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Research Article

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INFLUENCE OF THE PITUITARY AND THE RENIN–ANGIOTENSIN SYSTEM ON THE SECRETION OF ALDOSTERONE, CORTISOL, AND CORTICOSTERONE

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There is evidence suggesting that the control of aldosterone secretion is relatively independent of the pituitary gland. This is based on morphological studies in the rat (1), on indirect assessments of adrenal secretion during metabolic studies in man (2), and on direct estimations of adrenal hormone secretion in the rat (3, 4) and dog (5–7). The morphological studies of Deane and Masson (8), which indicate that renin may stimulate the adrenal cortex in the rat, the results of Genest and his colleagues (9), which suggest that arterial hypertension may be associated with an increased excretion of aldosterone in man, and the demonstration by Genest (10, 11) and Laragh (12) and their colleagues that angiotensin II stimulates excretion (10, 11) and secretion (12, 13) of aldosterone suggest that the kidney may have some influence on the rate of secretion of adrenal hormones. Recently, Pronove, MacCardle, and Bartter (14) described a dwarfed, normotensive child who was suffering from the effects of excessive production of aldosterone. This was associated with a remarkable hypertrophy and hyperplasia of the juxtaglomerular apparatus in the kidney. The syndrome has been found subsequently in two other patients with hyperaldosteronism (15, 16). The observations suggested that the kidney itself was in some way responsible for the overproduction of aldosterone. The present experiments, performed in the dog, were designed to test the hypothesis that the normal kidney contains material capable of stimulating the adrenal cortex. This was found to be so (17, 18), in agreement with results simultaneously obtained by Mulrow, Ganong, Cera, and Kuljian (19) and by Davis and associates (20, 21). Accordingly, further studies were designed to see whether the pattern of response of the three important adrenocortical steroid hormones (aldosterone, cortisol, and corticosterone) was such as to suggest that this renal stimulus is involved in the mediation of aldosterone secretion physiologically.

MATERIAL AND METHODS

The effects of hypophysectomy in unanesthetized dogs were tested in eight dogs fed a diet containing 9 mEq of sodium and 186 mEq of potassium for 13 to 21 days. On day 1, the lumbo-adrenal vein was cannulated, and the right kidney was removed under Nembutal or ether anesthesia. On day 2, two adrenal blood samples were collected without anesthesia with the animal lying quietly. The pituitary was then removed under Nembutal or halothane anesthesia. The next morning (about 18 hours after hypophysectomy), two adrenal blood samples were again collected without anesthesia, with the animal lying quietly. All animals received 100 mg cortisone acetate intramuscularly on the day of hypophysectomy.

Infusion studies. Male mongrel dogs weighing between 16.4 and 26.5 kg were anesthetized with Nembutal, and the pituitary was removed by a buccal approach 2 to 4 hours before the beginning of each experiment, except as otherwise indicated. Both kidneys were removed between double ligatures on the renal pedicles, and a cannula was inserted into the right lumbo-adrenal vein by the method of Hume and Nelson (22). During surgery, 1.5 to 2.0 L of 0.15 M sodium chloride was given intravenously. After the abdomen was closed, the animal was left undisturbed for 1 to 2 hours. During the experiment, 20 to 30 ml of adrenal venous blood was collected over 4 to 20 minutes. This was immediately replaced by the iv infusion of dog blood taken from either saline-loaded normal animals or saline-loaded, hypophysectomized, nephrectomized animals.

As judged from the basal rate of secretion (not corrected for hematocrit) of Porter-Silber chromogen (less than 1.2 μg per minute) or that of cortisol (less than 500 mμg per minute), hypophysectomy appeared to have successfully prevented adrenocorticotropin (ACTH) release in all except dogs 7, 10, and 11. Femoral arterial and venous pressures were measured continuously with Statham strain gauges on a Sanborn recording system; an electrocardiogram was also recorded.

The materials being studied were dissolved or suspended in 0.15 M sodium chloride and infused intravenously by a Bowman pump. Changes in rate of administration were made by altering the concentration

TABLE I

Granularity of the juxtaglomerular cells in normal dogs given extra NaCl and desoxycorticosterone acetate and in hypophysectomized dogs deprived of NaCl

	Animals	Glomeruli counted	Glomeruli granulated		JGI*
	no.	*no.*	*no.*	*%*	
Na-deprived	2	689	13	1.9	7.1
Na-loaded	3	658	3	0.4	1.2

* Index of juxtaglomerular granulation (Hartroft, P. M., and W. S. Hartroft. Studies on renal juxtaglomerular cells. I. Variations produced by sodium chloride and desoxycorticosterone acetate. J. exp. Med. 1953, **97**, 415).

in the infusion fluid rather than the rate of infusion. Cortisol hemisuccinate was given intravenously throughout some experiments by a steady, slow drip.

