Norepinephrine Metabolism in Dogs with Chronic Renovascular Hypertension (34197)

LEWIS G. LEFER AND CARLOS R. AYERS
(Introduced by Allan M. Lefer)
University of Virginia, Virginia Heart Laboratory, Department of Medicine, Charlottesville, Virginia 22901

The role of catecholamines in the pathogenesis of experimental hypertension has recently received much attention. In rats made hypertensive by uninephrectomy and administration of DOCA plus NaCl, an increased norepinephrine turnover secondary to a decreased ability of the catecholamine storage granule to bind norepinephrine has been observed (1). However, both this storage defect and the elevation in blood pressure were reversed. Wistar rats which become spontaneously hypertensive, decreased norepinephrine turnover and decreased rates of synthesis were observed. There was no storage defect present (3). Little information is available about the role of the catecholamines in the pathogenesis of renovascular hypertension in the dog. This report will be concerned with subcellular localization of norepinephrine in various tissues in this experimental model.

Materials and Methods. Six trained adult mongrel dogs underwent right uninephrectomy and application of a Crutchfield clamp (4) to the contralateral renal artery. The mean arterial blood pressure (MABP) of the unanesthetized animals was obtained by needle puncture of the femoral artery and was measured with a Statham pressure transducer connected to a Sanborn recorder. Each experimental dog was studied preoperatively with 3–5 determinations of the MABP over the course of 1 week, and postoperatively with determinations of the MABP 2–3 times/week for 1–2 months until it stabilized at hypertensive levels. The MABP was followed until immediately prior to sacrifice. Six control dogs were studied similarly for 1 week prior to use in the study, and underwent no operative procedure. The dogs were maintained on a standard laboratory diet which contained 50 meq of sodium/day.

After 2-10 months of stable hypertension, 0.1 mC/kg of body weight of *dl*-^{3}H-norepinephrine HCl (54 mC/mg) was injected intravenously into hypertensive and control dogs. Twelve hr later they were sacrificed, and the heart liver, kidney, aorta, and spleen were removed and chilled on ice. The tissues were analyzed for endogenous norepinephrine by alumina column chromatography (5) and the trihydroxyindole fluorometric method of Von Euler and Lishajko (6), and for radioactive norepinephrine by liquid scintillation counting as described by De Champlain (5). The right and left ventricle, kidney, and aorta were also analyzed for subcellular localization of the endogenous and labeled norepinephrine. Ultracentrifugation of these tissues at 100,000g for 60 min yielded soluble cytoplasm and particulate fractions, the latter of which contains the catecholamine storage granules (1).

Results. The mean preoperative MABP of the 6 experimental dogs was 99 $\pm$ 5 mm Hg, and the postoperative stable MABP was 135 $\pm$ 10 mm Hg ($p<0.01$). The mean MABP of the six control dogs was 105 $\pm$ 7 mm Hg. Trained dogs are used in this laboratory and have yielded somewhat lower levels of normal and hypertensive blood pressure than reported by others. The endogenous norepinephrine values of the various tissues for both the unoperated (control) and postoperative (hypertensive) dogs are shown in Table I. The total endogenous norepinephrine was significantly reduced ($p<0.05$) in only the kid-

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TABLE I. Endogenous Norepinephrine[a] in Control (C) and Hypertensive (H) Dogs.

Tissue		Total	Soluble cytoplasm	Storage granules
Kidney	C	0.32 ± 0.07	0.09 ± 0.02	0.07 ± 0.02
	H	0.15 ± 0.03	0.03 ± 0.01[b]	0.05 ± 0.02
Right ventricle	C	0.65 ± 0.08	0.21 ± 0.02	0.05 ± 0.01
	H	0.57 ± 0.11	0.14 ± 0.01[b]	0.03 ± 0.01
Left ventricle	C	0.57 ± 0.05	0.18 ± 0.03	0.05 ± 0.02
	H	0.47 ± 0.08	0.09 ± 0.02[b]	0.06 ± 0.02
Aorta	C	1.02 ± 0.09	0.49 ± 0.16	0.21 ± 0.09
	H	1.02 ± 0.11	0.28 ± 0.05	0.16 ± 0.03
Spleen	C	1.69 ± 0.40		
	H	1.60 ± 0.34		
Liver	C	0.22 ± 0.05		
	H	0.25 ± 0.04		

[a] (μg/g of tissue wet wt); values expressed are the means of 6 animals ± SEM.
[b] $p < 0.05$.

ney of the hypertensive dogs, and the reduction was localized to the soluble cytoplasm. Significant reductions ($p<0.05$) in the soluble cytoplasm were also found in the right and left ventricle. There was no deficiency in endogenous norepinephrine in the catecholamine storage granular fraction in any of the tissues studied.

The accumulation of tritiated norepinephrine (in dpm/g tissue) is shown in Table II. There was a significantly lower ($p<0.02$) accumulation in only the soluble cytoplasm of the left ventricle.

