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STIMULATION OF ALDOSTERONE SECRETION BY ANGIOTENSIN II

A Preliminary Report†

INTRODUCTION

The role of the renin-angiotensin system in renal hypertension has been investigated for many years but the relationship of the renin-angiotensin system to adrenocortical function has received less attention. In 1951, Deane and Masson,[4] using the rat as an experimental animal, reported that encapsulation of the kidney, a procedure which results in hypertension presumably through release of renin, and injections of crude renin preparations both produced enlargement of the zona glomerulosa of the adrenal gland, the site of aldosterone formation. Hartroft and co-workers[6,16] noted that salt loading in the rat decreased both the granulations of the juxtaglomerular apparatus (the probable site of renin formation) and the content of renin in the kidney, while sodium deprivation increased the granulations and the content of renin. An excellent review by Tobian[19] has summarized the data concerning the possible role of the renin-angiotensin system in electrolyte metabolism and hypertension. Recently prolonged infusions of synthetic angiotensin into man have been reported as producing a pressor response and an increase in aldosterone excretion and secretion.[5,10] It is a possibility that the aldosterone effect was an indirect one through angiotensin stimulating ACTH release from the pituitary. In fact, Genest[5] found that the angiotensin infusion also increased urinary tetrahydrocortisol excretion. Experiments conducted independently by Davis and co-workers[8] and the authors[13] have implicated the kidney and the renin-angiotensin system in the increased aldosterone secretion that occurs fol-

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lowing hemorrhage in the hypophysectomized dog. The present report concerns a preliminary study of the effect of angiotensin II on adrenocortical secretion in the hypophysectomized, nephrectomized dog.

PROCEDURE AND METHOD

Angiotensin II Experiments. Mongrel male dogs weighing about 12 kilograms underwent a left nephrectomy one to four weeks prior to the experimental day. On the experimental day, each dog was anesthetized with Nembutal, acutely hypophysectomized by the transbuccal route, following which the right kidney was removed and the right lumboadrenal vein cannulated by the method of Nelson and Hume.[14] The femoral artery was cannulated for continuous blood pressure recording using a Grass Model 5 Polygraph and a Statham strain gauge, and the femoral vein for administration of blood or angiotensin II. During the control period, two 10-15 ml. samples of adrenal vein blood were collected at 20-minute intervals. The dog was then hemorrhaged 15 ml./kg. body weight and three post-hemorrhage samples of adrenal vein blood collected at 20- to 30-minute intervals. Following the last sample, isotonic saline solutions containing 3.3 μg of synthetic angiotensin II* per ml. were infused through a constant infusion pump. Two more adrenal vein blood samples were collected, one during the infusion and the other one-half hour after its completion.

In one experiment (#224), following the last post-hemorrhage sample, 10 μg of angiotensin II in 3 ml. of isotonic saline was administered intravenously over one minute and adrenal vein blood collected for 18 minutes. One-half hour after the angiotensin II injection, 5 milliunits of Upjohn ACTH in 1 ml. of isotonic saline were administered intravenously over one minute and adrenal vein blood was collected for 18 minutes.

ACTH experiments. The effect of graded doses of Upjohn ACTH on adrenocortical function was studied. On the experimental day, Dog #111 was anesthetized with Nembutal, hypophysectomized through the transbuccal route, and the right lumboadrenal vein cannulated. At one-half hour intervals a dose of ACTH (5 mμ. per ml. of saline) was injected intravenously and adrenal vein blood collected from four to fourteen minutes following the injection.

