Cardiac Effects of Angiotensin II

By EUGENE E. VOGIN* and JOSEPH P. BUCKLEY

The injection of angiotensin II, 1.0 mcg./Kg., into pentobarbital anesthetized dogs produced a significant increase in systolic and diastolic blood pressures and myocardial blood flow, a slight increase in the force of right ventricular contraction, and no significant effects on heart rate. Cardiac output was initially decreased; but within 80 to 100 seconds after angiotensin II administration, a secondary rise was noted. The rise in diastolic pressure was significantly greater than that of the systolic pressure at the 5 per cent level. The coronary flow increase appeared to be dependent entirely on the increase in diastolic blood pressure. In coronary perfusion studies, angiotensin II produced myocardial vasoconstriction when injected either intravenously or intra-arterially. These effects were mediated by both direct musculotropic actions and the sympathetic nervous system since acute sympathectomy reduced, but did not abolish, the activity of angiotensin II. The sympathetic component of the cardiovascular effects of angiotensin II appear to be *via* the alpha receptors since nethalide markedly potentiated the activity of angiotensin II. The effect of pretreatment with phenoxybenzamine, atropine, or dichloroisoproterenol on the cardiovascular responses to angiotensin II was also evaluated.

THERE HAVE BEEN several conflicting reports concerning the effects of angiotensin II on coronary blood flow. Investigators have reported that angiotensin decreases coronary blood flow (1), produces coronary vasoconstriction (2, 3), produces a transient reduction in coronary flow followed by a transient increase in flow (4), increases coronary vascular resistance without altering coronary flow (5), produces no alteration in coronary flow (6), and produces an increase in coronary blood flow (7). This paper reports a group of studies designed to facilitate a better understanding of certain cardiovascular actions of angiotensin II.

EXPERIMENTAL

Mongrel dogs of either sex, weighing between 10.2 and 18.6 Kg., were anesthetized by an intravenous injection of pentobarbital sodium (35 mg./Kg.) and the trachea cannulated. Femoral arterial blood pressure was recorded with a Statham pressure transducer (model P23AC) following cannulation of the right femoral artery. Heart rate was determined from the blood pressure tracing.

Cardiac output flow measurements were performed utilizing an electromagnetic flowmeter (Medicon FM-6), described by Olmsted (8). The animals were placed on artificial respiration with a Harvard respirator (model 606) utilizing a mixture of 95% O_2 and 5% CO_2. The thoracic cavity was entered at the fourth intercostal space using electrocautery to minimize bleeding. The lungs were retracted and the pericardium incised and secured to the chest wall with wound clips to create a pericardial sac whereby the heart and aorta were easily accessible. The ascending aorta was carefully isolated from the surrounding tissue, and Medicon flo-probe (12 or 15 mm. i.d.) was placed around the vessel. The left common carotid artery was cannulated and connected to the inflow tubing of a Shipley-Wilson rotameter; a 7- to 10-mm. segment of the left anterior descending coronary artery was isolated for subsequent cannulation and the animal heparinized (1000 units/Kg. i.v.). The left descending coronary artery was perfused as described by Buckley *et al.* (9).

In another series of animals, flow to the cannulated coronary artery was maintained constant by a Sigmamotor pump (TM10) and changes in coronary perfusion pressures measured as described by Fowler and Holmes (2). Changes in ventricular force were estimated with a Walton-Brodie strain gauge arch (10) sutured to the right ventricle.

All physiological measurements were recorded on an Offner dynograph or Grass polygraph. Coronary vascular resistance was calculated from the formula of Wegria *et al.* (11)

$$\text{Resistance} = \frac{\text{mean blood pressure (mm. Hg)}}{\text{mean coronary flow (ml./minute)}}$$

Acute cardiac denervation was performed in two dogs according to the method described by Morrow *et al.* (12). This consisted of bilateral cervical vagotomy and excision of the stellate and upper four sympathetic ganglia. The following compounds were administered: synthetic angiotensin II,[1] 1.0 mcg./Kg.; phenoxybenzamine hydrochloride, 5.0 mg./Kg.; atropine sulfate, 0.25 mg./Kg.; dichloroisoproterenol hydrochloride (DCI), 5.0 mg./Kg.; and nethalide hydrochloride,[2] 5.0 mg./Kg.

