.86
Mean	0.17	0.17	0.33	0.34	0.70	0.66
Unoperated dogs						
1	0.15	0.16	0.30	0.35	0.45	0.42
2	0.11	0.07	0.30	0.16	0.44	0.31
3	0.24	0.24	0.76	0.81	0.82	0.84
4	0.13	0.13	0.21	0.24	0.51	0.51
Mean	0.16	0.15	0.39	0.39	0.56	0.52

* These figures represent the average of values determined during 3 control periods.

excretion. In 4 dogs pretreated with Catron, tyramine excretion averaged 8.80 (1.32 to 17.73) μg per hour compared to a value of 0.40 (0.24 to 0.55) μg per hour in control dogs.

Renal denervation and NE excretion. Chronic left renal denervation did not result in a marked alteration in renal hemodynamics. In Figure 4 the results of clearance measurements of creatinine and PAH are shown with average values from 3 control and 6 left renal denervated dogs. In the control dogs, clearances of creatinine and PAH were comparable in the 2 kidneys. In the dogs with left renal denervation these values from the control and denervated kidney were also comparable with only a very small increment in the clearance of creatinine in the denervated kidney. The clearances of PAH from both kidneys in the denervated dogs were somewhat lower than the measured clearances in the unoperated dogs. This reduction may have been due to an alteration of the extraction ratio in these animals, but extraction ratios were not determined in these studies. In contrast to clearance values, renal NE concentration was reduced strikingly in the denervated left kidney. In this tissue, the NE averaged 0.01 (0 to 0.02) μg per g compared to 0.32 (0.24 to 0.37) μg per g in the unoperated right kidney. In control dogs NE concentrations were comparable in the two kidneys.

Endogenous NE excretion rates in the 6 renal denervated dogs showed no consistent differences between the control and the denervated kidney (Table III). In fact the mean NE excretion, calculated by averaging the 3 control periods in all 6 experiments, was identical, 0.17 μg per hour. During the infusion of tyramine, at both 10 and 20 μg per kg per minute, there was a striking increase in NE excretion from both kidneys. The rate of excretion remained essentially equal from the innervated and denervated sides, averaging, respectively, 0.33 and 0.34 μg per hour at the lower dose of tyramine and 0.70 and 0.66 μg per hour at the higher dose. In one experiment a difference of NE excretion was noted in all 3 control periods (dog 1). However, NE excretion was virtually identical in this experiment during tyramine infusion. The results of similar studies in 4 unoperated dogs (Table III) showed that in these animals there was also equality in the NE excretion between the right and left kidneys. This was true both during the control periods and during tyramine infusion.

Epinephrine excretion was equal in the two kidneys whether or not the innervation to the left kidney was preserved. During the control periods the excretion rates for epinephrine averaged 0.06 and 0.08 μg per hour from the left and right kidneys, respectively, in the dogs with denervated left kidneys and 0.03 and 0.04 μg per hour in the unoperated dogs. During tyramine infusion these values fell to undetectable levels in all experiments.

Discussion

In these studies it has been possible to measure the renal excretion of an administered vasoactive amine, NE, without affecting circulatory hemodynamics by infusing trace quantities of radioactive NE (NE- ^3H) of a very high specific activity. Although the excretion of NE- ^3H represented a small portion of the total radioactivity in the urine, it was possible to measure the NE fraction separately without contamination of the radioactive metabolites. The clearance of NE- ^3H from the circulating plasma was found to be consistently less than the glomerular filtration rate. Since all of the NE- ^3H in plasma was found to be ultrafiltrable *in vitro* and presumably filterable at the glomerulus, this clearance of NE- ^3H must indicate either net active reabsorption, passive diffu-

sion back into the blood, or metabolism in the tubular cells. These findings definitely exclude the possibility of net tubular secretion of NE in the dog, a process previously found to be operative for both NE and 5-hydroxytryptamine in the avian species (10, 11). These findings are in contrast to those previously reported for epinephrine in the dog, in which tubular secretion was demonstrated (24, 25). However, in these earlier studies clearances of the catecholamine were based on chemical measurement of high blood and urine levels induced by the administration of pharmacologic amounts of epinephrine. Therefore, it is difficult to draw any conclusion about renal clearances of physiologic quantities of such a vasoactive amine. The possibility of interference of tubular transport of NE- ^3H by creatinine was ruled out by one experiment in which inulin was used to measure glomerular filtration. Similarly, interference by PAH is unlikely since 7 of 13 experiments were performed without it and no differences were found.

