PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 259-263 (1977)

Hemodynamic Effects of an Angiotensin II Antagonist in Rabbits with Perinephritis Hypertension[1] (39785)

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Studies of experimental renal hypertension in rats (1) and dogs (2, 3) have indicated that increases in cardiac output may play an important role in the development of hypertension. Studies of human renovascular hypertension have indicated that the total peripheral resistance (TPR) is increased while the cardiac output is either elevated or reduced (4-6). In a recent report from this laboratory (7) infusion of the angiotensin II antagonist, 1-sarcosine-8-alanine angiotensin II, into dogs with cellophane perinephritis hypertension failed to lower the arterial pressure, which provided strong evidence that the renin-angiotensin system was not involved in maintaining the elevated arterial pressure. This report also pointed out that a rise in arterial pressure occurred initially during infusion of the angiotensin II analog, and that this rise in pressure was greater than in either normotensive dogs or in dogs with hypertension produced by renal artery stenosis. The purpose of the present study was to examine the role of the renin-angiotensin system in cellophane perinephritis hypertension in another animal species, the rabbit. This study also investigated the influence of the renin-angiotensin system on cardiac output and TPR in this form of hypertension.

Methods. Eleven male New Zealand rabbits, weighing 2.5 to 3.5 kg, were used in these studies. All rabbits were fed a commercial rabbit diet (Purina rabbit chow) which contained 0.168 mEq of Na and 0.467 mEq of K per gram. Water was available *ad libitum*.

The rabbits were divided into two groups. One group of five rabbits served as normotensive, unoperated controls; cellophane perinephritis hypertension was produced in the other group of six rabbits. The rabbits in the latter group were anesthetized with halothane (5% with nitrous oxide). With sterile surgical procedures, the left kidney was exposed by an abdominal incision; the kidney was decapsulated and was wrapped in a sheet of cellophane (215-PD, E. I. DuPont de Nemours and Co.) which was tied loosely around the renal hilus. Two weeks later each rabbit was again anesthetized as before and the right kidney was removed. The experiments were performed 4 weeks after the nephrectomy. On the day prior to an experiment each rabbit was anesthetized with halothane and nitrous oxide, and catheters of polyvinyl tubing (Fr. 5 infant feeding tubing) were placed in the jugular vein and in the femoral artery and vein. The rabbit was then placed in a rectangular box small enough to limit his movement, and he remained in the box until the conclusion of the experiment. The following morning a catheter (PE 50 tubing) was placed in the marginal ear vein for infusion of the angiotensin II antagonist. All experiments were performed on conscious, unanesthetized rabbits.

The end of the femoral arterial catheter was connected by a three-way stopcock to a densitometer cuvette (Model DC-410, Waters Instrument Co.) for determination of the concentration of indocyanine green in the blood; the cuvette was attached to a Model TD-1 densitometer (Waters Instrument Co.). The distal end of the cuvette was fastened to a 60-cm length of polyvinyl tubing (2.5-mm i.d.) which was passed through

[1] Supported by the Medical Research Service of the Veterans Administration, and by USPHS Grant HL 17366.

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ISSN 0037-9727

a roller pump (Holter pump, Model 911, Extracorporeal Medical Specialties) and back to the femoral venous catheter. The pump circuit was filled with sterile isotonic saline prior to connecting it to the rabbit; the volume of the tubing in the circuit was 3.5 ml. The stopcock on the arterial catheter was also attached to a pressure transducer (Statham P23Db) so that either the arterial pressure or the cardiac output could be determined by turning the stopcock. The arterial pressure and the dye concentration were recorded on a Hewlett-Packard recorder. At the start of the experiment a 2-ml sample of arterial blood was obtained; 1 ml was placed in a tube containing ethylenediaminetetraacetate (EDTA) in an ice bath, for the determination of plasma renin activity (PRA), and the remainder was placed in a clot tube for serum urea nitrogen determination. Each rabbit then received 3000 units of heparin iv. Mean arterial blood pressure was recorded for 15 min, then the heart rate was determined by recording the pulsatile pressure for 20 s at a fast paper speed. Control values for cardiac output also were obtained. For cardiac output determinations a 0.2-ml sample of indocyanine green dye (Cardio-green; Hynson, Westcott, and Dunning, Inc.) with a dye concentration of 2.5 mg/ml was placed in a jugular venous catheter. While the arterial blood was pumped through the densitometer cuvette at a rate of 10.0 ml/min the dye was flushed through the catheter with 0.7 ml of isotonic saline. The area under the resulting dye concentration curve exclusive of recirculation was determined and the cardiac output was calculated by dividing the amount of dye injected by the minute-concentration of dye in the arterial blood. All cardiac output measurements were made in triplicate and the average of the three measurements was accepted as the cardiac output value. Individual cardiac output measurements varied by an average of less than 4%.

