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THE NATURE OF THE ALDOSTERONE-STIMULATING FACTOR IN DOG KIDNEYS *

By PATRICK J. MULROW, WILLIAM F. GANONG, GERALD CERA AND ALBERT KULJIAN

(From the Medical Service, VA Hospital, West Haven, and Department of Medicine, Yale University School of Medicine, New Haven, Conn., and Department of Physiology, University of California School of Medicine, San Francisco, Calif.)

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Previous studies (2–4) have demonstrated that nephrectomy lowers control aldosterone secretion rates and prevents the increase in aldosterone secretion that follows hemorrhage. Injection of saline extracts of kidneys (3, 4) from normal dogs into hypophysectomized, nephrectomized dogs stimulated aldosterone secretion markedly while also stimulating 17-hydroxycorticoid and corticosterone secretion. A marked pressor response also occurred. These observations suggested that the kidney secreted an aldosterone-stimulating factor. The stimulation of glucocorticoid secretion by the saline kidney extracts raised the possibility that ACTH was present in the kidney extract. This possibility appeared to be supported by the finding of Richards and Sayers (5) that exogenous ACTH accumulated preferentially in the kidney. On the other hand, the pressor response produced by the saline kidney extracts suggested that the extracts contained renin. Considerable indirect evidence (6) suggested that the renin-angiotensin system may play an important role in the regulation of aldosterone secretion.

To investigate the nature of this kidney factor, the effect of several substances on adrenocortical secretion of hypophysectomized, nephrectomized dogs has been studied. Saline extracts of kidneys from hypophysectomized dogs, and dog anterior pituitaries, commercial ACTH, semipurified renin extracts, and synthetic angiotensin II have been assayed. The results indicate that the aldosterone-stimulating factor in the dog kidney is renin.

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METHODS

Under pentobarbital anesthesia, a cannula was placed in the right lumboadrenal vein of 51 male mongrel dogs (weight 9.2 to 18.6 kg) which had been subjected to left nephrectomy 3 or more days before. The remaining right kidney was removed during cannulation of the lumboadrenal vein and the dog hypophysectomized via the transbuccal route. Starting 1 hour after hypophysectomy, two or more control samples of adrenal venous blood were collected at 20-minute intervals. Thereafter, collections were made every half hour and immediately before injection of the substance to be assayed. Usually, a 10-minute adrenal venous blood sample was collected, starting 4 minutes after the beginning of the intravenous injection. Each sample of adrenal venous blood was replaced by an equal volume (12 to 40 ml) of bank blood from nephrectomized, hypophysectomized donor dogs. Blood pressure was recorded continuously with a Grass model 5 polygraph and the Statham strain gauge.

Blood samples were centrifuged immediately after collection and the plasma frozen for subsequent analysis. Aldosterone and corticosterone were measured by the isotope derivative technique of Kliman and Peterson (7) and 17-hydroxycorticoids by the Silber-Porter method (8). Secretion rates for each of these hormones were calculated from their concentrations in the adrenal venous plasma and the plasma flow per minute. All hypophysectomies were found to be complete by gross examination of the pituitary region at autopsy. Serial histological sections of the hypophyseal region were made in 14 dogs (nos. 17, 46, 68, 70, 85, 142–145, 148, 215, 224, 249, 280). No anterior pituitary tissue was seen.

Saline extracts of kidneys removed from dogs hypophysectomized 4 hours previously were prepared by dicing, mixing with isotonic saline, and filtering through cheesecloth. The filtrate was centrifuged at approximately 3,000 rpm for 20 minutes, and the supernate (100 ml volume) injected into assay dogs over a 1-minute period. Each dog received the extract of one kidney.

Saline extracts of the anterior lobe of dog pituitaries were obtained from normal dogs sacrificed by injecting an overdose of pentobarbital. The pituitary was removed within 3 minutes after injection of the pentobarbital. The anterior lobe was separated from the posterior, and homogenized in isotonic saline. The solution was

centrifuged, diluted to a concentration of 1/100 anterior pituitary per ml, and frozen for subsequent injection into assay animals.

Semipurified extracts of renin were prepared from kidneys of normal dogs and dogs hypophysectomized 4 hours previously. The kidneys were removed surgically, immediately frozen and stored at −20° C. The kidneys were later thawed, freed of fat, the pelvis, and capsule, and weighed and extracted for renin by the method of Haas and Goldblatt (9), which involved purification by selective acid denaturation of proteins, precipitation of the renin with 2.2 M ammonium sulfate, and dialysis of the precipitate against distilled water for 24 hours in the cold. The final volume of the extract of one kidney was between 10 and 13 ml. Although an approximately 15-fold purification was achieved, as judged from the wet weight of the initial and final extracts, the extract was still relatively impure. However, all readily dialyzable substances should have been eliminated. Amounts equivalent to various fractions of one kidney were injected over a 1-minute period into nephrectomized, hypophysectomized assay animals. The wet weight of the kidneys ranged from 20 to 40 g. In dogs that received more than one injection, at least 1 hour elapsed between injections.

Synthetic angiotensin II [1] in isotonic saline was administered by constant infusion. The volume injected per minute was always less than 0.6 ml. The infusion was stopped at the end of the collection; i.e., approximately 14 minutes after the start of the injection. At least 35 minutes elapsed between infusions of angiotensin II in the same dog.

In two dogs after two control adrenal venous samples were obtained, angiotensin II was infused continuously at a rate of 0.167 μg per minute. Samples were collected beginning 4 minutes after the start of the injection and at half-hour intervals thereafter for 4 hours. Five-mU doses of ACTH were then given while the angiotensin was being infused, and adrenal venous collections started 4 minutes later.

[1] Valine-5 angiotensin II (CIBA).

Adrenocorticotrophin (Upjohn) was administered by single injection in 2 ml or less of saline. The administration of ACTH was frequently preceded by angiotensin II or renin injections or saline kidney extracts which had terminated about 0.5 hour prior to the ACTH injection, except for the two dogs receiving the prolonged angiotensin II infusion.

