PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 560–564 (1976)

Role of the Renin-Angiotensin System in Dogs with Perinephritis Hypertension[1] (39440)

J. ALAN JOHNSON, DAVID STUBBS, AND W. FORD KEITZER

Research Service, Harry S Truman Memorial Veterans Administration Hospital and Departments of Physiology and Surgery, University of Missouri, School of Medicine, Columbia, Missouri 65201

The development of analogs of angiotensin II which effectively antagonize the vascular actions of angiotensin II has provided a means of evaluating the role of angiotensin II in maintaining the arterial pressure in various hypertensive models. The compound 1-sarcosine-8-alanine angiotensin II has been shown to be an effective competitive antagonist to angiotensin II in a variety of animal species, including the dog (1). The present study utilized this angiotensin antagonist to investigate the role of the renin–angiotensin system in maintaining the elevated arterial pressure in dogs with experimentally induced perinephritis hypertension.

Materials and methods. Nine mongrel, female dogs, ranging 18 to 23 kg in body weight, were used in these studies. All dogs were fed a standard diet which provided a daily intake of 65 mEq of sodium and 55 mEq of potassium. Water was available *ad libitum*. Arterial pressures were routinely measured three times each week by placing the conscious dog supine on a table and puncturing the femoral artery with a 22 gauge syringe needle attached to a pressure transducer; the arterial pressures were recorded on a Sanborn recorder. Only those dogs that consistently had control arterial pressures of less than 130 mm Hg were used in these studies.

The dogs were divided into two groups. The first group of five dogs was the normotensive control group. The second group of dogs consisted of those in which hypertension was produced by cellophane perinephritis. Each dog in the hypertensive group was anesthetized with sodium pentobarbital, 35 mg/kg body weight iv, and the left kidney was exposed by a flank incision with sterile surgical procedures. A sheet of cellophane (no. 215 PD, E. I. DuPont de Nemours and Co.) was placed around the kidney and was held in place by a silk suture tied loosely around the renal hilus. The incision was closed and the animal was allowed to recover. Two weeks later each dog was again anesthetized as before and the right kidney was removed through a flank incision by sterile surgical procedures. After recovery from surgery the routine measurements of arterial pressures were resumed.

After approximately 2 months, when the hypertension was well established, or after about 3 weeks of control arterial pressure measurements in the normotensive group, each dog was again anesthetized and a catheter of polyvinyl chloride (Fr No. 5) was inserted into the carotid artery toward the heart, for measurement of arterial pressure. The incision was closed, with the end of the catheter sutured just beneath the skin. Three to five days later the experiment was performed.

On the morning of the experiment the dog's rectal temperature was measured. A polyethylene catheter (PE 50) was inserted percutaneously into the saphenous vein for the administration of angiotensin II or the angiotensin II analog. The end of the arterial catheter was exposed and was connected by means of an extension tube to a pressure transducer for recording mean arterial blood pressure. Throughout the experiment the conscious dog was lying on the floor of the laboratory, tied loosely to a table leg, and appeared to be undisturbed by the procedures.

After the dog was completely prepared for the experiment and was lying calmly for 30 min, a 6 ml blood sample for plasma renin activity was obtained through the arterial catheter and was placed in a tube containing 0.06 ml of a 10% solution of ethylenediaminetetraacetate (EDTA); a 6 ml arterial

[1] Supported by USPHS Grant No. HL 17366.

blood sample was also collected for serum urea nitrogen and plasma concentrations of sodium and potassium. The arterial blood pressure was recorded continuously while 2.5 μg of angiotensin II (1-asparagine-5-isoleucine angiotensin II, Schwarz-Mann, Inc.) in 1 ml of isotonic saline was injected iv through the venous catheter. After the pressor response had subsided the arterial pressure was recorded continuously for two periods of 15 min each. The angiotensin II analog 1-sarcosine-8-alanine angiotensin II was then infused iv at a rate of 6 μg/min/kg of body weight for 45 min; the analog was dissolved in isotonic saline and was infused through the saphenous catheter by means of a variable-speed syringe pump at a flow of approximately 0.5 ml/min. During infusion of the angiotensin II analog the mean arterial blood pressure was recorded continuously. Additional blood samples for plasma renin activity were obtained after 15, 30, and 45 min of analog infusion. Immediately after stopping the analog infusion the pressor response to 2.5 μg of synthetic angiotensin II was again recorded. One hour after stopping the infusion of the angiotensin II analog the mean arterial blood pressure was again recorded and a final blood sample for plasma renin activity was obtained.