STATISTICAL METHODS

Because of the wide variation in control values, all results were calculated as their percentage change from control, then analyzed statistically by the following methods. To compare the effects of angiotensin prior to surgical or drug treatment, the mean percentage difference was calculated and probabilities determined using the Student *t* test for

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[1] Kindly supplied as Hypertensin by Dr. W. Wagner, Ciba Pharmaceutical Co., Summit, N. J.

[2] Kindly supplied as Ay 6204 by Dr. A. Sahagian-Edwards, Ayerst Laboratories, New York, N. Y.

TABLE I.—EFFECTS OF ANGIOTENSIN (1 mcg./Kg.) ON CARDIAC ACTIVITY

	Systolic Pressure	Diastolic Pressure	Coronary Blood Flow	Heart Rate	Right Ventricular Force	Coronary[a] Perfusion Pressure	Cardiac Output
Intravenous Administration							
Mean % change	48	70	63	6	39	27	−23
S.E.[b]	4	6	8	5	4	3	3
N[c]	21	21	15	21	6	5	21
P	<0.001	<0.001	<0.001	>0.05	<0.001	<0.001	<0.001
Intra-arterial Administration							
Mean % change	40	72	...	3	37	33	−23
S.E.[b]	4	11	...	5	7	3	3
N[c]	6	6	...	6	6	6	5
P	<0.001	<0.001	...	>0.05	<0.01	<0.001	<0.001

[a] Flow to coronary bed was maintained constant by a Sigmamotor pump. [b] ± Standard error. [c] Number of animals.

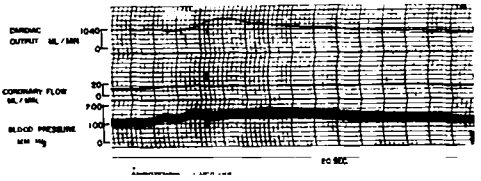

Fig. 1.—The effect of intravenous injection of angiotensin II (1 mcg./Kg.) on blood pressure, coronary flow, and cardiac output.

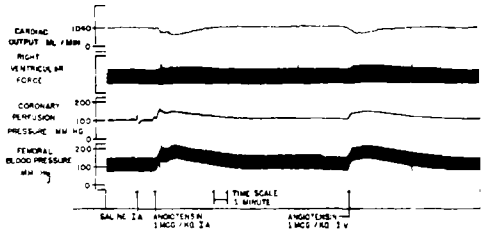

Fig. 2.—The effect of intra-arterial and intravenous injections of angiotensin II on blood pressure, coronary perfusion pressure, right ventricular force, and cardiac output.

paired results (13). To determine the effects of surgical sympathectomy or drug treatment on the response to angiotensin, the data were analyzed for homogeneity (13). All homogeneous results were then subjected to an analysis of variance (13); if significant differences existed, the means were compared using Harter's modification of Duncan's multiple-range test (14–16).

RESULTS

The effects of angiotensin II injected either intravenously or intra-arterially are summarized in Table I (Figs. 1 and 2). These results show that there were significant increases in systolic pressure, diastolic pressure, coronary blood flow, right ventricular force, and coronary perfusion pressure. The coronary flow increase occurred concomitantly with the increase in diastolic pressure throughout its entirety. Heart rate increased in several animals while in others it decreased; no definite pattern of effect could be established in these pentobarbital anesthetized animals when monitoring this parameter.

During the first minute following angiotensin injections, cardiac output decreased. However, in several animals cardiac output increased between the second and third minutes after injection. Coronary vascular resistance calculations showed that in the autoperfusion studies, angiotensin neither increased nor decreased this value. However, the increase in coronary perfusion pressure indicated that the myocardial vessels were constricted as a result of angiotensin administration. The rise in diastolic pressure was significantly greater than that of the systolic pressure at the 0.1% level following intravenous injections and at the 5% level following intra-arterial injections of angiotensin.

Systolic and Diastolic Blood Pressure.—Tables II and III depict the results of surgical sympathectomy or various drug pretreatments on the blood pressure responses to injected angiotensin. Only in the surgical sympathectomized animals were changes in the systolic blood pressure response noted. The reduction in responsiveness to angiotensin was significant at the 0.1% level.