RESULTS

Saline extracts of kidneys from hypophysectomized dogs. The effects of injection of saline extracts of kidney from hypophysectomized dogs are shown in Table I. The extracts increased aldosterone secretion in only two dogs. No change in 17-hydroxycorticoid secretion occurred. The slight rise in systolic blood pressure may have been due partly to the volume of saline administered. In three control hypophysectomized, nephrectomized dogs, intravenous administration of 100 ml of saline produced a 17 ± 9 (SE) mm Hg rise in systolic blood pressure. Aldosterone and 17-hydroxycorticoid secretion did not change.

These results with injections of saline extracts from kidneys of hypophysectomized dogs are in striking contrast to the results obtained with five first-injection saline extracts from kidneys of normal dogs, as found previously (3). Injection of these extracts produced marked rises: in aldosterone secretion, 43 ± 6 mμg per minute (mean ± SE); in blood pressure, 91 ± 10 mm Hg systolic pressure, 81 ± 14 mm Hg diastolic pressure; and a slight rise in 17-hydroxycorticoid secretion: 2.1 ± 0.8 μg per mm.[2]

[2] These results are slightly higher than those in the report which included both first and second injections

TABLE I

The effect of crude saline extracts from kidneys of hypophysectomized dogs on adrenocortical secretions of hypophysectomized, nephrectomized dogs

Assay dog	Dose of kidney*	Δ Blood pressure† S/D	Steroid secretion: Aldosterone Pre‡	Aldosterone Post‡	17-OH corticoids Pre	17-OH corticoids Post
		mm Hg	*mμg/min*		*μg/min*	
225	1	0/0	1	0	0.2	0.5
205	1	10/0	11	13	0	0
206	1	25/15	5	5	2.7	2.2
212	1	5/10	10	22	0	0
148	1	20/20	17	31	0.5	0.3

* The extract was made from the kidney of a hypophysectomized dog.

† Δ Blood pressure represents the differences in systolic and diastolic pressure immediately before and at the peak of the rise after the injection of the extract.

‡ Pre and post indicate the secretion rates immediately before and during approximately a 10-minute period starting 4 minutes after injection of the extracts.

TABLE II

The effect of ACTH upon adrenocortical secretions in hypophysectomized, nephrectomized dogs

Assay dog	ACTH dose	Steroid secretion: Aldosterone Pre	Aldosterone Post	Corticosterone Pre	Corticosterone Post	17-OH corticoids Pre	17-OH corticoids Post
	mU	*mμg/min*		*μg/min*		*μg/min*	
227	2	20	29	0.25	1.21	1.1	3.7
280	2	23	24	0.19	0.39	1.2	1.2
3	2	58	51	0.21	0.96	0.6	5.2
39	2	4	7	0.02	0.31	0.4	1.7
224	5	17	3	0.30	0.49	1.4	3.2
227	5	0	0	0.30	0.51	0.7	1.8
217	5	3	7	0.028	0.46	0	5.6
250	5	19	15	0.39	1.77	2.0	5.5
225	5	0	1	0.13	0.35	1.4	3.2
5	5	2	13	0.041	1.80	0	6.6
64*	5	28	34	0.16	1.94	0	9.7
65	5	4	9	0.047	0.80	0	4.6
74*	5	3	2			0.1	3.6
79	5	1	3	0.017	0.65	0	3.4
280	10	22	18	0.28	2.25		
225	10	5	0	0.085	0.54	0.8	5.9
227	10	23	20	0.48	2.54	2.5	6.9
3	10	65	49	0.90	2.90	1.5	11.4
5	10	69	76	0.31	3.72	0.5	15.4
76	10	1	6	0.10	0.80	0.3	5.8
281	50	2	6	0.018	0.72	0	5.1
61	50	3	5			0	6.1
51	100	28	54	0.06	1.34	0	5.6
128	100	16	46	0.20	4.13	0	8.9
70	1,000	3	58	0.09	2.18	0.7	8.6
85	1,000	6	81	0.36	3.06	2.6	9.4
142	1,000	84	32	0.35	1.40	1.4	9.9
143	1,000	69	43	0.41	1.85	2.4	6.1
144	1,000	47	93	0.76	4.35	0.5	9.5
145	1,000	90	68			1.5	8.9
148	1,000	5	108			0.2	8.4
206	1,000	23	51			0.4	10.8
249	1,000	12	42	0.031	1.77	1.3	6.9
4	1,000	16	41	1.3	1.75	6.8	7.7

* ACTH administered during prolonged angiotensin II infusion.

This alteration in the pressor and steroidogenic properties of the kidneys by hypophysectomy raised the possibility that hypophysectomy removed the secretion of a substance, presumably ACTH, which accumulated in the kidney. In order to investigate this possibility the effects of commercial ACTH and saline extracts of dog anterior pituitary on adrenocortical secretions were studied.

ACTH experiments. The effects on adrenocortical secretion of various doses of ACTH are summarized in Table II. In contrast to the saline extract of normal dog kidneys, small doses of ACTH (2, 5, and 10 mU) stimulated corticosterone and 17-hydroxycorticoid secretion markedly but had no consistent or striking effect on aldosterone secretion even when the pre-ACTH secretion rates of aldosterone were very low. Doses of 10 mU ACTH produced near maximal secretion of 17-hydroxycorticoids and corticosterone. However, large doses of ACTH (100 and 1,000 mU) increased aldosterone secretion markedly after 9 of 12 injections. The three failures were probably due to the fact that the pre-ACTH secretion rates were already very high

of kidney extracts into the same dog (3). The increments in blood pressure and steroids were less after the second injections.

(89, 69, and 90 mμg per minute), owing to marked stimulation of aldosterone secretion by a prior injection of a saline extract from a normal dog kidney.

None of the ACTH injections had any significant effect on blood pressure.

Dog anterior pituitary extracts. Since it was possible that dog anterior pituitary produced an ACTH that was different chemically and physiologically from commercial ACTH, saline extracts of dog anterior pituitary were assayed. The results are shown in Table III. Doses equivalent to 1/100 and 1/400 of a dog anterior pituitary produced a greater rise in 17-hydroxycorticoids and corticosterone than did saline extracts of normal dog kidneys but had no striking or consistent effect on aldosterone secretion. No pressor response was observed.