The blood samples for plasma renin activity were centrifuged in a cold environment (3°) and the plasma samples obtained were stored frozen. The renin determinations involved processing the plasma samples for the generation of angiotensin I by the method of Schneider *et al.* (2). The angiotensin concentration was determined by bioassay in the pentolinium-blocked, pentobarbital-anesthetized rat, with changes in arterial pressure as the response parameter; synthetic angiotensin II (Hypertensin-Ciba) was used as a standard. Plasma renin activity was expressed as nanograms of angiotensin generated by 3 hr of incubation per milliliter of plasma. All samples were assayed in triplicate and the average of the three determinations was the value recorded. Plasma concentrations of sodium and potassium were determined by flame photometry. Serum urea nitrogen determinations were performed by the clinical chemistry laboratory of the University Hospital.

Results. All four dogs in the hypertensive group developed a pronounced increase in arterial pressure. The mean arterial pressure in this group averaged 170 ± 11 (SEM) mm Hg as compared to the average mean arterial pressure of 100 ± 10 mm Hg for the five normotensive dogs. The arterial pressures in these two groups of dogs were significantly different ($P < 0.01$) when analyzed by Student's t test for group comparisons (3). The values for rectal temperature, serum urea nitrogen, and plasma concentrations of sodium and potassium, which are summarized in Table I, were the same in these two groups of animals.

The effect of the angiotensin II analog on mean arterial pressure in the normotensive dogs is summarized in Fig. 1. Infusion of the angiotensin antagonist in these dogs resulted in a slight initial increase in arterial pressure of 5–10 mm Hg, which returned to the preinfusion levels before 5 min of infusion. After the initial pressor response had subsided, no further changes in arterial pressure were observed during the 45 min of analog infusion. One hour after stopping the infusion of the analog the arterial pressure was still unchanged. Infusion of the angiotensin II antagonist into dogs with cellophane perinephritis hypertension resulted in a pronounced initial rise in arterial pressure

TABLE I. VALUES FOR RECTAL TEMPERATURE, SERUM UREA NITROGEN, AND PLASMA CONCENTRATIONS OF SODIUM AND POTASSIUM IN NORMAL DOGS AND IN DOGS WITH PERINEPHRITIS HYPERTENSION.[a]

	Rectal Temp. (°F)	Serum urea nitrogen (mg%)	Plasma Na concentration (mEq/liter)	Plasma K concentration (mEq/liter)
Normal dogs (n = 5)	101.9 ± 0.3	14 ± 2	145.2 ± 0.7	4.5 ± 0.2
Perinephritis hypertensive dogs (n = 4)	101.5 ± 0.2	17 ± 3	143.0 ± 0.6	4.3 ± 0.1

[a] Values are means ± SEM. There were no statistical differences between the two groups when compared by Student's t test.

which did not subside nearly as rapidly as in the normotensive dogs (see Fig. 2). Comparing the arterial pressure after 15, 30, and 45 min of analog infusion with the arterial pressure during the control period by Student's *t* test for paired observations revealed no statistically significant differences at any of these time periods. The arterial pressure also was unaltered 60 min after stopping the infusion of the analog. In no instance did a fall in arterial pressure occur with angiotensin II analog infusion in the hypertensive dogs. Prior to infusion of the analog the arterial pressure was stable in both groups of dogs, as indicated by the averages for the mean arterial pressures during the two control periods. The iv injection of 2.5 μg of angiotensin II during the control period produced a rise in mean arterial pressure averaging 78 and 71 mm Hg for the control and hypertensive dogs, respectively; at the termination of the analog infusion this dose of angiotensin II did not result in any changes in arterial pressure.