Nethalide at a dose which blocked the chronotropic effect of isoproterenol (1 ml. of 10^{-6} solution) potentiated the rise in diastolic blood pressure to an extent greater than any of the other treatments. This potentiation was significant at the 0.1% level following intravenous angiotensin. Neither surgical sympathectomy nor other drug pretreatments were able to alter significantly the diastolic blood pressure response to intravenous angiotensin; nor did nethalide significantly alter the response to intra-arterial injections of this polypeptide. As in the untreated animals, the increase in diastolic pressure was significantly greater than that of the systolic pressure in the sympathectomized, atropine pretreated, and nethalide pretreated animals at the 1% level and at the 5% level in the DCI pretreated animals. Phenoxybenzamine pretreated animals did not show this greater increase in diastolic pressure.

Coronary Blood Flow, Coronary Vascular Resistance, and Coronary Perfusion Pressure.—Nethalide potentiated the effect of angiotensin in increasing coronary blood flow to an extent greater than any of the other treatments (Tables II and III). The increase in coronary blood flow following this agent differed from the nontreated animals at the 0.1% level. None of the other agents altered the coronary blood flow response to angiotensin significantly. It should be noted that in the surgically sympathectomized animals, the coronary blood flow response to angiotensin was significantly reduced ($p < 0.001$) when compared to the nontreated animals.

TABLE II.—EFFECT OF SURGICAL SYMPATHECTOMY OR DRUG PRETREATMENT ON THE CARDIOVASCULAR RESPONSES TO ANGIOTENSIN (1 mcg./Kg. i.v.)

Treatment	Systolic Pressure	Diastolic Pressure	Coronary Blood Flow	Cardiac Output
Surgical Sympathectomy				
Mean % change	18	48	18	−18
S.E.[a]	8	2	2	6
N[b]	2	2	2	2
Phenoxybenzamine, 5 mg./Kg.				
Mean % change	46	64	49	−13
S.E.[a]	9	13	6	8
N[b]	5	5	5	5
Atropine, 0.25 mg./Kg.				
Mean % change	52	80	62	−10
S.E.[a]	4	1	2	9
N[b]	2	2	2	2
Dichloroisoproterenol, 5 mg./Kg.				
Mean % change	52	87	56	+19
S.E.[a]	4	8	5	2
N[b]	3	3	3	3

[a] ±, Standard error. [b] Number of animals.

TABLE III.—EFFECT OF NETHALIDE (5 mg./Kg. i.v.) PRETREATMENT ON THE CARDIOVASCULAR RESPONSES TO ANGIOTENSIN (1 mcg./Kg.)

	Systolic Pressure	Diastolic Pressure	Coronary Blood Flow	Right Ventricular Force	Coronary Perfusion Pressure	Cardiac Output
Intravenous Angiotensin						
Mean % change	50	125	103	25	21	−20
S.E.[a]	6	20	5	3	4	5
N[b]	7	7	2	5	4	7
Intra-arterial Angiotensin						
Mean % change	53	106	...	27	26	−25
S.E.[a]	9	35	..	6	11	7
N[b]	5	5	...	5	3	2

[a] ± Standard error. [b] Number of animals.

As in the untreated animals, neither surgical sympathectomy nor drug pretreatment significantly altered the effects of angiotensin on coronary vascular resistance. Nethalide pretreatment did not alter significantly the increase in coronary perfusion pressure produced by either intravenous or intra-arterial injections of angiotensin.

Heart Rate.—Similar to the results obtained in untreated animals, neither surgical sympathectomy nor drug pretreatment significantly altered the effects of angiotensin on heart rate.

Right Ventricular Force.—Nethalide pretreatment attenuated the increase in intravenous or intra-arterial right ventricular force of contraction produced by angiotensin to a slight extent, but this antagonistic activity was not statistically significant ($p > 0.05$).

Cardiac Output.—The effect of surgical sympathectomy or drug pretreatment on the cardiac output response to angiotensin is summarized in Tables II and III. Following surgery or nethalide pretreatment, the decrease in cardiac output to intravenous angiotensin was still statistically significant ($p < 0.05$). Pretreatment with phenoxybenzamine or atropine, however, attenuated this effect. Nethalide pretreated animals showed only a decrease in cardiac output following angiotensin with no secondary increase as seen in the untreated animals. DCI pretreatment caused subsequent angiotensin injections to produce an initial highly significant elevation in cardiac output ($p < 0.01$) and only a negligible decrease at the end of 3 minutes. Figures 3 and 4 illustrate the difference in the cardiac output response to angiotensin in the nethalide and DCI pretreated animals.