Renin extracts. The effects on aldosterone, corticosterone, and 17-hydroxycorticoid secretion of 28 renin injections into 13 hypophysectomized, nephrectomized dogs are shown in Table IV. The values represented are the secretory rates immediately before and during approximately a 10-minute period starting 4 minutes after the injection. The preinjection values were not always basal values for hypophysectomized, nephrectomized dogs, since the effect of a previous injection sometimes persisted despite the lapse of 1 hour between injections. This was especially true when more than one injection of a large dose of an extract was given to the same dog. The renin extracts were made from 14 dog kidneys individually extracted. Seven of the kidneys were from normal dogs and seven from dogs hypophysectomized 4 hours prior to the removal of the kidneys. The kidneys weighed between 20 and 40 g.

In contrast to the crude saline extracts of kidneys from hypophysectomized dogs, the semipurified renin extracts of hypophysectomized dog kidneys had a striking effect on steroid secretion. There were no significant differences between the extracts from normal or hypophysectomized dogs on adrenocortical secretion or blood pressure. Although the effects of the same extracts varied considerably between dogs, in the one dog (no. 19) in which equal doses of the same renin extract were asayed 1 hour apart, the effects on steroid secretion and blood pressure were quite similar.

There appeared to be two patterns of response of adrenocortical secretions, depending upon the dose of renin injected. The injection of a renin extract from 0.33 to 1 dog kidney was followed by a marked pressor response and an increase in 17-hydroxycorticoids, corticosterone, and aldosterone secretion. After six of nine of the large doses of renin, the greatest increase in aldosterone secre-

TABLE III

The effect of saline extracts of dog anterior pituitaries upon adrenocortical secretions of hypophysectomized, nephrectomized dogs

Assay dog	Dose*	Preparation	Steroid secretion					
			Aldosterone		Corticosterone		17-OH corticoids	
			Pre	Post	Pre	Post	Pre	Post
			mμg/min		*μg/min*		*μg/min*	
39	1/400	E	4	12	0.043	1.58	0	9.1
39	1/400	F	8	7	0.038	1.57	0	8.0
60	1/400	G	3	4		0.13	0	0.8
250	1/100	A	9	14	0.086	1.54	0	4.6
249	1/100	B	10	21			0.3	12.8
4	1/100	C	20	21	0.10	0.93	0	4.0
19	1/100	E	3	16	0.27	2.16	1.1	13.7
60	1/100	F	3	11	0.065	1.67	1.1	11.6
61	1/100	G	4	4			0	4.2
250	1/20	A	13	10	0.95	1.24	2.4	3.2
60	1/20	F	5	26	0.10	1.95	0.8	11.5
53	1/20	E	10	46			0.3	11.5

* Each extract was made from the anterior pituitary of a single dog. The dose indicates the fraction of the extract injected.

TABLE IV

The effect of renin extracts upon adrenocortical secretion in hypophysectomized, nephrectomized dogs

Assay dog	Dose*	Prep.†	Δ Blood pressure‡ S/D	Steroid secretion: Aldosterone Pre§	Aldosterone Post§	Corticosterone Pre	Corticosterone Post	17-OH corticoids Pre	17-OH corticoids Post
			mm Hg	*mμg/min*		*μg/min*		*μg/min*	
280	1	N 3	100/65	2	17	0.029	0.89		
280	1	H 3	45/30	13	30	0.28	0.91	0.8	2.4
6	1	H 2	110/65	4	28	0.031	0.55	0	2.6
3	1	H 4	160/145	3	35	0.049	1.61	0	4.9
3	1	N 4	50/40	30	67	0.20	0.87	1.9	5.2
5	0.33	N 5	80/65	13	52	0.66	0.91	0.2	3.7
4	0.33	N 6	75/50	4	34	0.064	0.37	0	1.5
281	0.33	H 5	125/100	4	19	0.036	0.75	0.2	4.4
46	0.33	H 7	145/135	5	23‖	0.025	0.41‖	0	2.1‖
24	0.05	N 5	20/15	16	33			4.5	0
31	0.05	N 7	30/20	1	30	0.030	0.046	0	0
51	0.05	N 8	20/25	8	41	0.022	0.065	0.1	0
54	0.05	N 9	30/25	5	18	0.29	0.38	1.1	2.6
24	0.05	H 5	35/30	3	23			1.4	0.9
51	0.05	H 7	25/20	4	32	0.022	0.042	0.6	0.4
54	0.05	H 8	25/35	1	20	0.090	0.50	1.6	0.6
61	0.05	H 9	25/25	1	7	0.053	0.083	0.5	0.2
19	0.033	N 6	30/30	3	22	0.16	0.33	1.8	1.6
19	0.033	N 6	20/25	6	20	0.21	0.25	0.9	1.4
24	0.020	N 5	20/25		32			0	0
31	0.020	N 7	15/10	2	25	0.038	0.061	0	0
51	0.020	N 8	25/25	2	5	0.24	0.19	0	0
54	0.020	N 9	30/25	2	3	0.12	0.13	0.8	1.2
24	0.020	H 5	30/30	4	16			0.4	0.7
51	0.020	H 7	10/5	1	15	0.13	1.12	0.3	0
54	0.020	H 8	20/25	2	13	0.19	0.25	1.8	1.0
61	0.020	H 9	15/15	0	4	0.027	0.039	0.3	0.5
24	0.008	H 5	15/5	4	26			0	0

* Each renin extract was made from a whole dog kidney. The amount injected is represented as a fraction of the total wet weight of the kidney.

† Preparation denotes whether the extract was made from the kidney of a normal (N) or hypophysectomized (H) dog.

‡ Δ Blood pressure represents the difference in the systolic/diastolic pressures between the pressures immediately before and the peak pressures after the renin injection. The peak pressures occurred from 3 to 5 minutes after the injection.

§ Pre and post indicate the secretion rates immediately before and during approximately a 10-minute period starting 4 minutes after the renin injection.