Monoamine oxidase has been shown to play an important role in the metabolism of NE (26). Since the clearance of NE- ^3H was not altered by inhibition of this enzyme, we suggest that metabolism of NE in the tubular urine is not of major importance. Therefore, it seems likely that either active reabsorption or passive diffusion is responsible for the removal of approximately 40% of the filtered NE- ^3H . However, such a tubular mechanism has not been identified with certainty in these studies since the possibility of metabolic degradation by means of catechol-o-methyl transferase or by conjugation was not excluded; suppression of this metabolism would require potent and nontoxic inhibitors of these enzymatic processes that are not currently available. The absence of a marked change in NE- ^3H clearance during mannitol diuresis suggests that passive diffusion back into the blood is not the principal process responsible for the removal of the filtered NE- ^3H .

Since these studies were carried out with the racemic mixture of NE, the possibility must be considered that the renal excretion of the *d* species may be less effective than that of the *l* isomer. Although the neuronal binding of *d*-NE is less than that of *l*-NE (27) and this species is less active at the receptor site (28), there is no reason to believe that a tubular transport mechanism

would be sufficiently specific to differentiate between these two optical isomers. Tubular transport processes have been shown previously to be less specialized and to involve certain organic bases as a class rather than as specific compounds (9).

Although the excretion of many weak organic acids and bases, such as urobilinogen, indolacetic acid, ammonia, quinine bases, and other similar compounds, has been shown to be affected by changes in the concentration of hydrogen ion in the urine, in the present experiments the excretion of NE- ^3H was not altered significantly by maximal changes in urine pH. Although a pH dependence was suggested in the studies in which monoamine oxidase was blocked, the effect was minimal and not significant. It appears unlikely, therefore, that the hydrogen ion exerts any major effect on NE excretion. The absence of such an effect may result from the number of hydroxyl groups on the molecule, which would produce a more hydrophilic character in the molecule, thereby minimizing its lipid solubility even in alkaline urine. On the other hand, the excretion of tyramine, a monohydroxy aromatic amine, has been found in other studies to be influenced by urinary pH, but in these experiments actual clearances of tyramine were not determined (29), and it is not possible to make any inferences about the renal tubular excretion of this amine.

The source of urinary NE has been identified in the present investigation. Previous studies of the excretion of this neurotransmitter hormone have not defined its origin in the body and have assumed that this material is cleared from the blood, having originated from NE secreted by sympathetic nerve endings throughout the body (1). However, the possibility had to be considered that the renal nerves themselves may contribute a significant amount to the urinary NE. Sympathetic nerve fibers have been identified in close approximation to the nephron, and this proximity might permit entry of released NE into tubular urine (30, 31). In studies of patients with heart failure in which increased excretion of NE has been observed (5, 6), it was possible that the augmented NE in the urine represented principally or solely increased activity of the renal nerves themselves. Indeed, a number of investigators have suggested that an augmentation of sympa-

thetic activity to the kidney is an important contributing factor to the renal retention of sodium in patients with heart failure (32-34). Thus, it was of major concern to define the origin of this catecholamine in urine.

The excretion of endogenous NE from the individual kidneys was virtually identical in all control periods, whether or not renal innervation remained intact. These control observations were carried out in animals in which it was assumed that a basal level of sympathetic activity was present. However, it was impossible to control the degree of reflex activity in these animals, and some variability of this function was manifest among the different animals due to variation in the anesthesia or in the amount of blood loss that had occurred. Increased sympathetic neurotransmitter release was produced by tyramine infusion (35) in order to evaluate the effect of this condition on the excretion of NE. This chemical mode of activation of the sympathetic nerves was utilized since it provides a more reliable and reproducible method than induction of reflex activity in anesthetized animals (36). Also, sympathetic neurotransmitter release induced by tyramine is more specific than induction of reflex activity since it is not associated with a concomitant augmentation of catecholamine secretion by the adrenal medulla. This statement is supported by the observed decrease in epinephrine excretion in all experiments as well as previous observations of plasma catecholamine concentrations after tyramine administration (37). The absence of a differential effect on NE excretion for the innervated kidney with either the small or large amount of tyramine suggests that the local renal nerves do not contribute significantly to urinary NE. Therefore, these studies have validated the use of measurements of NE excretion as a method of estimating the rate of secretion of the sympathetic neurotransmitter hormone of the total body. It has been shown that such measurements will not be affected by changes in urine pH and flow or by variation in the level of renal sympathetic activity.

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