DISCUSSION

The pressor effect of angiotensin has been reported to depend on arteriolar constriction largely in renal and splanchnic beds (17). This pressor response was characterized by Page *et al.* (18) as an initial sharp rise, followed by a small compensatory fall, then by a sustained rise lasting from 4 to 6 minutes. In addition to these observations, our results showed that the diastolic pressure rose significantly greater than the systolic pressure. While a slight vagal bradycardia has been reported to accompany the pressor response (19), this effect was not evident in our studies. A biphasic response in cardiac output was noted in a majority of our animals. The initial decrease was shown by Fowler and Holmes (2) to correspond to an increase in left atrial pressure and a decrease in left ventricular force during the initial impairment of cardiac function. These investigators also have shown that, following the initial depression, an improvement in cardiac performance persisted for 30 to 120 seconds. During this interval, their results showed that cardiac output increased above control values.

Since the right ventricle encountered a reduced resistance to outflow of blood (20), the initial depression noted in the studies of Fowler and Holmes were not seen in these experiments. On isolated cat papillary muscles, these investigators consistently obtained increases in inotropic force of contraction following addition of angiotensin to the bath. Similar inotropic responses were observed by Bianchi *et al.* (1) and Meier *et al.* (21).

Although angiotensin produced an increase in coronary blood flow, there was no change in coronary vascular resistance. Mandel and Saperstein (22) found no evidence of alteration in coronary flow during angiotensin infusion in rats. Forte *et al.* (7), using the nitrous oxide method, demonstrated an increase in coronary blood flow. Hill and Andrus (23) and Lorber (3) showed that angiotensin decreased coronary flow in the isolated perfused cat's heart, and Maxwell *et al.* (5) found that angiotensin increased coronary vascular resistance but did not alter coronary blood flow. Since in the present study the increase in myocardial blood flow was analogous to the increase in diastolic pressure, it was possible that the elevation in blood pressure and the increased perfusion pressure resulting therefrom masked the increase in coronary vascular resistance.

In those experiments in which coronary perfusion pressure was controlled and not dependent on the cardiac performance, both intravenous and intra-arterial injections of angiotensin demonstrated the vasoconstricting property seen in other vascular beds. These results were in agreement with those reported by Fowler and Holmes (2).

Bickerton and Buckley (24) reported that a pressor response to angiotensin could be mediated centrally *via* sympathetic pathways. Zimmerman (25) further showed that the peripheral vasoconstrictor effects of angiotensin could be reduced following surgical sympathectomy or cervical spinal transection, in which case there was a passive vasodilation due to the loss of sympathetic tone. Recently, Benelli *et al.* (26) demonstrated the involvement of peripheral sympathetic nerves in the action of angiotensin. They advanced the hypothesis that angiotensin acts at the peripheral nerve endings by promoting a greater output of norepinephrine. Following acute surgical sympathectomy, it was seen that although angiotensin still increased systolic and diastolic blood pressure and coronary blood flow, these effects occurred to a lesser extent than in the intact animal. Since there is some evidence (27) that the coronary flow is dependent to an extent on the sympathetic system, then it is probable that the decrease in cardiac activity following destruction of that system would result in a decreased responsiveness to angiotensin.

Bickerton and Buckley (24) reported that administration of the alpha adrenergic blocking agent, piperoxan, to the recipient's peripheral circulation prevented the central pressor response in the recipient animal elicited by the injection of angiotensin into the recipient's arterial inflow in the dog cross-circulation experiment. However, when angiotensin was given peripherally to the recipient animal, it still produced the potent pressor effect. Page and Bumpus (17) recently stated that sympathicolytic drugs in pentobarbital anesthetized dogs have little or no effect on the pressor action of angiotensin. Although our data obtained with angiotensin in phenoxybenzamine-treated animals are in agreement with the above reports, it should be noted that phenoxybenzamine pretreatment did prevent the decrease in cardiac output following angiotensin. This may be attributed to the fact that phenoxybenzamine possesses sympathomimetic activity of its own (28).