A test dose of synthetic angiotensin II (Schwarz-Mann, Inc.) was injected iv and the pressor response was recorded; 300 ng of angiotensin II was the test dose for the normotensive rabbits and 100 ng of angiotensin II was used in the hypertensive rabbits. After the pressor response had subsided, the angiotensin II antagonist, 1-sarcosine-8-alanine angiotensin II (Saralasin, Norwich Pharmacal Co.), was infused iv at a rate of 6 μg/min/kg body weight in normal saline at 0.1 ml/min for 30 min; during infusion of the angiotensin analog the mean arterial pressure was recorded continuously. After the 30 min of analog infusion the test dose of angiotensin II was administered again and the pressor response was recorded. Cardiac output and heart rate measurements were obtained at this time.

The plasma and serum samples for PRA and serum urea nitrogen were stored frozen until they were analyzed. PRA was determined by radioimmunoassay for generated angiotensin I, as described by Cohen *et al.* (8). Serum urea nitrogen determinations were performed as described by Fawcett and Scott (9).

Results. The values (means ± SEM) for mean arterial pressure, cardiac output, TPR, heart rate, stroke volume, PRA, and serum urea nitrogen for both the cellophane perinephritic and the normotensive control groups of rabbits are given in Table I. Cardiac output data are reported as milliliters per minute per kilogram body weight; stroke volume was calculated by dividing the cardiac output by the heart rate. The TPR values are expressed in the arbitrary units that result from dividing the mean arterial pressure in millimeters of Hg by the cardiac output in milliliters per minute per kilogram. Values for PRA are in nanograms of generated angiotensin I per hour per milliliter of plasma.

Mean arterial pressure in the cellophane perinephritis group of rabbits averaged 125 ± 6 (SEM) mm Hg, which was significantly ($P < 0.01$) higher than the mean arterial pressure of 87 ± 2 for the normotensive control group when tested by Student's t test for group observations (10). Cardiac output was significantly ($P < 0.01$) lower in the perinephritic rabbits, averaging 167 ± 12 ml/min/kg, while the cardiac output in the normotensive group averaged 263 ± 27; TPR values were significantly different ($P < 0.01$) for the two groups, averaging 0.79 ± 0.08 and 0.34 ± 0.04 for the hypertensive and normotensive groups, respectively. Heart rate and stroke volume were slightly

TABLE I. EFFECT OF AN ANGIOTENSIN II ANTAGONIST ON NORMOTENSIVE AND PERINEPHRITIS HYPERTENSIVE RABBITS.[a]

Parameter measured	Normotensive rabbits		Hypertensive rabbits	
	Before A-II antagonist	During A-II antagonist	Before A-II antagonist	During A-II antagonist
Cardiac output (ml/min/kg)	263 ± 27	261 ± 27	167 ± 12*	167 ± 12*
Arterial pressure (mm Hg)	87 ± 2	82 ± 2	125 ± 6*	120 ± 9*
Total peripheral resistance	0.34 ± 0.04	0.33 ± 0.04	0.79 ± 0.08*	0.77 ± 0.08*
Heart rate (beats/min)	256 ± 30	305 ± 22**	234 ± 23	292 ± 32
Stroke volume (ml/beat/kg)	1.09 ± 0.17	0.89 ± 0.14**	0.73 ± 0.04	0.61 ± 0.08
Plasma renin activity (ng/ml/hr)	5.7 ± 1.2		1.4 ± 0.3*	
Serum urea nitrogen (mg%)	21 ± 3		26 ± 3	

[a] Values are means ± SEM.

* $P < 0.01$ when compared with the corresponding value for normotensive rabbits by Student's t test for group observations.