Following a study of the effect of acute respiratory acidosis on adrenocortical secretion of a normal dog (Dog #13) the effect of intravenous administration of 10 and 1000 mμ of Armour ACTH in saline was studied. On the experimental day, the dog was anesthetized with Nembutal, the right lumboadrenal vein cannulated, and a tracheal cannula inserted for regulation of the dogs respirations by a respiratory pump. During the control period, 100 per cent oxygen was administered through the tracheal cannula. The dog was then respired with a mixture of 90 per cent oxygen and 10 per cent carbon dioxide for approximately one and one-half hours. Three samples of adrenal venous blood were collected in the control and the respiratory acidosis periods at one-half hour intervals. Following the respiratory acidosis period, the dog was respired with 100 per cent oxygen. After twenty minutes a "control' sample of adrenal venous

* Synthetic angiotensin II (Ciba-Hypertension II) was obtained through the courtesy of Ciba Pharmaceutical Inc.

blood was collected. Ten milliunits of Armour ACTH in 1 ml. of isotonic saline was then injected intravenously. Nine minutes later, a five-minute collection of adrenal venous blood was begun. Approximately seventeen minutes after the initial injection, 1000 milliunits of Armour ACTH in 1 ml. of isotonic saline was administered intravenously and collection of adrenal venous blood started five minutes later.

In all dogs, immediately after the collection of each sample of adrenal vein blood, an equal volume of dog donor blood was administered to avoid the effect of repeated small hemorrhages. There was no replacement of the blood removed in the large hemorrhage in the hypophysectomized, nephrectomized dogs.

TABLE 1. ANGIOTENSIN EXPERIMENT

Dog #215—12 Kg. Hypophysectomized, Nephrectomized

Time mins.	*B.P.* mm./Hg*	*Adrenal plasma flow** ml./min.*	*17-OH Corticoids† μg./min.*	*Corticosterone μg./min.*	*Aldosterone mμg./min.*
-40	65/25	0.9	0	0.058	19
-10	70/30	0.8	0	0.048	17
0-17	Angiotensin infusion 1.67 μg./min.			Total 28μg.	
6	150/100	0.7	2.2	0.67	44
53	100/60	1.4	0	0.078	17
58-71	Angiotensin infusion 0.42 μg./min.			Total 6μg.	
64	145/100	1.1	0.8	0.27	37
107	105/55	1.5	0	0.055	18
112-123	Angiotensin infusion 0.17 μg./min.			Total 2μg.	
118	120/70	1.3	0	0.056	15
160		1.0	0	0.067	26

* Blood pressure.
** Adrenal plasma flow was calculated from the adrenal blood flow and hematocrit.
† 17-OH corticoids is used for 17-hydroxycorticoids.

Corticosterone and aldosterone were measured in adrenal vein plasma by the isotope derivative method of Kliman and Peterson, 17-hydroxycorticoids by the Silber-Porter method.[17] Secretory rates were calculated from the concentration and the plasma flow per minute.

RESULTS

In Dog #111 only are the results of the entire experiment shown. The effect of hemorrhage on adrenocortical secretions of the hypophysectomized, nephrectomized dogs will be published elsewhere as part of a larger series of experiments. The possibility that the prior experimental conditions influenced the pattern of response of the adrenal cortex to angiotensin or ACTH cannot be excluded.

Angiotensin II experiments. The infusion of approximately 30μg of Angiotensin II stimulated secretions of 17-hydroxycorticoids, corticosterone, and aldosterone in three of the hemorrhaged, hypophysectomized, nephrectomized dogs (Tables 1, 2, 3). Cessation of the infusion resulted in the return of the secretory levels to the base line. In each dog, a lower dose of Angiotensin II was found which reduced the increment in 17-hydroxycorticoids and corticosterone secretion. However, aldosterone secretion was stimulated to approximately the same extent. In fact, in Dog #223 (Table 3) an infusion of 3μg of Angiotensin II stimulated only aldosterone secretion. In the hemorrhaged, hypophysectomized, nephrectomized Dog #224, a comparison between the effects of a single injection of Angiotensin II and 5 milliunits of ACTH on adrenocortical secretions was made. Angiotensin stimulated all three parameters, 17-hydroxycorticoid, corticosterone and aldosterone secretion, while ACTH stimulated only 17-hydroxycorticoid and ACTH secretion (Table 4).