‖ Collection of adrenal venous blood began 30 minutes after the injection.

tion occurred in the second collection period, approximately 30 minutes after the injection, even though the greatest increase in 17-hydroxycorticoids and corticosterone occurred in the first collection period. In Table IV only the steroid secretion rates of the first collection period beginning 4 minutes after the injection are shown. Figure 1 demonstrates the results of an experiment in Dog 3. The renin extract from one hypophysectomized dog kidney produced a marked rise in blood pressure and in 17-hydroxycorticoid, corticosterone, and aldosterone secretion. Despite a decrease in blood pressure, the aldosterone secretion rose further and remained high. In contrast, 17-hydroxycorticoid and corticosterone secretion decreased toward control levels. Injection of 10 and 5 mU of ACTH produced a greater rise in 17-hydroxycorticoids and corticosterone than did renin yet did not increase aldosterone secretion further. An injection of the renin extract from one normal dog kidney produced less of a rise in blood pressure but a similar pattern of adrenocortical secretion. After an initial rise in 17-hydroxycorticoid and corticosterone secretion, their secretion rates decreased with time, yet aldosterone secretion remained markedly elevated. In both dogs that received two injections of a large dose of renin extract, the second injection re-

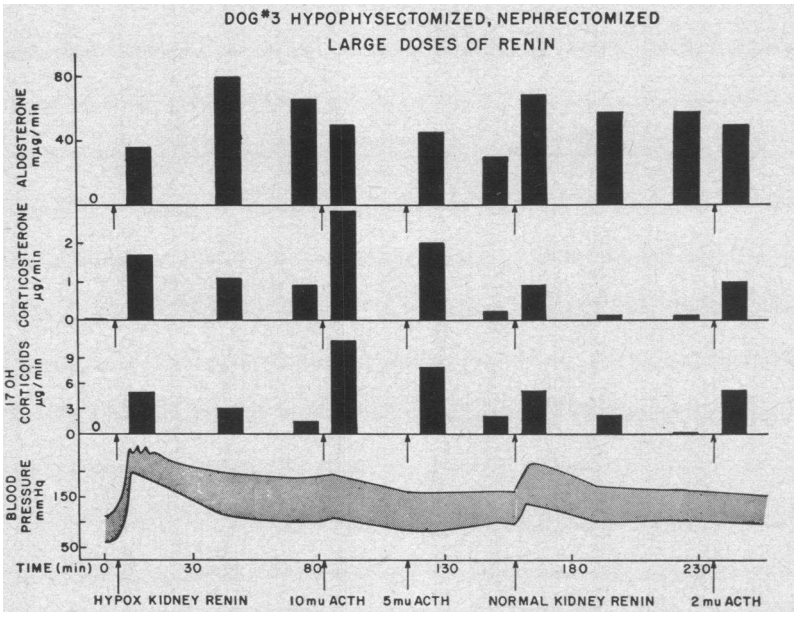

FIG. 1. A COMPARISON OF THE RESPONSE OF ADRENOCORTICAL SECRETIONS TO ADMINISTRATION OF THE ENTIRE RENIN EXTRACTS OF KIDNEYS FROM A NORMAL AND A HYPOPHYSECTOMIZED DOG, AND SMALL DOSES OF ACTH.

sulted in a much smaller rise and peak level of blood pressure attained, possibly owing to tachyphylaxis. After large doses of renin there was usually a slight decrease in adrenal blood flow.

Doses of renin, equivalent to an extract from 0.05 to 0.008 of a dog kidney, markedly increased aldosterone secretion. In contrast to the large doses of renin, the small doses did not stimulate 17-hydroxycorticoid secretion. Corticosterone secretion was generally increased but to a much

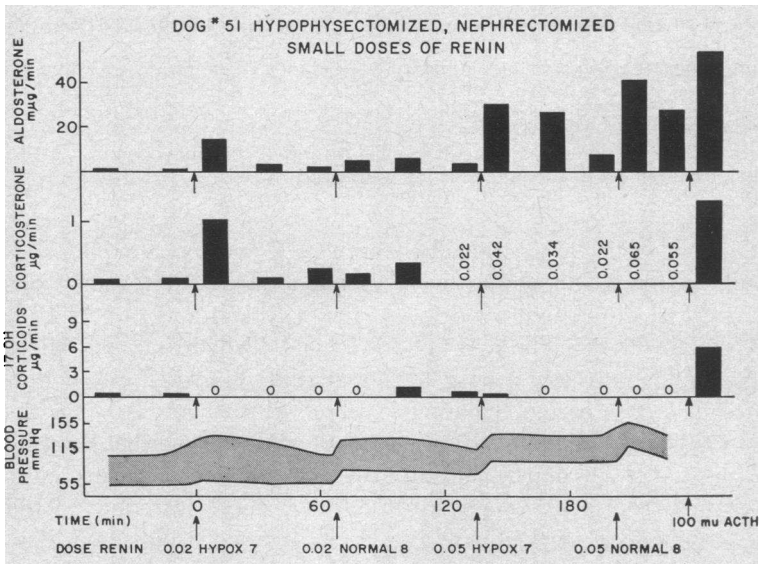

FIG. 2. A COMPARISON OF THE ADRENOCORTICAL RESPONSE TO THE ADMINISTRATION OF SMALL DOSES OF THE RENIN EXTRACTS OF THE KIDNEYS FROM A NORMAL AND A HYPOPHYSECTOMIZED DOG. Two different doses of each extract were administered.

lesser percentage than was aldosterone. Moreover, the postinjection secretion rates of corticosterone were well within the range found in hypophysectomized dogs, except after the dose 0.005 of the preparation Hypox. no. 7 (see Table IV).

With the smaller doses of renin, the peak effect on steroid secretion occurred in the first postinjection period. This explains the apparently greater increase in aldosterone secretion after the 0.05 dose of renin extract no. 5 assayed in Dog 24, than after the 0.33 dose assayed in Dog 281. After the latter dose the peak aldosterone secretion rate occurred in the second postinjection collection period 30 minutes later, and was 33 mμg per minute.