Renin was prepared by the method of Haas and Goldblatt (23). For the experiments shown in Figures 4 and 5, a lyophilized preparation was used, whereas for the experiments shown in Table IV, the renin was freshly prepared from the kidneys of dogs treated with Mercuhydrin and a low-sodium diet for the preceding 4 days. The potency of both preparations was assayed by the method of Goldblatt, Katz, Lewis, and Richardson (24). The renin used in the experiment shown in Figure 5 was inactivated by prolonged storage.

The angiotensin used was a synthetic preparation supplied as the valine-5 aspartic β-amide.[1]

The studies on the patients mentioned above suggested an effect of the juxtaglomerular apparatus on the adrenals. As the granulation in juxtaglomerular cells may be altered experimentally (25), we prepared extracts from kidneys with few granules and others from kidneys with many granules in the juxtaglomerular cells. They were made by homogenizing the whole kidney with approximately twice its weight of 0.15 M sodium chloride in a Waring Blendor. Each recipient dog received in succession extract of kidneys from two donor dogs. There was an hour's wait between infusions. Donors of group A had been fed a normal dog diet supplemented with 170 mEq of sodium chloride daily (total sodium intake about 186 mEq daily) and injected with 10 mg of desoxycorticosterone acetate intramuscularly daily for 13 to 19 days. In one experiment the dog was hypophysectomized 2 hours before removal of the kidney. Donors in group B had been hypophysectomized 14 to 34 days previously and had received a diet containing 9 to 16 mEq of sodium chloride and approximately 100 mEq of potassium for the previous 14 days. Hypophysectomized dogs were given cortisone, 25 mg a day, intramuscularly. As shown in Table I, granulation of the juxtaglomerular cells was effectively altered by these procedures ($p < 0.05$).[2]

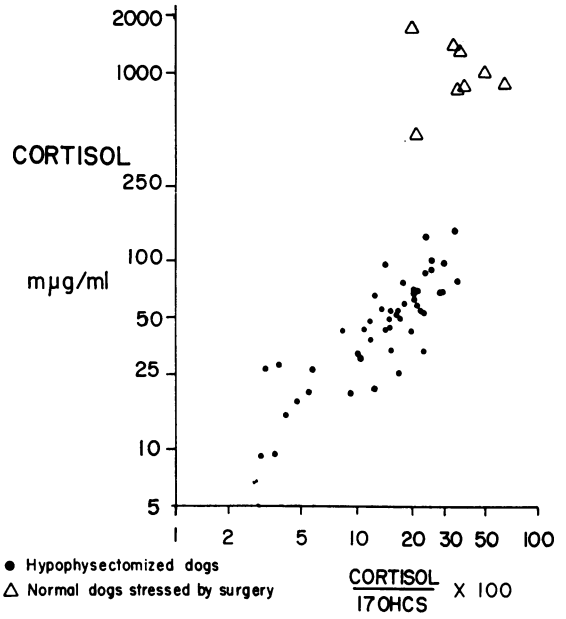

FIG. 1. RELATIONSHIP BETWEEN THE CONCENTRATION OF CHROMATOGRAPHICALLY PURE CORTISOL AND PORTER-SILBER CHROMOGEN IN THE ADRENAL VENOUS PLASMA OF HYPOPHYSECTOMIZED DOGS AND OF NORMAL DOGS STRESSED BY SURGERY. Data illustrate the limitation of using the Porter-Silber chromogen as an index of cortisol concentration.

The homogenate was centrifuged for 30 minutes at $2000 \times g$ and the supernatant fluid was infused at a rate delivering the extract from 0.85 to 3.2% of the whole kidney per minute.

Hog ACTH was used either as a lyophilized powder[3] or as a gel,[4] and the adrenocortical response was measured at the end of a 3- to 4-hour experiment in nephrectomized dogs whose pituitary glands had been removed on the morning of the same day. The effect of ACTH was measured in blood samples started 4 minutes after the beginning of the infusion. The ACTH preparation made by the National Drug Company had lost much of its stated potency, so that the doses actually administered were calculated from the results of bioassay in hypophysectomized rats.[5]

Chemical estimations. 17-Hydroxycorticosteroids (17-OHCS) were measured in 2 to 3 ml of adrenal venous plasma by the method of Porter and Silber as modified by Peterson, Karrer, and Guerra (26). This method does not measure cortisol specifically, so that although it provides a reasonable index of cortisol secretion at high cortisol concentrations, it fails to do so at the very low

[1] The authors wish to thank Drs. R. Gaunt and N. Sullivan of Ciba and Co., Summit, N. J., for generous supplies of angiotensin.

