Effect of Angiotensin on the Denervated Kidney in the Dog* (33904)

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It has been suggested that the sympathetic nervous system participates in the renal vasoconstrictor response to angiotensin. In the dog, denervation by section of the renal nerves or by cervical cord transection has been reported to reduce or to abolish completely the renal vascular reactivity to angiotensin injection (1, 2). Likewise, pharmacological denervation achieved by the administration of adrenergic blocking agents, such as guanethidine, bretylium, or hydralazine seemed to modify markedly the vasoconstrictor response to single injections of angiotensin (2). However, when infusions, instead of single injections of angiotensin were employed, pretreatment with adrenergic blocking agents did not cause a consistent reduction of the renal vasoconstrictor effect of the octopeptide (3). In the same experiments the only consequence of reserpine–guanethidine infusion was a modification of the natriuretic response to angiotensin infusion. Indeed, treatment with adrenergic blocking agents seemed to facilitate the natriuretic effect of angiotensin. The experiments presented here were designed to evaluate further the influence of the renal nerves on the vascular and natriuretic response of the kidney to angiotensin infusion.

Methods. Experiments were performed on mongrel dogs of either sex, ranging in weight from 10.8 to 19.8 kg, and anesthetized with sodium pentobarbital, 30 mg/kg. Both ureters were isolated by a left flank incision and catheterized with polyethylene tubing. Intravenous infusions were administered through a catheter in a femoral vein and jugular vein. One femoral artery was cannulated to record blood pressure with a pressor transducer, and to collect blood samples. After a priming dose of inulin and PAH an intravenous infusion containing these substances in adequate amounts was started through the femoral vein. Five dogs received an isotonic saline solution (0.15 *M* NaCl) at 3.2–4.2 ml/min. Three dogs received an hypotonic solution made up with 0.1 *M* glucose + 0.02 *M* NaCl. For this latter group the rate of infusion varied between 6 to 8 ml/min. In addition, all dogs received an infusion of 0.15 *M* NaCl into the jugular vein at 0.5 ml/min.

Renal denervation was accomplished 60–90 min after the beginning of the infusion. Through a left flank incision, all visible nerves leading to the left kidney and those around the left renal artery were divided. After all nerves were cut, the renal artery was carefully stripped of its adventitia. Twenty min later urine was again collected for 4–6 periods of 10 min each, and urine flow from both kidneys was compared with the diuresis observed before denervation. The denervation was considered as successful according to the following arbitrary criteria: Of the dogs infused with isotonic saline only those which showed an increase of urine flow on the denervated side of at least 50% as compared to the control side were used. Renal deneravation in water diuresis may produce an increase in sodium excretion without any apparent change in urine flow. Therefore, in dogs infused with a hypotonic solution, only those displaying an increase in sodium excretion on the denervated side larger than 30% as compared to the control kidney were used.

Two or three control clearance periods of 10–20 min (depending on the urine flow) were measured before starting the infusion of angiotensin. Blood samples were withdrawn 1 min before the midpoint. Angiotensin II (Hypertensin, Ciba) was administered at 0.025, 0.050, or 0.100 μg/kg·min through the jugular vein in isotonic saline infused at 0.5

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TABLE I. Effect of Angiotensin on Renal Function of Denervated and Innervated Kidneys.[a]

Dog	Wt (kg)	Infusion (ml/min)	Period (μg/kg • min)	MBP	V (ml/min)		$U_{Na}V$ (μeq/min)		C_{IN} (ml/min)		C_{PAH} (ml/min)		ΔC_{PAH} (ml/min)	
					I	D	I	D	I	D	I	D	I	D
1	11.5	Iso 4.2	Control	148	.72	1.39	151	294	31.0	34.5	74.1	70.1		
			Angio. 25	178	1.07	1.28	207	136	35.5	36.0	57.8	58.4	—16.3	—11.7
4	12.0	Iso 3.2	Control	150	.66	1.52	170	355	26.9	28.7	70.8	74.0		
			Angio. 50	210	.43	.37	105	95	27.6	25.2	49.2	45.2	—21.3	—28.8
13	12.7	Iso 3.2	Control	108	.71	1.35	152	192	41.2	38.2	82.6	74.9		
			Angio. 50	145	.60	.70	119	111	36.1	36.6	62.9	61.7	—19.7	—13.2
15	19.8	Iso 3.2	Control	120	.59	.99	145	211	45.0	50.6	104.9	118.0		
			Angio. 50	193	.92	1.23	119	145	47.9	47.0	79.4	81.1	—25.5	—36.9
1	11.5	Iso 4.2	Control	145	.68	1.75	159	336	31.8	33.0	83.2	84.5		
			Angio. 100	215	3.37	3.83	604	691	32.0	33.0	65.8	65.3	—17.4	—19.2
5	11.0	Iso 3.2	Control	105	.40	.87	96	185	24.3	26.3	70.7	74.8		
			Angio. 100	170	1.65	1.95	347	379	32.2	28.1	64.3	56.9	— 6.4	—17.9
10	13.0	Hypo 6.0	Control	112	1.65	3.61	66	128	51.9	54.8	113.8	119.8		
			Angio. 50	165	4.68	5.15	58	67	49.3	54.1	92.5	93.3	—21.3	—26.5
8	10.8	Hypo 8.0	Control	125	4.71	5.18	90	123	34.4	33.4	89.7	91.0		
			Angio. 50	170	2.86	2.91	90	92	33.9	32.5	62.8	62.2	—26.9	—28.8
11	11.8	Hypo 8.0	Control	110	2.98	3.96	182	254	33.2	34.9	81.5	84.4		
			Angio. 50	160	4.16	4.78	210	254	34.3	36.0	68.9	70.0	—12.6	—14.4
												Mean	—18.6	—21.9
												SE	± 2.1	± 2.3
													$p > .2$	