In Figure 2 are shown the effects on steroid secretion of small doses of renin extract from the kidneys of a normal and a hypophysectomized dog. Two different doses of each extract were assayed. The smaller dose was equivalent to the renin from 0.02 of a dog kidney, the large dose to 0.05. The 0.05 dose had a greater effect on aldosterone secretion than had the smaller 0.02 dose. Secretion of 17-hydroxycorticoid was not altered. The effect on corticosterone secretion was erratic. Except for the unusually marked rise in corticosterone secretion after the first injection, the per cent change in corticosterone secretion was much less than that of aldosterone. Also, the levels of corticosterone secretion attained after the larger doses of renin were well below the control values, while the aldosterone secretion rates were much greater. Injection of 100 mU of ACTH resulted in marked increases in aldosterone, corticosterone, and 17-hydroxycorticoid secretion.

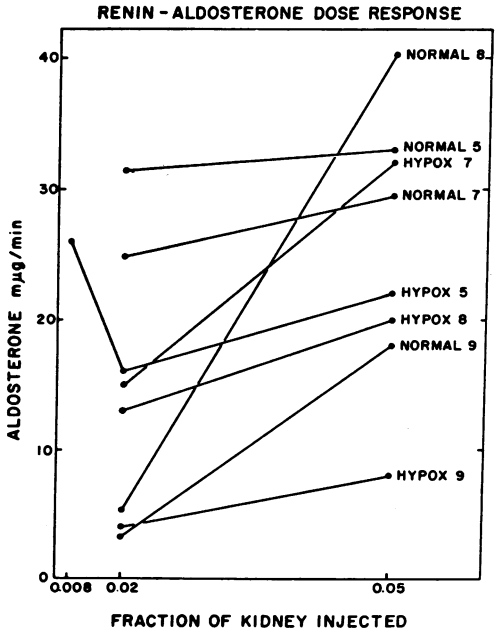

FIG. 3. A COMPARISON OF THE ALDOSTERONE SECRETION RATES ATTAINED AFTER ADMINISTRATION OF DIFFERENT DOSES OF EACH RENIN EXTRACT ASSAYED IN THE SAME HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOG. The figure was plotted from the data in Table IV.

In Figure 3 the secretion rates of aldosterone after injection of two or more small doses of each kidney extract in the same dog are compared. In each dog, the larger dose of renin extract resulted in a higher aldosterone secretion level

TABLE V

The effect of spleen and liver extracts upon adrenocortical secretion in hypophysectomized, nephrectomized dogs

Assay dog	Dose*	Preparation†	Steroid secretion					
			Aldosterone		Corticosterone		17-OH corticoids	
			Pre	Post	Pre	Post	Pre	Post
			mμg/min		*μg/min*		*μg/min*	
68	0.3	Spleen 2	0	0	0.023	0.028	0	0
68	0.3	Spleen 5	5	0	0.028	0.024	0	0
68	0.3	Spleen 1	1	5	0.059	0.062	0	0
69	0.3	Spleen 1	0	0	0.040	0.075	1.1	0.9
69	0.3	Spleen 3	0	2	0.17	0.27	1.2	2.3
76	0.3	Liver 1	1	1	0.12	0.10	0.1	0.2

* Wet weight of the spleen tissue extracted ranged from 26 to 39 g. The liver tissue weighed 36 g. The dose indicates the fraction of the "renin" extract that was injected.

† All the extracts were prepared from spleens or liver of normal dogs.

than did the smaller dose except in Dog 24–61. Despite this dose effect on aldosterone secretion, there was no consistent difference of effect on blood pressure between the 0.02 and 0.05 dose of renin extract. Since the smaller dose was administered first and 1 hour elapsed between injections, tachyphylaxis probably did not influence the blood pressure response.

Injection of spleen extracts and a single liver extract, prepared and assayed in the same fashion as the renin extracts, had no consistent or striking effect on aldosterone, 17-hydroxycorticoid, or corticosterone secretion (Table V). Blood pressure changes after injection of these extracts ranged from − 5 to + 20 mm Hg systolic and from 0 to + 10 mm Hg diastolic.

Angiotensin II experiments. In Table VI are represented the effects on aldosterone, corticosterone, and 17-hydroxcorticoid secretions of infusion of different doses of angiotensin II over approximately a 14-minute period. The pre-angiotensin values are the secretion rates immediately before the onset of the infusion. More than one infusion was administered to most dogs. Approximately 35 minutes elapsed between the termination of one infusion and the start of another. Despite this delay period, the effect of the preceding angiotensin infusion on aldosterone secretion persisted, especially after the larger doses. This may account for the failure to obtain a significant rise in aldosterone secretion after the 1.67 μg per minute dose in Dog 250, and after the 0.42 μg per minute dose in Dog 218. Despite these two failures to show a significant increase, the postinfusion aldosterone secretion rates were still very much higher than the control

TABLE VI

The effect of infusions of angiotensin II upon adrenocortical secretions of hypophysectomized, nephrectomized dogs

Assay dog	Dose	Δ Blood pressure S/D	Steroid secretion: Aldosterone Pre*	Aldosterone Post*	Corticosterone Pre	Corticosterone Post	17-OH corticoids Pre	17-OH corticoids Post
	μg/min/dog	mm Hg	mμg/min		μg/min		μg/min	
249	0.042	10/0	2		0.22	0.45	2.6	2.5
250	0.042	0/0	0	3	0.28	0.31	0	0
2	0.042	20/15	0	3			0.1	0.1
74	0.042	5/5	2	1			0.2	0.7
79	0.042	0/0	3	2	0.053	0.029	1.0	0
249	0.083	5/0	4	13	0.13	0.30	0.5	0.8
250	0·083	20/25	3	17	0.012	0.069	0	0
244	0.167	35/40	15	27			1.5	4.1
249	0.167	20/10	6	17	0.16	0.39	0.8	1.3
250	0.167	40/50	9	24	0.027	0.16	0	0
2	0.167	25/25	1	5			0.1	0.1
64	0.167	30/30	3	20	0.077	0.29	0	0
215	0.167	10/20	18	15	0.055	0.056	0	0
223	0.167	30/50	4	33	0.028	0.010	0.4	0.2
65	0.167	20/20	5	11	0.052	0.12	0.8	0
249	0.42	15/15	7	25	0.43	1.22	0.6	4.6
250	0.42	60/50	18	36	0.071	0.47	0	0.6
49†	0.42	90/75	1	3	0.010	0.45	0.1	2.3
50	0.42	65/65	8	18	0.11	0.19	0	0.2
251	0.42		16	37	0.78	0.27	0	0.8
223	0.42	40/45	15	61	0.010	0.57	0.1	3.0
249	1.67	55/45	10	26	0.110	1.33	0.6	7.2
250	1.67	80/85	24	26	0.11	0.69	0	2.2
215	1.67	100/90	17	44	0.48	0.67	0	2.2
218	1.67	95/90	1	23	0.044	0.81	0	2.6
223	1.67	55/100	6	30	0.017	0.40	0	2.1
224	1.67	50/50	1	47	0.043	1.14	0.1	5.7
17	1.67	100/90	14	42	0.081	1.31	0.6	6.5

* Pre and post indicate the secretion rates immediately before and during approximately a 10-minute period starting 4 minutes after the start of the infusion.