[2] We are grateful to Dr. Ross C. MacCardle for providing these data. Fresh kidney slices were fixed in 5% Zenker's formol for 48 hours. Sections were cut 4 μ thick and colored by Bowie's method with ethylviolet-Biebrich scarlet (Anat. Rec. 1935-36, **64**, 357-367).

[3] Upjohn Co., Kalamazoo, Mich.

[4] National Drug Co., Philadelphia, Pa.

[5] Kindly performed by Dr. G. Liddle.

concentrations seen after hypophysectomy. Figure 1 shows the true cortisol content (see below) of adrenal venous plasma collected under varying experimental conditions expressed as a proportion of the Porter-Silber chromogen content. Because of their low specificity when cortisol values are low, measurements of the Porter-Silber chromogen were used only for assessing the response to maximal amounts of ACTH and angiotensin.

Aldosterone, cortisol, and corticosterone were measured in 5 ml of adrenal venous plasma by a double-isotope derivative method using tritium-containing acetic anhydride with a specific activity of 100 mc per mmole. The dichloromethane (10:1) extract of plasma was washed successively with 0.05 N NaOH, 0.1 N acetic acid, and water and then treated with 10% H^3-acetic anhydride in pyridine (redistilled over glacial acetic acid) for 72 hours at 37° C. The acetylated products were then purified by paper partition chromatography, with the three systems described by Kliman and Peterson (27). Twenty μg of a standard solution of each steroid acetate was added for its identification by ultraviolet light absorption. The three spots on the first chromatogram (run in benzene: cyclohexane: methanol: water, 50: 100: 100: 25) were cut out, and the one corresponding to aldosterone diacetate was run separately in cyclohexane: dioxane: methanol: water, 100: 100: 50: 25, oxidized with 0.5% chromic acid for 10 minutes and finally run again on the first system. The cortisol monoacetate and corticosterone monoacetate spots on the first chromatogram were combined and run on the second system, which gave good separation of the two compounds. In most instances, the cortisol and corticosterone monoacetates were then oxidized with 0.5% chromic acid for 5 minutes. The resulting 11-dehydrocorticosterone and cortisone monoacetates were usually run separately on the cyclohexane: dioxane: methanol: water system and a carbon tetrachloride: methanol: water (100: 100: 25) system, respectively.

In experiments 1 to 8, free 4-C^{14} steroid was added to the initial plasma sample. Unfortunately, the specific activity of these standards was such that acetylation of the internal standard often contributed a considerable proportion of the final tritium counts (for example, standard solution contributed 76% of counts when only .03 μg of aldosterone was contained in sample). This introduced a greater error than that resulting from the unmeasured, but almost certainly small, losses incurred before and during acetylation. Accordingly, in all other experiments, internal standards labeled with C^{14} in position 4 were added as the acetates after acetylation, according to the initial procedure described by Kliman and Peterson (27).

Calculation of steroid secretion rate. The results are expressed as millimicrograms per adrenal per minute. The adrenal plasma flow was calculated from the total blood flow and the hematocrit of the adrenal venous blood. The red blood cells of the dog were found to take up 7 H^3-aldosterone, 4-C^{14}-cortisol, and 4-C^{14}-corticosterone from dog plasma giving a plasma: red blood cell distribution approaching 1: 1. (The uptake requires less than one minute.) Therefore, since the relevant steroids were measured only in samples of plasma, the real secretion rates of these steroids are approximately double the values reported in this paper. A similar qualification applies to the result obtained by others (28, 29). In these experiments, variations of hematocrit were

