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The Role of the Renin–Angiotensin System in Experimental Renal Hypertension in Dogs[1] (38347)

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A possible role of the renin–angiotensin system in the pathogenesis of renal hypertension has recently been investigated by the use of analogs of angiotensin II which act as competitive antagonists of endogenous angiotensin II. A major problem has been to obtain an angiotensin analog which is sufficiently specific and potent and which lacks agonistic properties. The angiotensin II analog, 1-sarcosine-8-alanine angiotensin II, which was first reported by Pals *et al.* (1) to be an effective competitive antagonist of angiotensin II in the rabbit and rat, has been shown also to antagonize the effects of endogenous angiotensin II in the dog with little or no agonistic effects (2, 3). In the dog with chronic unilateral renal hypertension secondary to renal artery stenosis there is a paucity of data (3) from the use of angiotensin II antagonists and no data are available from the use of converting enzyme inhibitors. The present report describes recent experiments on the use of 1-sarcosine-8-alanine angiotensin II to study the possible participation of the renin–angiotensin system in dogs with malignant and with chronic renal hypertension both secondary to renal artery constriction.

Materials and Methods. Female hounds (14–24 kg body wt) were used in this study. All were fed a diet which provided 65 mEq of sodium and 55 mEq of potassium per day; water was available *ad libitum*. Prior to and during the acute experiment, arterial pressure was measured routinely three times each week by puncturing the femoral artery with a 22 Ga needle attached to a pressure transducer. Pressures were recorded on a Sanborn recorder. Only dogs that consistently had mean arterial pressures of less than 130 mm Hg during a control period of 2–3 weeks were used in these experiments.

Two groups of dogs were studied. The first group consisted of six normal dogs. The second group of eight dogs was subjected to a right nephrectomy and 10–14 days later the left renal artery was partially constricted by means of a Goldblatt clamp (4). Three of these dogs developed a chronic hypertension in which the only apparent abnormality was an elevated arterial pressure. Five of the dogs with renal artery constriction, on the other hand, developed a malignant form of hypertension[2] within 10 days; these dogs became lethargic and hyper-reflexic and died within 3 days of developing the syndrome. Additional evidence to aid in classifying the type of hypertension as chronic or malignant was provided by the rectal temperature, serum urea nitrogen (SUN), plasma renin activity (PRA), and the plasma concentrations of sodium and potassium.

In the dogs with renal artery constriction and chronic hypertension, the experiments were performed 2–7 weeks after placing the renal artery clamp. In the dogs with renal artery stenosis that developed malignant hypertension, the experiments were performed as soon as the syndrome was well established. The normal control dogs underwent experimentation after 2–3 weeks of

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[2] The term malignant hypertension is used to describe a hypertensive syndrome which occurs in some dogs following constriction of the renal artery. It is characterized by hypertension, a decrease in rectal temperature, elevations is serum urea nitrogen, plasma renin activity, and plasma potassium concentration; the condition runs a rapid, fulminating course and leads to death of the animal within a few days. For further description see Brown *et al.* Circ. Res. **18,** 475 (1966).

routine arterial pressure measurements. Two days prior to the experiment each dog was anesthetized with pentobarbital and a chronic catheter was inserted into the brachial artery to allow continuous measurement of arterial pressure during the acute experiment. In the dogs that developed the malignant form of hypertension, arterial pressure was measured by a catheter of polyethylene (PE) tubing (#50) inserted percutaneously into the femoral artery through a syringe needle (#18T) at the time of the experiment. Immediately prior to the experiment a catheter (PE 50) was inserted percutaneously into the saphenous vein for infusion of the angiotensin II analog. All experiments were performed on conscious dogs; during each experiment the dog was lying quietly on the floor of the laboratory restrained only by a rope loosely fastened around the neck.

At the beginning of each experiment the rectal temperature of the dog was measured and an arterial blood sample was obtained for determination of SUN, PRA, and plasma concentrations of sodium and potassium. After a 15 min control period of continuous arterial pressure measurement, a second blood sample was obtained for PRA. An intravenous infusion of the angiotensin II analog was begun at a rate of 6 μg/min/kg of body wt (0.6 ml/min in isotonic saline) and was continued for 45 min; additional blood samples were obtained at 15, 30, and 45 min. At this point the infusion of the angiotensin analog was stopped in most of the experiments; however, in the malignant hypertensive dogs and in two of the dogs with chronic hypertension, the rate of infusion of the angiotensin II analog was increased to 12 μg/min/kg for an additional 15 min. Recovery observations were made at 45 and 60 min after stopping the infusion of the analog in the chronic hypertensive dogs with renal artery constriction. The plasma samples for PRA were processed for the generation of angiotensin I by the method described by Schneider *et al.* (5) and were bioassayed in the pentobarbital-anesthetized, pentolinium-blocked rat; changes in arterial pressure were used as the response parameter with synthetic angiotensin II as a standard. Plasma electrolyte concentrations were determined by flame photometry. SUN levels were determined by the clinical chemistry laboratory of the University Hospital.