** $P < 0.05$ when compared with the same group of rabbits prior to infusion of the antagonist, by Student's t test for paired observations.

less in the hypertensive group, but the values were not significantly different. PRA in the cellophane perinephritic group of rabbits averaged 1.4 ± 0.3 ng of angiotensin I/ml/hr, which was significantly ($P < 0.01$) less than the PRA of 5.7 ± 1.2 for the normotensive rabbits. Serum urea nitrogen levels were the same in the two groups.

Effect of infusion of the angiotensin II antagonist. Administration of the angiotensin II analog produced an increase in arterial pressure which was maximal after about 3 min of infusion. This rise in mean arterial pressure averaged 7 ± 1 mm Hg for the normotensive group and 15 ± 2 mm Hg for the hypertensive group of rabbits, values which were significantly different ($P < 0.05$). After 30 min of analog infusion the arterial pressure averaged 82 ± 2 and 120 ± 9 mm Hg for the normotensive and hypertensive groups respectively; these values were not significantly different from the preinfusion values when compared by Student's t test for paired observations (10). These data are summarized in Figs. 1 and 2. Infusion of the angiotensin II analog did not alter the cardiac output or the TPR in either group of rabbits. The heart rate increased and the stroke volume decreased as a result of infusion of the angiotensin antagonist, but these changes were statistically significant ($P < 0.05$) only for the normotensive group. Prior to the infusion of the angiotensin antagonist the test doses of angiotensin II produced pressor responses averaging 13 ± 3 mm Hg for the normotensive group and 17 ± 2 mm Hg for the hypertensive group; at the termination of the analog infusion the responses to these test doses were completely blocked.

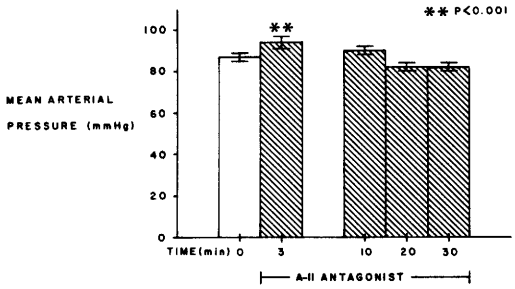

FIG. 1. Effect of infusion of angiotensin II (A-II) antagonist, 1-sarcosine-8-alanine angiotensin II, on mean arterial pressure in five normotensive rabbits. Values are means ± SEM.

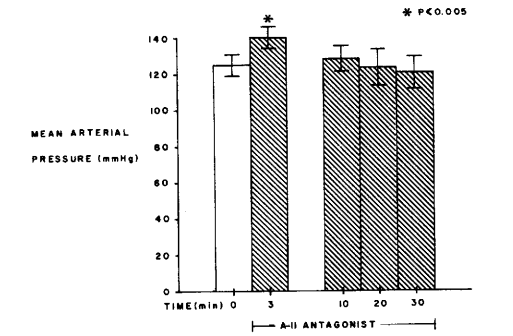

FIG. 2. Effect of infusion of angiotensin II (A-II) antagonist, 1-sarcosine-8-alanine angiotensin II, on mean arterial pressure in six perinephritis hypertensive rabbits. Values are means ± SEM.

Discussion. Cellophane perinephritis as a

model of hypertension was first described by Page (11) in 1938. The mechanisms involved in producing the elevated arterial pressure in this model, however, still are not understood. Ferrario *et al.* (2, 3) reported that the hemodynamic changes in dogs with perinephritis hypertension were similar to those in dogs with hypertension produced by renal artery stenosis. In both models the hypertension initially was associated with an increase in both the cardiac output and TPR; in later stages the cardiac output returned toward control levels and the elevated blood pressure was sustained by a progressive further increase in TPR. Similar hemodynamic changes have been observed in rats (1). In human renovascular hypertension there is an increase in TPR and the cardiac output is frequently increased, but may be reduced (4–6). In the present study the cardiac output was significantly reduced and the TPR was greatly increased in the rabbits made hypertensive by cellophane perinephritis of 30-days duration. The decreased cardiac output resulted from the combination of a decrease in heart rate and in stroke volume, but the mechanisms producing these decreases are not known. Recently, Simon *et al.* (12) reported observing a decrease in venous compliance in dogs with chronic perinephritis hypertension; they also suggested that the return of the cardiac output to normal in the chronic stage of hypertension was due to mechanisms other than the return of venous compliance to normal. On the other hand, Averill *et al.* (13), in studies of rats with chronic renal hypertension, found that cardiac function was impaired and suggested that this might be related to either diminished cardiac contractility or reduced left ventricular compliance, even though cardiac output was normal. For the present study it can be speculated that the reduced cardiac output in the hypertensive rabbits could have been the result of an impairment in cardiac function related to these two factors. It is obvious, however, that in rabbits with chronic hypertension produced by cellophane perinephritis, increased cardiac output did not contribute to the elevated arterial pressure, and the hypertension was maintained solely by increased TPR.