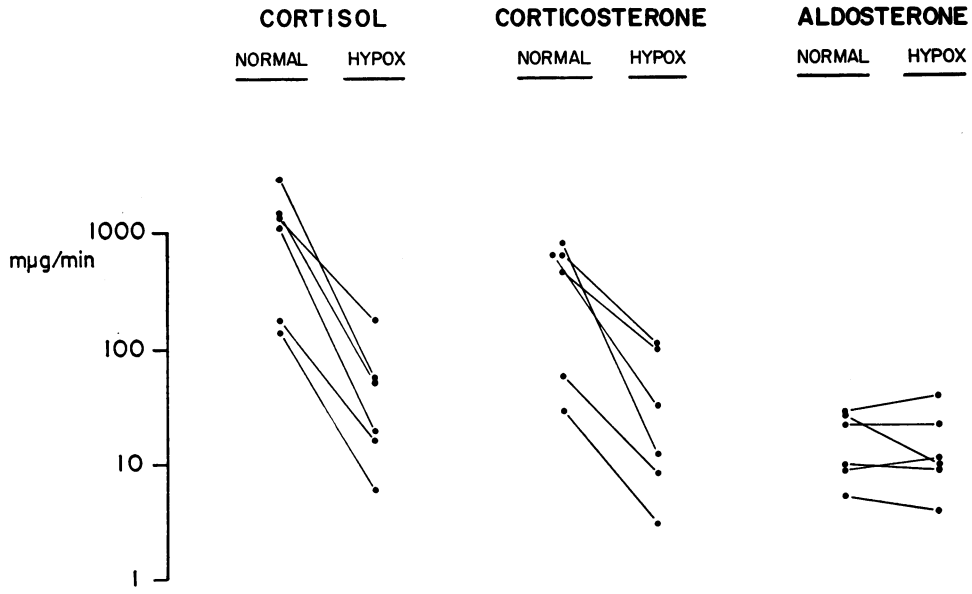

FIG. 2. RATE OF ADRENAL STEROID HORMONE SECRETION BEFORE AND 18 HOURS AFTER HYPOPHYSECTOMY IN SIX CONSCIOUS OR LIGHTLY ANESTHETIZED DOGS ON LIMITED NaCl INTAKE. Figures are plotted logarithmically to emphasize proportional changes.

TABLE II

Effect of hypophysectomy 18 hours previously on the rate of cortisol, corticosterone, and aldosterone secretion in six dogs deprived of NaCl

Dog	Cortisol		Corticosterone		Aldosterone	
	Before	After	Before	After	Before	After
	mμg/min		*mμg/min*		*mμg/min*	
Joe	1,092	26	834	12	23.7	8.5
Rover	2,828	74	614	108	20.1	19.7
Jeff II	1,414	70	623	31	9.0	8.6
Randy	198	23	56	8	8.3	12.1
Walker	136	8	28	3	4.7	11.4
Jack	1,312	233	442	99	25.3	31.9

small and random, so that corrections for red-cell uptake have not been made. A detailed study of the uptake of adrenocortical steroids by red blood cells will be published separately.

Limitations of Student's t test. These experiments and those of others (28, 29) have been designed so that each animal acts as its own control. The results should, therefore, be analyzed by the paired *t* test. But the magnitude of the response to any stimulus is much less variable for aldosterone than for corticosterone or cortisol. Since Student's *t* test depends not only on the difference of the means but also on the scatter of values, the use of the paired *t* test will give lower p values in groups showing a small scatter even when the mean change is smaller. When the scatter of the magnitude of the response is considerable, this leads to the paradoxical situation whereby removal of the animals showing the greatest response improves the statistical significance of the change for the group as a whole. For example, angiotensin at 0.17 to 0.5 μg per minute stimulated the production of corticosterone in all eight animals and of cortisol in six animals, but the responses were not significant ($p < 0.2 > 0.1$) when assessed by the paired *t* test. If the two animals showing the greatest rise (dogs 10 and 16) are excluded, however, the rise of corticosterone secretion in the remaining six animals becomes significant ($p < 0.05 > 0.02$), although the mean increase is one-sixth as great. If, on the other hand, all eight studies are analyzed by comparing the logarithms of the results (ratio change), the effect is significant ($p < .01$ and $p < .05$ for corticosterone and cortisol, respectively). In keeping with the usual practice, we have, nevertheless, analyzed our data by Student's *t* test, as it does not appear that more sophisticated statistical treatment of the data would change their physiological significance.