The average values for plasma renin activity are given in Table II. During the control period the values for plasma renin activity were similar in both groups of dogs, and no changes in plasma renin activity occurred during infusion of the angiotensin analog in either group.

Discussion. In recent years several analogs of angiotensin II have been developed which will antagonize the effects of angiotensin II in biological systems. The ability of the compound 1-sarcosine-8-alanine angiotensin II to block the vascular action of angiotensin II was first reported by Pals *et al.* (4) who studied the effect of this compound on the rabbit aortic strip *in vitro* and *in vivo* in the rat. Studies on dogs by this laboratory (5) have shown that infusion of this angiotensin analog into two preparations with elevated levels of plasma renin activity and increased aldosterone secretion, i.e., sodium-depleted dogs and dogs with a constriction of the thoracic inferior vena cava, resulted in a fall in arterial pressure and a decrease in the adrenal secretion rate of aldosterone; these results indicated that

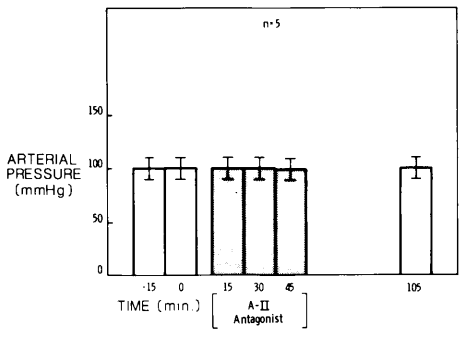

FIG. 1. Effect of iv infusion of 1-sarcosine-8-alanine angiotensin II, at 6 μg/min/kg of body weight, on arterial pressure in five normotensive dogs.

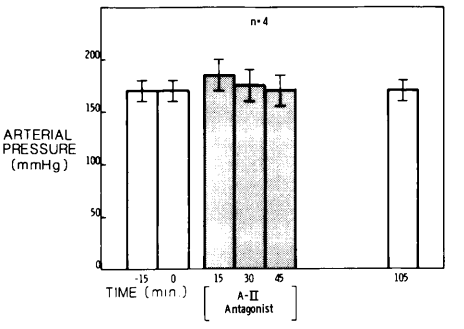

FIG. 2. Effect of iv infusion of 1-sarcosine-8-alanine angiotensin II, at 6 μg/min/kg of body weight, on arterial pressure in four dogs with cellophane perinephritis hypertension.

TABLE II. EFFECT OF THE INFUSION OF THE ANGIOTENSIN II ANALOG 1-SARCOSINE-8-ALANINE ANGIOTENSIN II ON PLASMA RENIN ACTIVITY IN NORMAL DOGS AND IN DOGS WITH PERINEPHRITIS HYPERTENSION.[a]

		Minutes of infusion of analog			
	Control	15 min	30 min	45 min	Recovery
Normal dogs (n = 5)	5.9 ± 0.8	6.8 ± 1.2	5.6 ± 1.3	6.4 ± 1.5	6.1 ± 1.0
Perinephritis hypertensive dogs (n = 4)	5.4 ± 1.0	5.5 ± 1.4	5.0 ± 1.4	5.5 ± 1.0	5.5 ± 1.3

[a] Values are means ± SEM. Renin values are nanograms of angiotensin generated during 3 hr incubation, per milliliter of plasma.

this compound blocked both the vascular and the steroidogenic effects of endogenously produced angiotensin II in the dog. This compound has been used to study the role of angiotensin II in maintaining the elevated arterial pressure in dogs with experimental hypertension produced by renal artery stenosis and contralateral nephrectomy. Dogs prepared in this way that developed a chronic hypertension with normal levels of plasma renin activity did not show a reduction in arterial pressure during infusion of 1-sarcosine-8-alanine angiotensin II (6, 7); however, in those dogs that developed a malignant form of hypertension with elevated levels of plasma renin activity, the infusion of this analog resulted in a reduction in arterial pressure. Similar results have been reported by Sweet *et al.* (8) for dogs with renal artery stenosis and chronic hypertension receiving another angiotensin antagonist, 1-sarcosine-8-isoleucine angiotensin II.