Results. The arterial pressure for the control group averaged 119 mm Hg (±2 S.E.M.); the arterial pressures for the two groups of hypertensive dogs were significantly elevated, averaging 173 (±6) mm Hg for the chronic hypertensive dogs and 170 (±8) mm Hg for the dogs with malignant hypertension. The dogs with chronic hypertension showed no abnormalities in rectal temperature, SUN, PRA, or in plasma electrolyte concentrations. However, the dogs with the malignant form of hypertension had significant elevations in PRA, SUN, and plasma potassium concentration, decreased rectal temperature, and hyponatremia (see Table I). Infusion of the angiotensin II antagonist into normal conscious dogs for 45 min did not alter the arterial pressure or the plasma renin activity although initially a small (5–10 mm Hg) transient rise in arterial pressure occurred for 4–5 min. Likewise, the chronic hypertensive dogs showed a small initial increase in arterial pressure during infusion of the angiotensin analog, but returned to the preinfusion level within 5 min and remained at this control level for the duration of the infusion (Fig. 1); also, PRA was unchanged. In two of the chronic hypertensive dogs, the infusion rate of the angiotensin II analog was increased to 12 μg/min/kg of body wt; no change in arterial pressure was observed during infusion of this larger dose of the angiotensin antagonist. Infusion of the angiotensin antagonist into dogs with malignant hypertension (Fig. 2), however, resulted in a significant lowering of the arterial pressure and an increase in PRA; increasing the dose of the angiotensin antagonist to 12 μg/min/kg did not result in any further decrease in arterial pressure.

Discussion. Earlier studies from this laboratory (6) demonstrated that administration of 1-sarcosine-8-alanine angiotensin II at 6 μg/min/kg of body wt blocked both the pressor and steroidogenic effects of synthetic angiotensin II infused at a rate of 1.5 μg/min in normal anesthetized dogs. Also, infusion of the analog at this same rate and for the same duration in dogs with thoracic caval constriction or in sodium-depleted dogs (6), conditions in which the PRA and presumably the plasma angiotensin II levels were elevated, produced pronounced decreases in arterial pressure and aldosterone secretion (2, 6); these findings demonstrated that this analog of angiotensin II effectively antagonized both the vascular and the steroidogenic effects of the endogenous an-

TABLE I. Means (±Standard Errors) of Several Factors Measured in Normal Dogs and in Dogs with Chronic or Malignant Experimental Hypertension.

	Mean Arterial pressure (mm. Hg)	Plasma renin activity (ng. Ang/ml)	Rectal Temp. (°F)	Serum urea nitrogen (mg%)	Plasma Na conc. (mEq/l)	Plasma K conc. (mEq/l)
Normal Dogs	119	6	101.6	14	145	4.7
($n = 6$)	±2	±1	±0.3	±2	±0.5	±0.1
Chronic Hypertensive	173	7	101.4	30	143	4.4
Dogs	±6	±2	±0.2	±9	±1.5	±0.2
($n = 3$) P[a]:	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
Malignant Hypertensive	170	42	98.9	228	135	6.8
Dogs	±8	±11	±0.5	±33	±3	±0.8
($n = 5$) P:	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05

[a] P-value when compared to the group of normal dogs by Student's t test for group comparisons. Not significant (N.S.) = $P > 0.05$.

giotensin II in the dog. In the present study, infusion of 1-sarcosine-8-alanine angiotensin II in normal conscious dogs did not alter appreciably either the arterial pressure or the PRA, which indicates that this angiotensin analog had little or no agonistic action on the vascular receptor in this species; similar results were obtained earlier in normal, anesthetized dogs (2).

Infusion of the angiotensin II analog in dogs with malignant hypertension, however, resulted in a prompt and significant lowering of arterial pressure. These results provide strong evidence that increased activity of the renin–angiotensin system is a significant factor in maintaining the elevated arterial pressure in this type of experimental hypertension. However, it is of interest that the average arterial pressure of 137 mm Hg observed during infusion of the angiotensin analog in these experiments was substantially higher than the prehypertensive pressures, which averaged 110 mm Hg in these dogs; this suggests that some other factor in addition to be increased, angiotensin II is acting to elevate the arterial pressure in malignant renal hypertension. The rise in PRA from already elevated levels to yet higher values during infusion of the angiotensin antagonist in these experiments could have resulted from the decrease in arterial pressure, or could have occurred as a result of an antagonism of the direct inhibitory effect which angiotensin II has on renin release (7, 8); similar results were observed during infusion of this analog in dogs with caval constriction and in sodium-depleted dogs (2, 6).