[a] Each value represents the mean of two or three clearance periods. Iso = isotonic saline (0.15 *M* NaCl); Hypo = 0.1 *M* glucose + 0.02 *M* NaCl; MBP = mean blood pressure; $\Delta C_{PAH} = C_{PAH}$ control period $- C_{PAH}$ angiotensin period; I = innervated kidney; and D = denervated kidney.

TABLE II. Influence of Angiotensin on the Clearance Ratios Denervated/Innervated.[a]

Dog	Infusion (ml/min)	V		$U_{Na}V$		C_{IN}		C_{PAH}	
		Control	Angio.	Control	Angio.	Control	Angio.	Control	Angio.
1	Iso. 4.2	1.93	1.19	1.95	.66	1.11	1.03	.95	1.01
4	Iso. 3.2	2.30	.86	2.14	.90	1.06	.92	1.04	.92
13	Iso. 3.2	1.90	1.16	1.27	.93	.92	1.02	.91	.98
15	Iso. 3.2	1.68	1.34	1.46	1.22	1.13	.98	1.12	1.02
1	Iso. 4.2	2.57	1.13	2.13	1.14	1.07	1.03	1.01	1.00
5	Iso. 3.2	2.18	1.18	1.93	1.09	1.08	.87	1.05	.89
10	Hypo. 6.0	2.18	1.09	1.91	1.15	1.06	1.10	1.05	1.01
8	Hypo. 8.0	1.09	1.01	1.37	1.02	.97	.96	1.02	.99
11	Hypo. 8.0	1.33	1.14	1.40	1.21	1.05	1.05	1.04	1.01
	Mean	1.90[b]	1.12	1.73[b]	1.04	1.05	.99	1.02	.98
	± SE	.16	.05	.19	.06	.02	.04	.02	.02

[a] Each value represents the mean of that parameter for the denervated kidney divided by the mean for the innervated kidney.

[b] The mean is statistically different from zero and from that mean during angiotensin infusion; in both cases $p < .001$.

ml/min. When blood pressure stabilized, two clearance periods of 10 or 20 min each were obtained during angiotensin infusion.

Urine flow (V), sodium excretion ($U_{Na}V$), clearance of inulin (C_{IN}) and clearance of para-aminohippuric acid (C_{PAH}) were determined for each period.

Chemical methods were as follows: PAH with the method of Smith *et al.* (4); inulin according to Walser and Davidson (5); sodium by flame photometry.

Results. The results presented in Table I show clearly that denervation of the kidney by section of all visible renal nerves does not affect the renal hemodynamic response to angiotensin, as measured by the variation of C_{PAH} and C_{IN}.

In the control periods, the mean increase in water and sodium excretion of the denervated kidney compared to the innervated side was 0.84 ml/min and 96 meq/min, respectively. Denervation, however, did not enhance significantly the mean values for C_{IN} or C_{PAH}. Angiotensin, infused for at least 2 hr after denervation, produced, in all experiments, a fall of C_{PAH} in both kidneys. The mean decrease of C_{PAH} for the denervated side (21.9 ± 2.2 ml/min) was not significantly different ($p > 0.2$) from the fall measured for the innervated kidney (18.6 ± 2.1 ml/min).

Following angiotensin, V changed in the same direction in all experiments except for dog no. 1a. For $U_{Na}V$ the same was true in all but Expts. 1a, 8, and 11. In none of the experiments did the denervated kidney respond with a greater natriuresis than the innervated side. Moreover, during angiotensin administration, V and $U_{Na}V$ from the denervated side tended to become equal to the excretion of the controlateral innervated kidney. Table II shows this clearly. Before the infusion of angiotensin the ratios of V and $U_{Na}V$ of the denervated kidney divided by that for the innervated kidney were all greater than 1.0. During angiotensin infusion these ratios approached 1.0; and in fact the mean ratio during angiotensin infusion for V and $U_{Na}V$ was not significantly different from 1.0. This phenomenon appeared when angiotensin produced either an increase or a decrease in water and sodium excretion, and was unrelated to the magnitude of C_{PAH} or C_{IN} variation.