Earlier studies have demonstrated that immunization of rabbits to angiotensin II did not prevent the development of hypertension following renal artery stenosis or perinephritis (14–16). Also, previous work from this laboratory (17) has shown that in rabbits with renovascular hypertension due to renal artery stenosis, when the PRA was normal the infusion of the angiotensin II antagonist, 1-sarcosine-8-alanine angiotensin II, did not lower the arterial pressure. Similar results also have been observed in dogs with perinephritis hypertension (7). In the present study the cardiac output was measured in addition to arterial pressure; this allowed us to evaluate the effect of angiotensin II antagonism on vascular resistance in addition to its effect on arterial pressure, which was the only factor examined in earlier studies. Thus, these studies provided evidence that angiotensin II probably does not play a role in maintaining the increased peripheral vascular resistance responsible for the elevated arterial pressure in perinephritis hypertension in rabbits. The observation that the PRA was significantly lower in the rabbits with perinephritis hypertension than in the normotensive control rabbits is an additional argument against angiotensin II having a causative role in this hypertensive model. A similar suppression of PRA has been reported by others for rabbits (18) and dogs (19) with perinephritis hypertension.

Infusion of the angiotensin II analog resulted in an initial increase in the arterial pressure in both the normotensive and the perinephritic hypertensive rabbits, but the response was considerably greater in the hypertensive rabbits. Similar results have been reported for the infusion of this compound into dogs with perinephritis hypertension (7). In contrast, rabbits and dogs with hypertension produced by renal artery stenosis did not have more of an initial increase in arterial pressure during infusion of the angiotensin II analog than did the normotensive animals (17, 20). The initial pressor effect of this angiotensin II analog is thought to be due in part to an agonistic action of the analog and in part to the release of catecholamines from the adrenal medulla (21), because it is known that angiotensin II will

stimulate the release of catecholamines from the adrenal medulla (22) and an aromatic C-terminal amino acid is not necessary for this effect of angiotensin II (23). Also, the initial pressor response to 1,8-substituted angiotensin II analogs in rats is attenuated by adrenalectomy or by α-adrenergic blocking agents (21). Therefore, the greater initial pressor response of the perinephritic hypertensive rabbits to the analog may have been due to a greater release of adrenal catecholamines or to an increased reactivity of the vasculature to catecholamines. Increased pressor responsiveness to catecholamines in experimental renal hypertension has been reported previously (24). In the present study the hypertensive rabbits also exhibited a much greater pressor response to the test dose of angiotensin II than did the normotensive rabbits, which necessitated using a lower dose of angiotensin II in the hypertensive animals.

Summary. Rabbits with cellophane perinephritis and hypertension had lower cardiac output values than normotensive rabbits, and the elevated arterial pressure was maintained by pronounced increases in total peripheral resistance. Plasma renin activity was decreased in the perinephritic hypertensive rabbits compared to the values in normotensive controls. Infusion of the angiotensin II antagonist, 1-sarcosine-8-alanine angiotensin II, failed to lower the arterial pressure or to decrease the peripheral vascular resistance in the perinephritic hypertensive rabbits, which was a strong indication that angiotensin II was not involved in maintaining the hypertension.

The excellent technical assistance of Ms. Omalou McBride and Mr. Jerry Benedict was sincerely appreciated. The authors are extremely grateful to Dr. Alan W. Castellion of Norwich Pharmacal Co. for providing the 1-sarcosine-8-alanine angiotensin II (Saralasin) used in these experiments. The cellophane used in these studies was supplied by E. I. du Pont de Nemours and Co. The indocyanin green dye (Cardio-green) was generously provided by Dr. T. R. Carski, of Hynson, Westcott and Dunning Pharmaceutical Laboratory. The anti-angiotensin I antiserum used in the radioimmunoassay for renin was provided by Dr. E. L. Cohen, of the University of Michigan School of Medicine.

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Received September 10, 1976. P.S.E.B.M. 1977, Vol. 155.