TABLE 2. ANGIOTENSIN EXPERIMENT

Dog #218—12 Kg. Hypophysectomized, Nephrectomized

Time mins.	*B.P.* mm./Hg*	*Adrenal plasma flow** ml./min.*	*17-OH Corticoids† μg./min.*	*Corticosterone μg./min.*	*Aldosterone mμg./min.*
-40	90/45	0.7	0	0.031	1.9
-10	100/50	0.6	0	0.044	0.7
0-19	Angiotensin infusion 1.67 μg./min.			Total 32μg.	
6	185/125	0.3	2.6	0.81	23
49	100/45		..	....	...
49-78	Angiotensin infusion 0.42 μg./min.			Total 12μg.	
54	150/95	0.2	0.2	0.14	18

* Blood pressure.
** Adrenal plasma flow was calculated from the adrenal blood flow and hematocrit.
† 17-OH corticoids is used for 17-hydroxycorticoids.

Angiotensin II, a potent pressor substance, always produced a rise in blood pressure, but not necessarily to levels abnormal for the dog. Two of the dogs were hypotensive prior to the initial angiotensin infusions, while the blood pressure of Dog #223 had returned to the pre-hemorrhage level of 150/100mm./Hg. In this dog, 3μg of Angiotensin II increased aldosterone secretin to 33 mμg./min. while only raising the blood pressure to 155/95 mm./Hg. Although this was an increase over the blood pressure

of 125/45 recorded immediately prior to the angiotensin infusion, the level is comparable to the "control" blood pressure of 150/100mm./Hg. when the aldosterone secretory rate was only 6mμg./min.

The effect of Angiotensin II upon adrenal blood flow was variable.

ACTH experiments. Commercial ACTH produced a dose response pattern of adrenocortical secretion different from Angiotensin II. Under different experimental conditions, both preparations of ACTH produced simi-

TABLE 3. ANGIOTENSIN EXPERIMENT

Dog #223—11 Kg. Hypophysectomized, Nephrectomized

Time mins.	*B.P.* mm./Hg*	*Adrenal plasma flow** ml./min.*	*17-OH Corticoids† μg./min.*	*Corticosterone μg./min.*	*Aldosterone mμg./min.*
-50	110/60	0.9	0	0.023	3
-20	150/100	0.5	0	0.017	6
0-18	Angiotensin infusion 1.67 μg./min.			Total 30μg.	
6	190/150	0.5	2.1	0.40	30
48	150/75	0.2	0	0.010	15
58-70	Angiotensin infusion 0.42 μg./min.			Total 5μg.	
64	190/145	1.4	3.0	0.57	61
106	100/30	1.3	0.4	0.028	4
110	125/45	..	..	....	..
111-127	Angiotensin infusion 0.167 μg./min.			Total 3μg.	
117	155/95	0.8	0.2	0.010	33

* Blood pressure.
** Adrenal plasma flow was calculated from the adrenal blood flow and hematocrit.
† 17-OH corticoids is used for 17-hydroxycorticoids.

lar results (Tables 4 and 5). The largest dose stimulated both glucocorticoid secretion (17-hydroxycorticoids or corticosterone). There were no changes in arterial blood pressure following ACTH administration.

The volumes of isotonic saline administered were small in all experiments and probably did not influence the results. Moreover, administration of 100 ml. of isotonic saline over one minute to each of three hypophysectomized, nephrectomized dogs by the authors had no significant effect on adrenocortical secretions.

DISCUSSION

Angiotensin is a decapeptide that is formed *in vivo* by the action of renin, an enzyme released from the kidney, on the α^2 globulins of blood. A specific

converting enzyme in plasma converts angiotensin I into the potent pressor octapeptide angiotensin II.[18]

The pattern of adrenocortical response to short term intravenous administration of synthetic angiotensin II depended upon the dose administered. In each of three dogs, a dose of about 30μg. of angiotensin II stimulated both glucocorticoid and aldosterone secretion while a lower dose stimulated primarily aldosterone secretion. The values of glucocorticoid secretion at-