Discussion. The results presented here fail to demonstrate that denervation of the kidney alters the vascular response to angiotensin. Although no significant difference was found in the change of C_{PAH} between innervated and denervated kidney, it is possible that true plasma flow differed. The E_{PAH}

might decrease in a denervated kidney perfused with antiotensin. This possibility would invalidate C_{PAH} as a direct estimate of renal plasma flow. However, the superimposition of angiotensin in a vasodilated kidney has increased rather than decreased E_{PAH} in the dog (6). In addition, in the rat angiotensin does not alter E_{PAH} (10). A clear interaction between the autonomic nervous system and the renal vascular response to angiotensin has only been demonstrated when the octapeptide was administered by single intravenous or intra-arterial injection (1, 2). Indeed surgical denervation or treatment with autonomic blocking agenst such as guanethidine (a drug supposed to act by inhibiting the release of neurotransmitter to adrenergic nerve activity) abolishes the renal vasoconstrictor response to angiotensin injection of 0.1–0.2 μg/kg (2). However, infusion of 0.0375 and 0.375 μg/kg·min delivered during 8–12 min produced a large decrease in the renal blood flow of reserpine–guanethidine treated dog (3). In our experiments denervated kidneys displayed the same vascular response to the infusion of angiotensin (0.025–0.100 μg/kg·min) as did the contralateral innervated kidneys. Thus the influence of denervation on the vasoconstrictor response to angiotensin seems to be detectable only when the renal vasculature is exposed for a very short period of time to the vasoactive substance. In experiments carried out in the dog, the renal vasoconstrictor response to sympathetic stimulation was potentiated by an infusion of angiotensin during the first seconds of the nerve stimulation (8). Hence the suggestion was advanced that only a small store of norepinephrine (the presumed neurotransmitter) might be made available for release by angiotensin (8). Such a small store of norepinephrine could also be liberated at the very beginning of the administration of angiotensin, and play a major role in the vasoconstrictor response observed with single injections. After this initial response, vasoconstriction would not depend upon the release of the adrenergic neurotransmitter, but on a direct effect of the octapeptide on the vascular smooth muscle. A direct action of angiotensin was repeatedly demonstrated on several kinds of vascular smooth muscles (9, 10). Although arterial resistance vessels isolated from the dog kidneys did not respond to angiotensin *in vitro* (11), such a finding does not imply that intactness of innervation is a critical factor for the renal vasoconstrictor response *in vivo*.

Treatment with adrenergic blocking agents has modified the effect of angiotensin on water and sodium excretion (3). After administration of reserpine and guanethidine the antidiuretic and antinatriuretic effect of angiotensin observed in normal dogs was converted into a diuretic and natriuretic response. Therefore, an interaction between the autonomic nervous system and agniotensin on the renal handling of sodium has been suggested (3). In our experiments denervation did not enhance the diuretic and natriuretic response to angiotensin. Thus, surgical denervation does not appear to bring about the modification of the natriuretic response to angiotensin which pharmacological denervation does. One explanation for these contradictory results might be related to the different alterations induced by both kinds of denervation on renal function. Administration of reserpine and guanethidine, in the aforementioned experiments (3), lowered CFR and RBF without altering sodium and water excretion. On the other hand, denervation by dividing the renal nerves, in our experiments resulted in an increase of V and $U_{NA}V$ without any significant change in GFR and RPF. The hemodynamic and excretory condition of the kidney prior to the infusion of angiotensin have influenced the effect of the octapeptide on water and sodium excretion in the dog (6, 12–14) and in the rat (8, 9, 15–17). Therefore any interpretation considering the specific influence of denervation on the renal excretory response to angiotensin should take into account the changes induced by the denervation itself on the renal functions. The finding showing that angiotensin tends to cancel out the difference in sodium and water excretion between denervated and innervated kidneys (Table II) remains to be explained. Whether or not this phenomenon

represents a specific response to angiotensin should deserve a comparative study with other natriuretic and antinatriuretic agents.

Summary. The renal effects of angiotensin were studied in anesthetized dogs with one kidney acutely denervated. Denervation was accomplished in each animal by sectioning all visible nerves leading to the left kidney. The response of the innervated kidney was compared to that of the denervated kidney before and during the intravenous infusion of angiotensin (0.025, 0.050, and 0.100 μg/kg·min). The kidneys were compared for urine flow (V), sodium excretion ($U_{NA}V$), glomerular filtration rate (C_{IN}) and effective renal plasma flow (C_{PAH}). During angiotensin infusion the mean decrease of C_{PAH} for the denervated side (21.9 $\pm$ 2.2 ml/min) was not statistically different from the fall measured for the innervated side (18.6 $\pm$ 2.1 ml/min). In all but one experiment, V and $U_{Na}V$ changed in the same direction for both kidneys during the infusion of angiotensin. The denervated kidney did not display a greater diuresis nor natriuresis than the control kidney. These data indicate that renal denervation does not alter the vascular or excretory response to angiotensin infusion in the dog.

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