Both DCI and nethalide have been reported to be beta adrenergic blocking agents (29, 30); however, nethalide differs from DCI because it apparently lacks sympathomimetic activity (30). These differences were noted in the present study since DCI-produced positive chronotropic activity as reported by Furchgott (31), whereas nethalide induced bradycardia in all instances as reported by Black and Stephenson (30). DCI also increased coronary blood flow, and nethalide did not significantly alter myocardial flow. Thus, these results could explain the difference encountered by the administration of angiotensin to either DCI or nethalide pretreated animals. Nethalide potentiated the vascular responses to angiotensin to an extent greater than did DCI. In addition, DCI

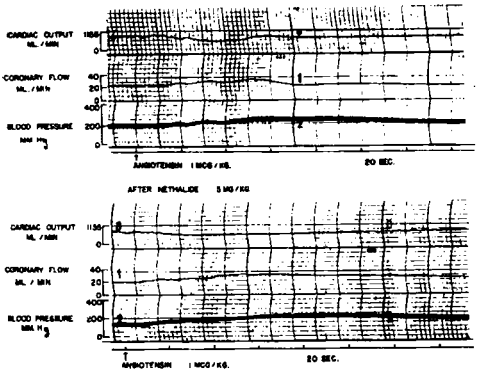

Fig. 3.—Effect of intravenous injection of angiotensin II (1 mcg./Kg.) on blood pressure, coronary flow, and cardiac output. Upper tracing shows the control response; lower tracing, after nethalide, illustrates the prolonged decrease in cardiac output and the prolonged increase in coronary flow.

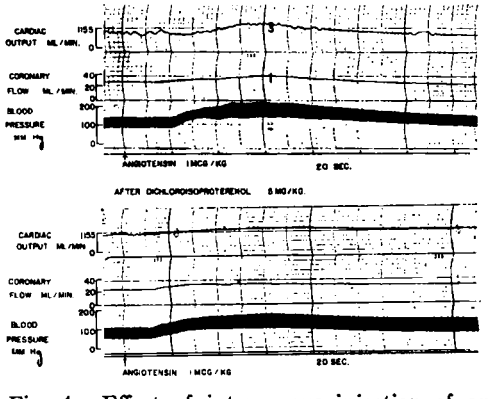

Fig. 4.—Effect of intravenous injection of angiotensin II (1 mcg./Kg.) on blood pressure, coronary flow, and cardiac output. Upper tracing depicts the control responses; lower tracing, after DCI, illustrates the prolonged increase in cardiac output. Coronary flow tracing retouched.

pretreatment caused an increase in cardiac output subsequent to angiotensin, whereas nethalide caused a further reduction in this parameter.

From these results, it seemed apparent that, in addition to direct musculotropic effects, the neurogenic component of the angiotensin-induced pressor response may be mediated in part *via* the alpha adrenergic receptor.

SUMMARY

1. The injection of angiotensin into pentobarbital anesthetized animals resulted in an increase in systolic and diastolic pressures (with the latter of greater magnitude), right ventricular force, and coronary perfusion pressure. The increase in myocardial blood flow was of approximately the same magnitude as that of the diastolic pressure; however, angiotensin had no effect on coronary vascular resistance or heart rate.

2. Angiotensin elicited a biphasic response on the cardiac output of the animals. There was an initial decrease, followed by an apparent compensatory increase.

3. The above effects were mediated *via* both direct musculotropic actions and the sympathetic nervous system since acute surgical sympathectomy reduced, but did not abolish, the effects of angiotensin.

4. The sympathetic component of the cardiovascular effects of angiotensin appears to be *via* the alpha receptors since nethalide greatly potentiated the activity of angiotensin.

5. The effect of pretreatment with phenoxybenzamine, atropine, DCI, or nethalide on the cardiovascular responses to angiotensin are discussed in an effort to clarify its mechanism of action.

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Aspirin Formulation and Absorption Rate I

Criteria for Serum Measurements with Human Panels

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Replication of serum salicylate level distribution in a human panel is obtained by rigorous adherence to properly selected protocols. The panel must be sufficiently large to represent all types of absorption variability after aspirin ingestion. The inherent variability of the human group can be measured. Thus, it is possible to calculate panel sizes required to provide the sensitivity, in terms of least significant differences, needed to evaluate factors of aspirin absorption. With the panels studied, random crossover testing offers no advantage over sequential testing. During the initial absorption period, serum salicylate levels existing at the time of ingestion, whether small artifacts or real and appreciable levels, may be treated as deductible blanks for the study of aspirin absorption.

IN RECENT medical and pharmaceutical literature there have been several papers related to some aspect of blood salicylate levels (1–10) or urinary recovery levels (11, 12) resulting from aspirin ingestion. There has been general agreement that the rate of absorption of drugs is reflected in the rate of increase of blood level and that in turn this level is reflected in the rate of urinary recovery. The results have not been in complete agreement, particularly on the blood salicylate levels resulting from different aspirin preparations; but not all the reasons for different levels have been established. The large varia-

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