† Adrenal blood flow was extremely low prior to the angiotensin II infusion.

secretion rates at the start of the experiments (0 and 1, respectively).

Like large doses of renin, the larger doses of angiotensin II (1.67 and 0.42 μg per minute) increased aldosterone, corticosterone, and 17-hydroxycorticoid secretion. However, the smaller doses of angiotensin II (0.083 and 0.167 μg per minute), like smaller doses of renin, did not increase 17-hydroxycorticoid secretion. Although increases in corticosterone secretion occurred, the levels obtained were within the range of untreated hypophysectomized dogs. Aldosterone secretion was significantly increased. The infusion of 0.042 μg per minute had no significant effect on aldosterone secretion. In general, the larger the dose of angiotensin II, the larger the pressor response.

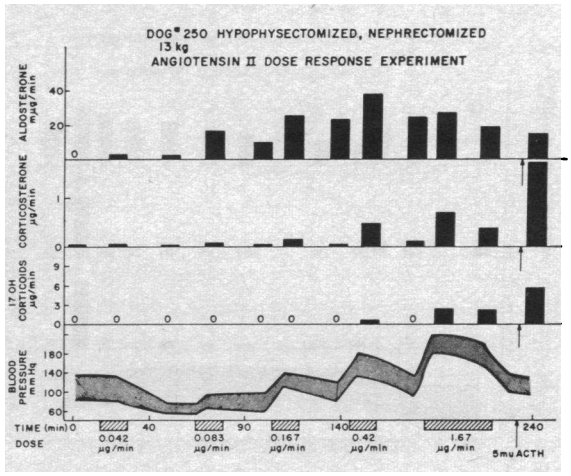

FIG. 4. THE EFFECT OF INFUSIONS OF DIFFERENT DOSES OF ANGIOTENSIN II UPON ADRENOCORTICAL SECRETIONS.

In Figure 4 are represented the results from sequential infusions of several different doses of angiotensin II into a hypophysectomized, nephrectomized dog, no. 250-60. Although each dose resulted in an increase in blood pressure above the preceding level, the absolute level of blood pressure was still below the control level during the infusion of 0.083 μg per minute, yet aldosterone secretion rose from 0 to 17 mμg per minute. The infusion of angiotensin II up to 0.167 μg per minute increased aldosterone and corticosterone secretion. However, the corticosterone secretory levels attained were still very low and probably of no physiological significance. The 17-hydroxycorticoid secretion was not affected. Infusions of angiotensin II at rates of 0.42 and 1.67 μg per minute had a more marked effect on corticosterone and 17-hydroxycorticoid secretion. Intravenous administration of 5 mU of ACTH increased corticosterone and 17-hydroxycorticoid secretion much more than did the largest dose of angiotensin II, but did not increase aldosterone secretion. Infusions of norepinephrine had no effect on adrenocortical secretion despite marked blood pressor responses (Table VII).

To determine whether the effects of angiotensin II were transient or persistent, 4.5-hour infusions of angiotensin II, at the rate of 0.167 μg per minute, were carried out in two hypophysectomized, nephrectomized dogs. The results from one of these animals are shown in Figure 5. No cumulative effect on corticosterone or 17-hydroxycorticoid secretion occurred. Aldosterone secretion rate rose several-fold immediately and continued well above the control secretion rate throughout the experiment. The 17-hydroxycorticoid secretion remained low and did not change significantly. Injection of 5 mU of ACTH increased 17-hydroxycorticoid and corticosterone secretion markedly while increasing aldosterone

TABLE VII

The effect of infusions of norepinephrine upon adrenocortical secretions of hypophysectomized, nephrectomized dogs

Assay dog	Dose	Δ Blood pressure S/D	Steroid secretion: Aldosterone Pre	Aldosterone Post	Corticosterone Pre	Corticosterone Post	17-OH corticoids Pre	17-OH corticoids Post
	μg/min/dog	*mm Hg*	*mμg/min*		*μg/min*		*μg/min*	
2–61	2.5	40/20	0	0	0.013	0.006	0.1	
2–61	12.5	135/85	0	1		0.011	0.1	0.1
49–61	12.5	160/85	3	3	0.019	0.014	0	0
50–61	12.5	120/80	4	7	0.062	0.085	0	0
42–61	12.5	40/25	0	0	0.25	0.31	1.2	1.5

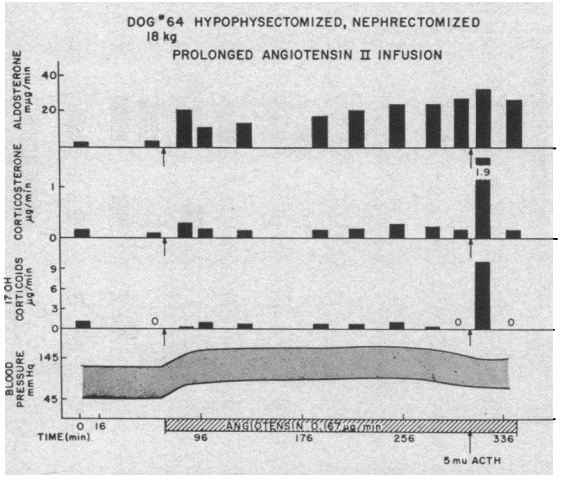

FIG. 5. THE EFFECT OF A PROLONGED INFUSION OF ANGIOTENSIN II UPON ADRENOCORTICAL SECRETIONS.

secretion only slightly. Similar results were obtained in the other dog.