The specific activity (SA) of the norepinephrine (dpm/μg of norepinephrine) in the various tissues is shown in Table III. There was a significantly higher ($p<0.05$) SA in only the cytoplasm of the kidney of the hypertensive dogs.

Discussion. We were unable to confirm the decreased levels of endogenous norepinephrine in the aorta or spleen which were previously reported in dogs with chronic renovascular hypertension (7). In rats made hypertensive by administration of DOCA plus NaCl, significant reductions in both endogenous norepinephrine and tritiated norepinephrine accumulation were reported in the spleen, kidney, and heart, and the decrease in specific activity was disproportionally greater in the catecholamine storage fraction than in the soluble cytoplasm of the hearts

TABLE II. Tritiated Norepinephrine[a] in Control (C) and Hypertensive (H) Dogs.

Tissue		Total	Soluble cytoplasm	Storage granules
Kidney	C	25.3 ± 2.6	6.3 ± 2.3	3.9 ± 0.8
	H	16.2 ± 3.9	3.2 ± 1.3	4.2 ± 1.4
Right ventricle	C	78.6 ± 14.2	21.6 ± 2.2	4.7 ± 0.9
	H	71.4 ± 14.3	17.5 ± 3.0	4.6 ± 1.2
Left ventricle	C	109.2 ± 13.2	37.7 ± 7.3	10.5 ± 4.8
	H	77.7 ± 12.5	14.9 ± 2.9[b]	10.8 ± 3.3
Aorta	C	3.8 ± 0.3	2.1 ± 0.4	1.9 ± 0.6
	H	3.8 ± 0.5	1.6 ± 0.3	0.9 ± 0.3
Spleen	C	141.7 ± 13.8		
	H	144.5 ± 28.2		
Liver	C	11.7 ± 0.7		
	H	9.1 ± 1.5		

[a] (dpm/g of tissue wet wt); values expressed are the means of 6 animals ± SEM.
[b] $p < 0.02$.

TABLE III. Specific Activity of Norepinephrine[a] in Control (C) and Hypertensive (H) Dogs.

Tissue		Total	Soluble cytoplasm	Storage granules
Kidney	C	91.5 ± 16.6	61.6 ± 8.6	57.9 ± 7.5
	H	118.5 ± 27.6	114.8 ± 18.1[b]	96.4 ± 25.6
Right ventricle	C	119.3 ± 12.4	105.2 ± 9.7	109.1 ± 12.2
	H	139.8 ± 27.8	122.7 ± 15.3	179.6 ± 40.5
Left ventricle	C	197.9 ± 27.1	243.0 ± 45.1	197.1 ± 28.2
	H	173.5 ± 40.6	186.6 ± 34.7	189.6 ± 34.7

[a] (dpm/μg of norepinephrine $\times 10^{-3}$); values expressed are the means of 6 animals ± SEM.
[b] $p < 0.05$.

(1). The authors postulated the presence of a defect in the ability of the granules to bind norepinephrine which was subsequently shown to be reversed by withdrawal of NaCl from the diet of the rats (2).

The present work in dogs with chronic renovascular hypertension has not revealed any changes in the levels of endogenous norepinephrine and accumulation of tritiated norepinephrine in the granules of the tissues studied. These findings indicate that a defect in the granular binding of norepinephrine does not exist in this experimental model.

It is to be noted that our data were obtained 12 hr after the injection of the tritiated norepinephrine into the dogs. Since no significant differences in immediate uptake of the catecholamine were noted in either the rats made hypertensive by DOCA plus NaCl administration (1) or in the spontaneously hypertensive rats (3), we assumed that there would be no difference in immediate uptake in the chronically hypertensive dogs used in this study. Thus, the increased specific activity in the cytoplasm of the kidneys indicates either a decreased rate of synthesis or a decreased turnover of norepinephrine. Although the decreased levels of endogenous norepinephrine in the kidney are consistent with a decreased rate of synthesis, we are currently unable to differentiate between these two possibilities. However, the net result of either mechanism would result in a decreased amount of physiologically active norepinephrine present at its receptor site. This would imply that variations in catecholamine dynamics are secondary and perhaps compensatory to, but not primarily the cause of, the hypertension in this experimental model. That chronic benign renovascular hypertension was established in the experimental dogs is shown by the postoperative MABP of 135 ± 10 mm Hg, a value consistent with previous observations in this laboratory of well trained animals. No sham operation was performed on the control dogs as others have shown that laparotomy does not significantly alter the MABP (7).

Summary. Analysis of several tissues (kidney, right ventricle, left ventricle, aorta spleen, liver) of dogs with renovascular hypertension revealed significantly less endogenous and tritiated norepinephrine in the soluble cytoplasm of the kidneys and in the right and left ventricle, but a higher specific activity of the tritiated compound in the soluble cytoplasm of the kidney. There was no deficit in the ability of the storage granules to bind norepinephrine. These findings indicate that changes in norepinephrine dynamics play only a secondary role in the pathogenesis of experimental renovascular hypertension.

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