RESULTS

Adrenal effects of hypophysectomy. The results of removing the pituitary gland 18 hours

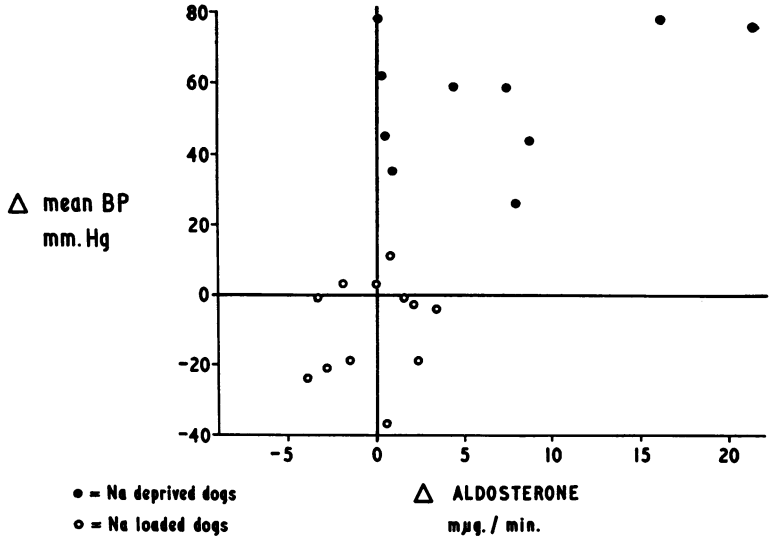

FIG. 3. RELATIONSHIP BETWEEN CHANGE OF MEAN SYSTEMIC BLOOD PRESSURE AND CHANGE OF ALDOSTERONE SECRETION IN RESPONSE TO AQUEOUS EXTRACTS OF KIDNEYS FROM NA-DEPRIVED DOGS OR NA-LOADED DOGS. Values are differences from the mean control value.

TABLE III

A comparison in four acutely hypophysectomized, nephrectomized dogs of some effects of an iv infusion of aqueous extracts of kidney made from (A) hypophysectomized dogs (experiments 1, 2, 4) or a normal dog (experiment 3) receiving NaCl and desoxycorticosterone acetate and (B) hypophysectomized dogs fed low-sodium diets

			High-salt kidney extract (A)					Low-salt kidney extract (B)				
			Before		Infusion			Before		Infusion		
Experiment		Min:*	−14 −25	−7 −13	4	15–20	24–31	−16 −18	−7 −10	0–4	13–21	28–41
	Aldosterone	mμg/min	2.6	2.5	2.5	4.6	0.6		0.5	9.2	1.0	
	Cortisol	mμg/min										
1e	Hematocrit	%	71.5	71.0	72.0	75.5	75.0		75	74.5	75.0	
6/6/61	Adrenal blood flow	ml/min	2.33	2.33	1.00	2.10	1.42		0.68	1.04	0.92	
	Mean arterial blood pressure	mm Hg	90	96	96	90	96		108	152	153	
	Aldosterone	mμg/min	5.1	5.7	2.6	3.9	1.5	2.1	1.5	2.7	9.7	36.8†
	Cortisol	mμg/min	50	56	29	12	26	75	25	131	207	153
2e	Hematocrit	%	47.5	47.5	48.5	49.0	49.5	57.5	58.0	59.0	60.5	61.5
6/8/61	Adrenal blood flow	ml/min	4.07	3.64	2.06	1.91	1.87	2.95	2.75	3.34	4.39	1.91
	Mean arterial blood pressure	mm Hg	51	51	30	33	27	54	51	87	78	45
	Aldosterone	mμg/min	1.0	1.9	2.2	2.0	3.8	2.1	1.5	2.1	6.2	9.7
	Cortisol	mμg/min	254	256	189	76	53	66	71	89	140	194
3e	Hematocrit	%	43.7	44.0	46.5	48.0	53.0	52.0	55.2	55.5	59.5	61.0
7/25/61	Adrenal blood flow	ml/min	4.57	4.38	3.18	2.32	1.94	3.88	3.65	3.00	4.00	3.67
	Mean arterial blood pressure	mm Hg	75	84	90	42	60	78	72	135	132	132
	Aldosterone	mμg/min	5.6	6.5	9.4	7.6	2.7	2.8	3.3	3.1	19.0	24.2
	Cortisol	mμg/min	392	405	393	374	298	32	43	379	548	564
4e	Hematocrit	%	58.5	59.0	59.0	59.0	60.0	47.0	47.0	49.0	51.0	53.0
7/27/61	Adrenal blood flow	ml/min	4.72	4.29	4.93	4.94	5.29	3.34	4.22	4.22	4.72	4.38
	Mean arterial blood pressure	mm Hg	128	120	120	123	123	57	57	135	135	129
	Aldosterone			3.86	+0.32	+0.67	−1.71		2.01	+2.27	+6.97	+14.9
Mean	Cortisol			236	−32	−82	−110		52	+148	+246	+327
figures	Hematocrit			55.3	+1.2	+2.6	+4.1		56.0	+3.5	+5.5	+1.0
	Adrenal blood flow			3.78	−.99	−.96	−1.15		3.11	−.21	+.40	+.91
	Mean arterial blood pressure			87	−3	−15	−11		68	+59	+56	+62

* Time of starting the collection of adrenal venous blood from beginning of infusion.
† These values have been omitted from Figure 3 as well as from the calculation of the mean changes because the animal died shortly afterwards.
‡ Infusion with kidney extract from Na-deficient animal given first.
§ These are the preinfusion figures followed by the changes during the infusion.