In Figure 6 are summarized the effects of large and small doses of renin and small doses (2 to 10 mU) of ACTH on adrenocortical secretions. The p values were calculated by the paired *t* test. The small doses of ACTH increased corticosterone and 17-hydroxycorticoid as much and even greater than did the large doses of renin yet did not increase aldosterone secretion. Although the mean of the aldosterone secretion rates prior to ACTH injections is high, it can be seen from Table II

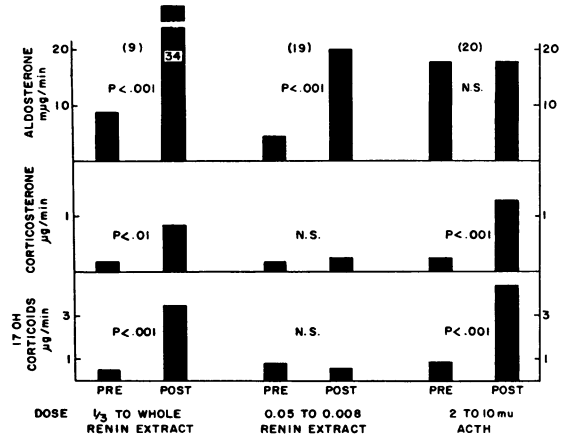

FIG. 6. A SUMMARY OF THE EFFECTS OF RENIN EXTRACTS AND SMALL DOSES OF ACTH UPON ADRENOCORTICAL SECRETION OF HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOGS. The dose of renin extract indicates what portion of the renin extract from a whole kidney was actually injected. The numbers in parentheses indicate the number of injections of each extract.

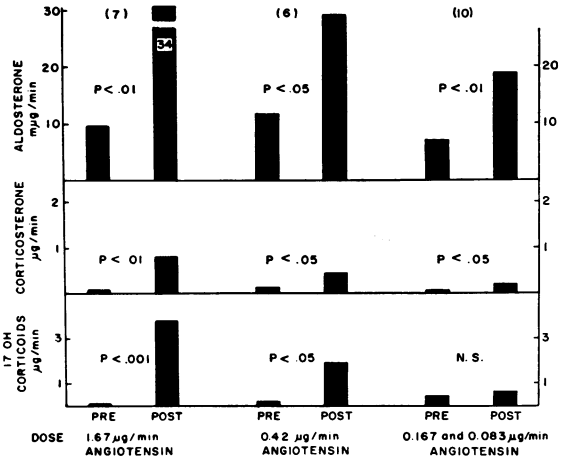

FIG. 7. A SUMMARY OF THE EFFECTS OF INFUSIONS OF DIFFERENT DOSES OF ANGIOTENSIN II UPON ADRENOCORTICAL SECRETIONS OF HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOGS. The numbers in parentheses indicate the number of infusions of each dose.

that even when the pre-ACTH secretion rates were low, small doses of ACTH did not increase aldosterone secretion significantly.

In Figure 7 are summarized the effects of different doses of angiotensin II on adrenocortical secretion. The pattern of response is similar to that following renin injections. The steroid secretion rates attained after the small doses of renin and angiotensin are very similar to those attained after hemorrhage in hypophysectomized dogs with intact kidneys (10).

DISCUSSION

The present data are evidence that the aldosterone-stimulating factor in kidney extracts is renin. Like the saline extracts of normal dog kidneys (3, 4) the large doses of renin extracts markedly stimulated aldosterone secretion and stimulated 17-hydroxycorticoids and corticosterone submaximally. There was a marked pressor response and a pressure curve typical for renin. Small doses of renin extract, as little as 0.008 of a dog kidney, also stimulated aldosterone secretion but not 17-hydroxycorticoid secretion. Although corticosterone was frequently increased, the per cent increase was less than aldosterone. If cortisol had been measured by the more sensitive double isotope derivative technique rather than 17-hydroxycorticoids by the Silber-Porter

method, an increase in cortisol secretion might have been detected. Nevertheless, the addition of 0.2 μg of cortisol to plasma of hypophysectomized dogs, which contained no measurable Silber-Porter chromogen, can be detected by the Silber-Porter method (11). Therefore it appears unlikely that any significant physiological change in cortisol secretion would go undetected.

Renin is an enzyme that acts upon a polypeptide substrate in plasma to form the decapeptide, angiotensin I. A specific converting enzyme in plasma converts angiotensin I into the potent pressor octapeptide, angiotensin II (12). Angiotensin II, therefore, should be able to duplicate the results of renin. The data demonstrate that the adrenocortical response to large and small doses of angiotensin II is similar to the response to large and small doses of renin.

The results obtained from renin and angiotensin are not due merely to pressor or nonspecific effects, since norepinephrine and control tissue extracts did not stimulate adrenocortical secretions.

Most of the evidence is against the possibility that ACTH in the kidney extracts was solely responsible for the adrenocortical response. Injections of both commercial ACTH and saline extracts of dog anterior pituitary produced a pattern of adrenocortical response different from that of the saline kidney extracts and the renin extracts; that is, small doses of ACTH increased 17-hydroxycorticoids and corticosterone secretion as much as did the large doses of renin, yet did not consistently or significantly increase aldosterone secretion. Nephrectomy does not alter the pattern of response or sensitivity of the adrenal cortex to ACTH in hypophysectomized dogs (4). Moreover, it is unlikely that the renin extracts of dogs hypophysectomized 4 hours prior to removal of the kidney would contain the same quantity of ACTH as the renin extracts of normal dog kidneys; yet the effect on adrenocortical secretion and blood pressure of the different types of extracts was about the same. The failure of injection of three of five saline extracts of hypophysectomized dog kidneys remains unexplained. Since the saline extracts were made from fresh, unfrozen tissue, only the readily available renin may have been extracted. It is possible that the extraction technique was inadequate, although the same technique was successful in extracting the active aldosterone-stimulating factor and pressor principle from five of five normal kidneys. On the other hand, the method of Haas and Goldblatt (9) for making renin extracts employs more vigorous extraction methods. Freezing and thawing of kidney tissue alone has been claimed to increase the extractable renin. Another possibility is that ACTH alters the availability of extractable renin. It has been claimed that granularity of the juxtaglomerular apparatus decreases after acute hypophysectomy, while administration of ACTH returns the granularity to normal (13). These latter findings, however, have been disputed (14).