TABLE 4. ANGIOTENSIN AND ACTH EXPERIMENT

Dog #224—10 Kg. Hypophysectomized, Nephrectomized

Time mins.	*B.P.* mm./Hg*	*Adrenal plasma flow** ml./min.*	*17-OH Corticoids† μg./min.*	*Corticosterone μg./min.*	*Aldosterone mμg./min.*
-60	125/70	1.1	0	0.067	0
-30	125/55	1.2	0	0.051	0
0	120/55	Angiotensin II 10 μg. over 1 minute			
0-18	151/93‡	1.5	1.4	0.30	17
18	120/65	..	..	....	..
30	125/60	ACTH¶ 5 mμ over 1 minute			
30-48	120/55	1.3	3.2	0.49	3
48	125/60	..	..	....	..
60	125/60	1.4	0.1	0.043	1

* Blood pressure.
** Adrenal plasma flow was calculated from the adrenal blood flow and hematocrit.
† 17-OH corticoids is used for 17-hydroxycorticoids.
‡ Average blood pressure for 18 minutes. Peak blood pressure 190/135 mm./Hg occurred within one minute, lasted for two minutes and gradually declined thereafter.
¶ Lyophilized Upjohn Adrenocorticotropin Hormone.

tained even with the 30μg. dose were still well below the maximum secretory rate. However, the aldosterone secretory rates attained were as high or higher than the secretory rates following hemorrhage in the hypophysectomized dog.[18] It should be kept in mind, however, that the response of the adrenal to short term infusions of angiotensin II may be different from the response to a prolonged infusion. A study by Hilton, *et al.*[7] using the perfused isolated dog adrenal showed that the stimulatory effect of a vasopressor infusion on cortisol secretion was transient and not sustained by a prolonged infusion.

Similarly the pattern of adrenocortical response to intravenous administration of ACTH is dose-dependent. Like angiotensin, the larger doses of ACTH stimulated both glucocorticoid and aldosterone secretion while,

in contrast to angiotensin, the smaller doses stimulated only glucocorticoid secretion. There were smaller doses of ACTH which even produced a higher glucocorticoid secretory rate than the 30μg of angiotensin yet did not stimulate aldosterone secretion. It should be pointed out that the experimental design of the angiotensin and ACTH experiments differed. It is possible that the metabolic state of the adrenal gland influenced the results. This possibility did occur in a previous study by the authors[18] in

TABLE 5. ACTH EXPERIMENTS

Treatment	*Time mins.*	*Adrenal plasma flow* ml./min.*	*17-OH Corticoids** μg./min.*	*Corticosterone μg./min.*	*Aldosterone mμg./min.*
		Dog #111—12 Kg. Hypophysectomized			
Control	—6	1.7	1.2	0.11	15
1 mμ.† ACTH‡	0	1.4	0.6	0.19	10
5 mμ. ACTH	30	1.4	2.5	0.81	8
10 mμ. ACTH	60	1.3	5.9	1.74	7
25 mμ. ACTH	90	1.2	7.1	2.17	10
2 mμ. ACTH	120	1.0	0.6	0.31	11
0	150	1.3	0.5	0.13	11
50 mμ. ACTH	180	1.4	9.3	3.14	33
		Dog #13 "Normal"			
Control	—8	0.9	..	1.1	40
10 mμ.† ACTH¶	0				
	9	1.4	..	3.2	31
1000 mμ. ACTH	17				
	22	1.3	..	4.1	116

* Adrenal plasma flow was calculated from the adrenal blood flow and hematocrit.
** 17-OH corticoids is used for 17-hydroxycorticoids.
† mμ. = International milliunits.
‡ Lyophilized Upjohn Adrenocorticotropin Hormone.
¶ Lyophilized Armour Adrenocorticotrophin Hormone.

which 1 unit of ACTH administered to hemorrhaged, normal dogs who were maximally secreting glucocorticoids, stimulated only aldosterone secretion. However, 1 unit of ACTH administered to hemorrhaged hypophysectomized dogs always stimulated both glucocorticoid and aldosterone secretion.