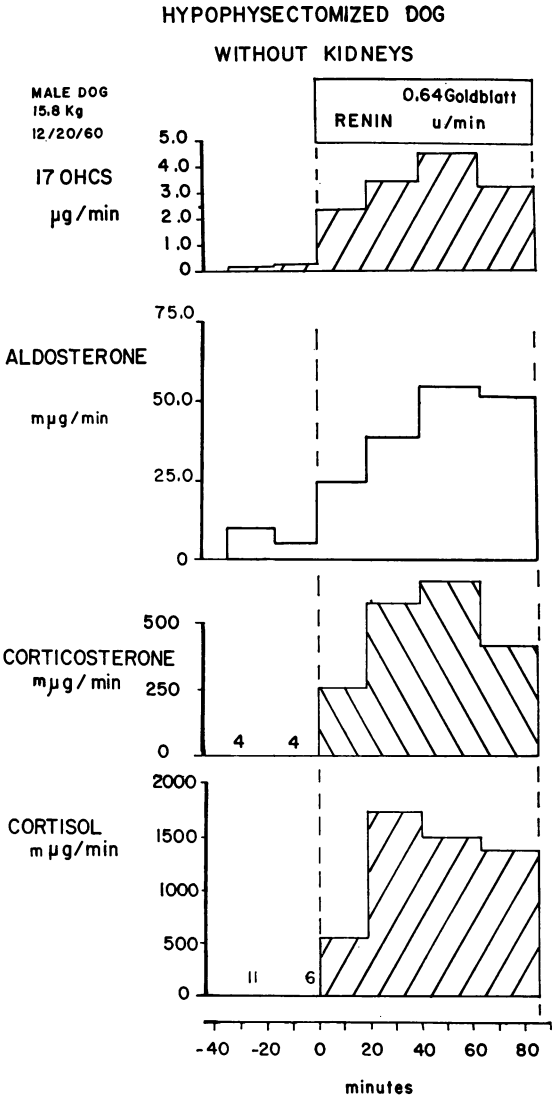

FIG. 4. ADRENAL EFFECTS OF A LARGE DOSE OF RENIN.

previously on six unanesthetized, sodium-deprived dogs are shown in Figure 2 and Table II. It is clear that the rate of aldosterone secretion did not change significantly ($p > .5$), despite a large fall in the rate of cortisol and corticosterone secretion.

Effects of kidney extracts. Table III shows the data from eight experiments performed in the four dogs infused at rates delivering more than 2.0% of the whole kidney per minute. Extracts of the kidneys from hypophysectomized dogs deprived of salt (B) consistently raised aldosterone and cortisol secretion as well as the mean arterial blood pressure. The mean figures 28 to 41 minutes after the beginning of the infusions show that

TABLE IV

Effect of a renin preparation infused slowly (0.16 Goldblatt U per minute) on steroidogenesis and arterial blood pressure in acutely hypophysectomized, nephrectomized dogs

Dog	Aldosterone			Cortisol			Corticosterone			Mean blood pressure		
	Before	4–28*	34–54*	Before	4–28*	34–54*	Before	4–28*	34–54*	Before	4–28*	34–54*
	mµg/min			*mµg/min*			*mµg/min*			*mm Hg*		
1R	2.6	2.3	1.6	24	39	31	18	22	20	84	104	104
2R	4.4	6.5	8.7	67	77	101	45	63	77	104	122	136
3R	1.1	3.0	7.3	17	28	42	7	12	33	80	96	92
4R	1.9	5.3	11.9	21	20	72	10	18	82	120	150	160
5R	8.2	11.7	14.1	24	60	35	5	17	68	130	140	139
6R	4.6	5.1	7.8	10	13	23	12	20	47	120	148	160
Mean	3.7	5.4	8.6	27	39	51	16	25	54	106	127	132
p†		<0.05	<0.05		<0.1	<0.02		<0.02	<0.02		<0.01	<0.01

* Time in minutes of blood sample collection after beginning of renin infusion.
† Estimated by the paired *t* test.