Results similar to those observed in the present study were reported recently by Pals and Masucci (3), who infused 1-sarcosine-8-alanine-angiotensin II into five malignant hypertensive dogs and three dogs with chronic hypertension. Bumpus *et al.* (9) recently reported that infusion of the angiotensin II analog 1-sarcosine-8-isoleucine angiotensin II into dogs with renal artery stenosis and hypertension of less than one week duration resulted in a decrease in arterial pressure; in contrast, two dogs with hypertension of 10 and 15 days duration failed to show this response. In the present study, infusion of the angiotensin analog, 1-sarcosine-8-alanine-angiotensin II in dogs with renal artery stenosis and chronic hypertension of 2–7 weeks duration failed to produce any decrease in arterial pressure; these results suggest that angiotensin II is not responsible for mainte-

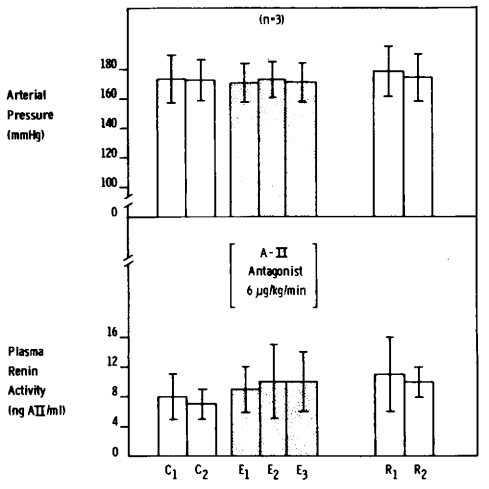

FIG. 1. Effects of the intravenous infusion of 1-sarcosine-6-alanine-angiotensin II on arterial pressure and plasma renin activity in conscious dogs with chronic renovascular hypertension. The abbreviations C_1, C_2, E_1, E_2, and E_3 represent two control and three experimental periods respectively, each of 15 min duration. R_1 and R_2 represent two recovery periods of 15 min each. All renin samples were obtained sequentially at 15 min intervals except R_1 was obtained 60 min after E_3.

nance of elevated arterial pressure in chronic renal hypertension in the dog. Thus, it seems probable that following renal artery constriction in the dog, the acute elevation in arterial pressure which occurs during the first week is influenced by an elevated plasma level of angiotensin II; however, the chronic hypertension which develops after the second week is the result of mechanisms other than increased angiotensin II. What role, if any, the increased plasma level of angiotensin II during the acute phase plays in the genesis of the chronic hypertension is unknown.

Studies in the rat have revealed that in chronic hypertension produced by constricting one renal artery and removing the opposite kidney infusion of 1-sarcosine-8-alanine angiotensin II failed to lower the arterial pressure (1, 10); however, in chronic hypertensive rats with one renal artery constricted and the contralateral kidney intact infusion of this angiotensin II antagonist resulted in a decrease in arterial pressure (10). In one-kidney rabbits made hypertensive by renal artery constriction, those rabbits that developed a normal renin chronic hypertension did not show a decrease in arterial pressure during infusion of 1-sarcosine-8-alanine angiotensin II, but in those rabbits that developed a high-renin form of hypertension infusion of this angiotensin II analog resulted in a pronounced fall in arterial pressure (11). Thus it appears that in the dog, as in the rat and the rabbit, when the experimental renal hypertension is associated with elevated values for plasma renin activity plasma angiotensin II plays a direct role in maintaining the hypertension, probably by acting directly on the smooth muscles of the arterioles. On the other hand, in the experimental renal hypertension that occurs with normal levels of plasma renin activity, the plasma angiotensin II apparently does not contribute to maintaining the elevated arterial pressure, and thus other mechanisms must be involved.

Summary. An angiotensin II antagonist, 1-sarcosine-8-alanine-angiotensin II, was infused intravenously into conscious dogs with experimental hypertension produced by renal artery constriction and unilateral nephrectomy. In dogs with malignant hypertension and elevated plasma renin activity, a decrease in arterial pressure was observed during infusion of the angiotensin II analog. However, in dogs that developed chronic hypertension of 2–7 weeks duration no decreases in arterial pressure occurred during infusion of this angiotensin II antagonist. Similarly, infusion of this compound into normal conscious dogs did not decrease arterial

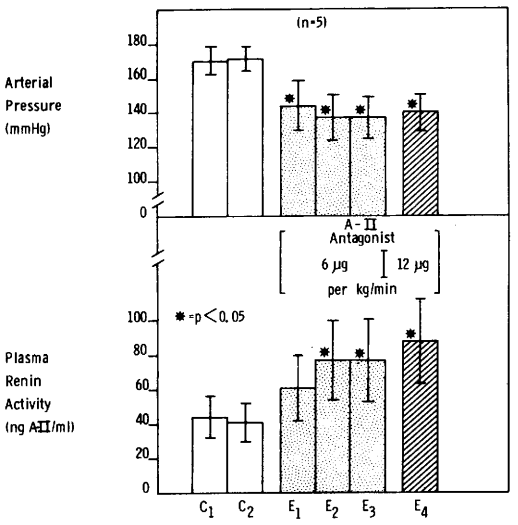

FIG. 2. Effects of the intravenous infusion of 1-sarcosine-8-alanine-angiotensin II on arterial pressure and plasma renin activity in conscious dogs with malignant renovascular hypertension. See Fig. 1 for abbreviations. All renin samples were obtained sequentially at 15 min intervals.

pressure. It is suggested that angiotensin II acts on receptors in arteriolar smooth muscle to increase peripheral resistance and arterial pressure in dogs with malignant hypertension, but chronic hypertension in the dog is maintained by other mechanisms.

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