Angiotensin II is the most potent vasopressor material known. Nephrectomy and hemorrhage increase the sensitivity of animals to angiotensin.[2] It is not surprising, therefore, that a dose as small as 3μg produced a pres-

sor effect. Although the present experiments do not rule out the possibility that the rise in blood pressure per se stimulated aldosterone secretion, several lines of evidence do not support this possibility. Laragh, *et al.*[10] have shown in man that the pressor agents norepinephrine, epinephrine, ephedrine, and vasopressin had variable effects on alosterone secretion except in sodium-depleted subjects with high aldosterone secretion in whom medullary type hormones generally produced a fall. Infusion of angiotensin II always produced a rise in aldosterone secretion. Moreover, Newman, *et al.*[15] demonstrated that norepinephrine does not increase aldosterone secretion in the dog, at least not in amounts which maintained the mean arterial pressure at 100 mm. Hg. Barter, *et al.*[1] actually dissociated alterations in blood pressure and aldosterone secretion in a specific experimental situation. They reported that bilateral constriction of the common carotid arteries in dogs with both thyrocarotid arterial junctions denervated, produced no increase in aldosterone secretion despite a rise in blood pressure due to an intact Hering reflex. Constriction, with the nerves to the thyrocarotid arterial junctions intact, resulted in increases in both aldosterone secretion and blood pressure. The work of Hilton, *et al.*[7] dissociated the cortisol stimulatory activity and the pressor action of several vasopressin peptides in the isolated perfused dog adrenal. Moreover, acetyl arginine (vasopressin) which has no pressor activity stimulated both cortisol and aldosterone in one experiment. These authors also stated that oxytocin and norepinephrine in doses sufficient to have pressor activity had no effect on cortisol secretion. Finally, in a study of the effect of hemorrhage on adrenocortical secretion in hypophysectomized dogs, the authors[13] found that transfusion of the shed blood did not cause a significant increase in adrenocortical secretions, including aldosterone, despite a modest elevation of blood pressure and increased adrenal blood flow. Nevertheless, these experimental situations do not duplicate the present experiments and it remains to be shown that the stimulatory effect on aldosterone secretion is specific for angiotensin.

It is tempting to speculate that angiotensin II is the elusive aldosterone-stimulating hormone. This hypothesis is supported by: (i) evidence[19] which suggests that the juxtoglomerular apparatus is a "volume" receptor; (ii) the hyperplasia of zona glomerulosa of the adrenal gland following renin injections;[4] (iii) the aldosterone-stimulating effect of angiotensin II in man[5, 10] and the dog;[8] and (iv) the increased secretions of aldosterone in man in malignant hypertension[11] and in some cases of hypertension due to unilateral renal vascular disease.[9] However, if angiotensin II is the aldosterone-stimulating hormone, a dose insufficient to have a pressor effect

should be able to stimulate aldosterone secretion, since several stimuli which increase aldosterone secretion are not associated with a pressor effect. Furthermore, a prolonged infusion of angiotensin II should maintain an increased secretory rate. Finally, and most important, it must be shown that the kidney and the renin-angiotensin system are necessary for the physiological stimuli which increase aldosterone secretion.

SUMMARY

The pattern of adrenocortical secretions produced by an infusion of angiotensin II was dose-dependent. A dose of approximately 30μg. of angiotensin II stimulated both glucocorticoid and aldosterone secretion. Reduction in dosage reduced the increment in glucocorticoid secretion without appreciably reducing the increment in aldosterone secretion. Angiotensin II always produced a pressor response.

The pattern of adrenocortical secretion produced by ACTH administration was also dose-dependent. Like angiotensin II, the larger doses of ACTH stimulated both glucocorticoid and aldosterone secretion. In contrast to angiotensin II, the smaller doses stimulated only glucocorticoid secretion. The possible role of the renin-angiotensin system in the regulation of aldosterone secretion is briefly discussed.

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