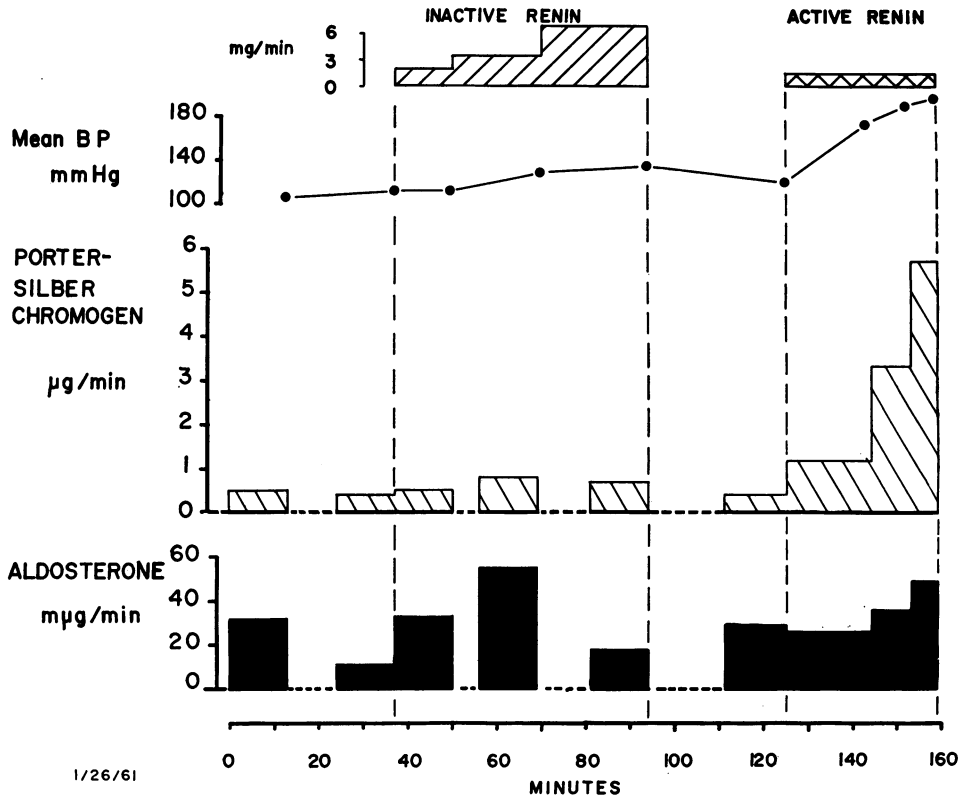

FIG. 5. COMPARISON BETWEEN INFUSIONS OF AN INACTIVE AND AN ACTIVE PREPARATION OF RENIN ON ADRENAL STEROID SECRETION AND ARTERIAL BLOOD PRESSURE IN A HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOG.

the secretion of aldosterone had risen over 7 times and that of cortisol over 6 times ($p < 0.02$ for aldosterone and $p < 0.01$ for cortisol by the paired t test). The mean blood pressure rose by 62 mm Hg, or 91%. Adrenal blood flow and hematocrit did not change significantly. In contrast, the perfusion of extracts of kidneys taken from salt-loaded animals (A), whether hypophysectomized or not, failed to increase the secretion of aldosterone or of cortisol. The secretion rate of cortisol actually fell considerably. The arterial blood pressure and adrenal blood flow both fell slightly. The effect on aldosterone secretion and on mean arterial blood pressure is shown in Figure 3. Nine other dogs received infusions at slower rates(delivering between 0.85% and 1.5% of the whole kidney extract per minute). Only one of these animals showed an increase of steroidogenesis or of arterial blood pressure when infused with extracts of kidneys taken from a hypophysectomized sodium-deprived dog.

Effects of renin. In the experiments described above, crude aqueous extracts of the kidney were capable of stimulating the adrenal cortex only if the granules in the juxtaglomerular cells of the extracted kidney were increased in quantity. Since these granules are thought to be the source of renin, and since renal extracts were effective only if they raised the blood pressure (an effect suggesting the presence of renin), the experiments illustrated in Figures 4 and 5 and Table IV were performed. Clearly, large amounts of renin stimulated the production of aldosterone and of both corticosterone and cortisol to levels not clearly different from those seen in this animal under maximal stimulation by ACTH (Figure 4). When a preparation of renin that had lost most of its hypertensive action was contrasted with that of an active sample (Figure 5), the adrenal effect, which appeared largely as an increase in the rate of 17-OHCS secretion, was again associated with the hypertensive effect. Table IV shows

the results obtained by infusing renin slowly (0.16 Goldblatt U per minute) into acutely hypophysectomized, nephrectomized dogs. The arterial blood pressure rose moderately in all animals, and there was a small but significant increase in the rate of secretion of cortisol and corticosterone as well as that of aldosterone. No clear evidence of an exclusive effect on aldosterone secretion could be detected. The rate of steroidogenesis and the level of the blood pressure were the highest during the second 30-minute period of the hour's infusion.