The fact that corticosterone secretion was frequently increased by even the small doses of renin extract and angiotensin II should not be construed as evidence against the importance of the renin-angiotensin system in the adrenocortical stimulation that follows hemorrhage. According to Davis and co-workers (2) hemorrhage increases corticosterone secretion slightly in hypophysectomized dogs but not in hypophysectomized, nephrectomized dogs. These authors also state that nephrectomy lowers control corticosterone secretion rates in hypophysectomized dogs. The present authors found a slight increase in corticosterone secretion after hemorrhage in four dogs (10), but the results were not statistically significant, possibly due to the small number of animals.

The renin extracts, moreover, stimulated aldosterone secretion by a greater percentage than they stimulated corticosterone secretion and, after injections of large doses of renin, the increases in aldosterone secretion persisted for some time despite a decrease in corticosterone secretion after its initial increase. In several instances this pattern was also true for angiotensin II infusions. Despite the fact that small doses of renin increased aldosterone secretion to levels greater than those attained after hemorrhage in hypophysectomized dogs, corticosterone secretion rates were still very low and well within the range seen in hypophysectomized dogs (2, 10). Moreover, these low levels and the increments are probably of little physiological significance.

Davis and colleagues have reported somewhat conflicting results on the effect of the administra-

tion of kidney extracts upon aldosterone, corticosterone, and 17-hydroxycorticoid secretion. Constant infusions of saline homogenates of kidneys from normal dogs into hypophysectomized, nephrectomized dogs stimulated only aldosterone secretion (2); infusions of saline homogenates of kidneys from dogs with thoracic inferior vena caval constriction reportedly increased aldosterone in all experiments and corticosterone and 17-hydroxycorticoid in 50 per cent of the experiments (15). Only one experiment was shown in detail and demonstrated only a slight change in corticosterone secretion, despite a 25-fold increase in aldosterone secretion. Constant infusions of pressor doses of partially purified renin extracts of normal dog kidneys were reported to increase aldosterone, corticosterone, and 17-hydroxycorticoid (16). These apparent differences between the various extracts may well have been due to differences in dose of "renin" actually administered.

These present data and the previous reports (1–4), demonstrating that nephrectomy prevents the increase in aldosterone secretion that follows hemorrhage, indicate that the kidney via the renin angiotensin system is a major mechanism by which hemorrhage stimulates aldosterone secretion in hypophysectomized dogs. This mechanism probably plays a role in normal dogs also but, because of the release of ACTH by hemorrhage and surgical trauma, it would be difficult to determine its role. Large doses of ACTH can stimulate aldosterone secretion. In fact, when glucocorticoid secretion is maximal, especially after surgical trauma in acute experiments, addition of more ACTH stimulates only aldosterone secretion, as if it were a specific aldosterone-stimulating hormone (10). For these reasons, therefore, it was necessary to study this mechanism in hypophysectomized dogs.

The mechanism of renin and angiotensin II stimulation of the adrenal is most likely direct. Angiotensin II does stimulate aldosterone secretion by direct adrenal perfusion in the dog (17) *in vivo* and by addition to bovine adrenal slices (18).

Considerable indirect evidence suggests that the kidney and renin-angiotensin system may play a role in other physiological stimuli associated with increased aldosterone secretion. Deane and Masson (19) in 1951 reported that encapsulation of one kidney in the rat results in hypertension and hypertrophy of the zona glomerulosa of the adrenal, the site of aldosterone formation. Pasqualino and Bourne (20) have demonstrated that constriction of one renal artery in the rat leads to hypertrophy of the zona glomerulosa within a few days. Both Genest (21, 22) and Laragh (23) and their co-workers, have noted increases in urinary aldosterone in severe hypertension, and Kahn, Skeggs, Shumway and Wisenbaugh (24) have reported increased circulating angiotensin in malignant hypertension.

There is evidence that the renin-angiotensin system plays a role in the hyperaldosteronism due to salt depletion. Hartroft, Hartroft, Pitcock and Newmark (25, 26), using rats, reported that salt depletion increased the granulation of the juxtaglomerular cells and the renin content of the kidney and caused hypertrophy of the zona glomerulosa. Salt loading had the reverse effect. During the preparation of this manuscript Davis, Ayers and Carpenter reported that nephrectomy lowered aldosterone secretion in hypophysectomized dogs with sodium depletion and thoracic inferior vena caval constriction (15).

These data, therefore, support the hypothesis that juxtaglomerular cells are volume- or baroreceptors which release renin in response to changes in intravascular volume or pressure (6). The renin augments blood pressure and increases volume by stimulating aldosterone secretion, which in turn increases salt and water retention by the kidney.

Although this scheme presents an appealing picture, the relative importance of this mechanism in the intact animal remains to be determined. The possibility that other mechanisms play as important or a more important role exists. Farrell (27) recently presented preliminary results indicating that a highly purified extract from the pineal gland is a potent stimulator of aldosterone secretion. These results await confirmation.

Another point that should be kept in mind is that angiotensin II is a potent pressor agent, yet many conditions associated with hyperaldosteronism have normal blood pressure. Although

Laragh (28) states that in cirrhotic patients with ascites there is a decreased sensitivity to the pressor effects of angiotensin, this may not be true in other conditions.

SUMMARY

The nature of the aldosterone-stimulating factor in dog kidneys has been studied. Injections of small doses of renin-containing extracts of dog kidneys and synthetic angiotensin II stimulated aldosterone secretion in hypophysectomized, nephrectomized dogs. Secretion of 17-hydroxycorticoids was not stimulated. A slight increase in corticosterone and blood pressure did occur. In contrast, small doses of ACTH stimulated 17-hydroxycorticoid and corticosterone but not aldosterone secretion. The effect of extracts of dog anterior pituitaries simulated ACTH injections. Control tissue extracts and norepinephrine infusions had no significant effect on aldosterone secretion. These data suggest that the aldosterone-stimulating factor in dog kidneys is renin.

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