Although the production of experimental renal hypertension by cellophane perinephritis in animals was first reported by Page in 1939 (9) and this renal hypertensive model has been studied by many investigators, the mechanisms whereby this procedure results in an elevated arterial pressure is still unknown. Studies reported by Ferrario *et al.* (10) showed an increase in cardiac output early during the course of the development of cellophane-perinephritis hypertension dogs; these workers postulated that the rise in cardiac output resulted from an increased venous return due to increased venous tone. Mogil *et al.* (11) found plasma renin activity to be normal or reduced in dogs with chronic hypertension produced by cellophane perinephritis, and concluded that the hypertension was not maintained by increased activity of the renin–angiotensin system. The present study likewise found normal values for plasma renin activity in dogs with perinephritis hypertension. Furthermore, the infusion of the angiotensin II antagonist 1-sarcosine-8-alanine angiotensin II into these hypertensive dogs did not produce a lowering of the arterial pressure in any of these hypertensive dogs. Similar findings have been reported by Bumpus *et al.* (12) who infused the angiotensin antagonist 1-sarcosine-8-isoleucine angiotensin II into dogs with perinephritis hypertension. These findings provide strong evidence that the renin–angiotensin system is not involved in maintaining the elevated arterial pressure in dogs with chronic hypertension produced by cellophane perinephritis.

The slight pressor response of 5–10 mm Hg seen initially during the infusion of the angiotensin analog in the normotensive dogs has been reported previously by this laboratory (5) and has also been observed by other workers (7). Also, dogs with renal artery stenosis and hypertension similarly exhibit only a slight pressor response initially during the administration of this compound (6). Presumably this pressor response is mediated by catecholamines, since it is well established that angiotensin II will stimulate the release of adrenal medullary catecholamines (13–15), and an aromatic C-terminal amino acid is not necessary for this effect of angiotensin II (16). Also, Muñoz-Ramírez *et al.* (17) found that the initial pressor response to 1,8-substituted angiotensin II analogs in rats was attenuated by adrenalectomy or by alpha-adrenergic blocking agents. This leads to speculation that dogs with cellophane perinephritis hypertension may release more adrenal medullary catecholamines in response to angiotensin than do normal dogs or dogs with renal artery stenosis and hypertension, or that in dogs with cellophane perinephritis hypertension there may be a greater vascular reactivity to catecholamines.

Summary. Cellophane perinephritis hypertension was produced in four dogs, while five additional dogs served as normotensive controls. A competitive antagonist of angiotensin II, 1-sarcosine-8-alanine angiotensin II, was infused iv into these conscious dogs at a rate of 6 μg/min/kg of body weight for 45 min. Arterial pressure averaged 170 $\pm$ 11 (SEM) mm Hg in the dogs with perinephritic hypertension, and was not altered significantly during infusion of the angiotensin antagonist. In the normal dogs the arterial pressure averaged 100 $\pm$ 10 mm Hg and likewise, did not change during administration of the angiotensin analog. Plasma renin activity values were essentially the same in these two groups of dogs and did not change

during infusion of the angiotensin antagonist. These studies provide strong evidence that the renin–angiotensin system is not involved in maintaining the elevated arterial pressure in dogs with chronic hypertension produced by cellophane perinephritis.

The authors gratefully thank Dr. A. W. Castellion of Norwich Pharmacal Company for supplying the 1-sarcosine-8-alanine angiotensin II used in these studies. The cellophane used to produce perinephritis hypertension was supplied by E. I. DuPont de Nemours and Company. Mr. Charles Payne assisted in the surgical procedures and the assays for plasma renin activity were performed by Ms. Jane Green.

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Received January 7, 1976. P.S.E.B.M. 1976, Vol. 152.