Angiotensin II. Much work has been done (30, 31) to show that the hypertensive action of renin depends upon its ability to form angiotensin II from plasma. From Table V, dogs 1 through 7, and Figure 6, it is clear that the adrenal effects of large amounts of synthetic valine-5 asparaginyl angiotensin II are very similar to those of hog renin; there is a considerable increase of the secretion of Porter-Silber chromogen as well as of aldosterone. The mean increase over the control value in blood samples started 3 to 21 minutes after the beginning of the infusion was nearly 400% for Porter-Silber chromogens, and 320% for aldosterone (Table V, dogs 1 through 7).

The duration of response. From Figure 6 it is clear that increased secretion of aldosterone and of Porter-Silber chromogen was maintained for the duration of the infusion (about 60 minutes) with large amounts of angiotensin, although there is a tendency for the response, like the blood pressure, to fall off.

Dose-response data. Figures 7 and 8 show a comparison of the effects of a small and a large dose of angiotensin in the same animal. In both studies, the smaller dose produced an effect on production of cortisol, corticosterone, or 17-OHCS; in one, it had a small effect on aldosterone

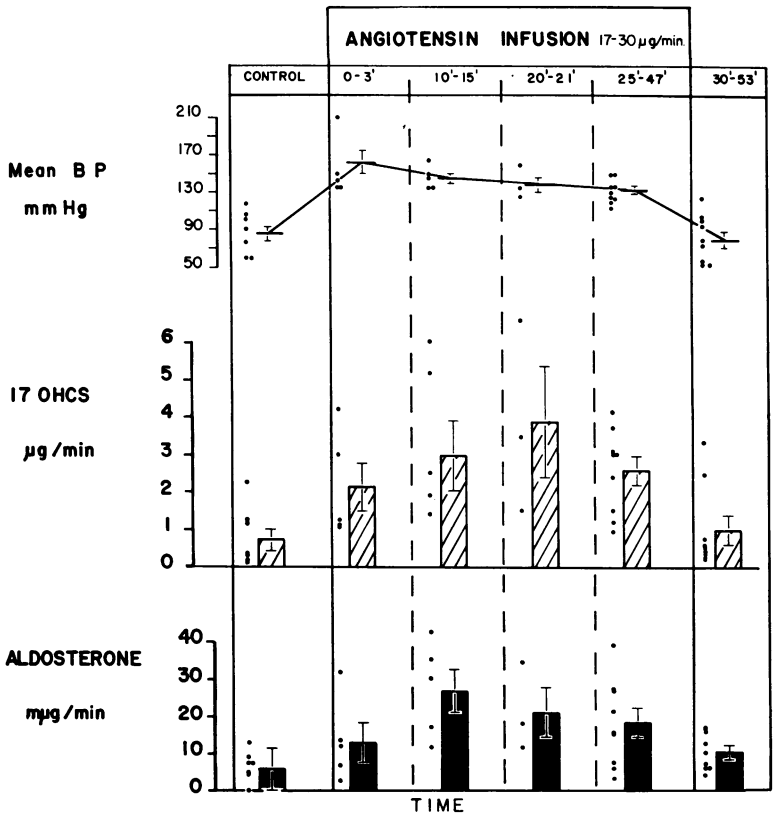

FIG. 6. EFFECT OF INTRAVENOUS INFUSIONS OF LARGE QUANTITIES OF ANGIOTENSIN II ON SYSTEMIC BLOOD PRESSURE AND ON ADRENAL STEROID SECRETION IN SEVEN HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOGS. ⊥ indicates SE of mean.

production; in the other it had none. To study this further, 28 angiotensin infusions at 5 different dose levels were given to 17 dogs. Details are shown in Table V.

The rate of 0.17 to 0.30 μg per minute was the lowest dose of angiotensin capable of increasing aldosterone production both significantly ($p < 0.05 > 0.02$) and consistently (7 out of 8 dogs). The mean increase of aldosterone production was just over 70%. The blood pressure also rose considerably in all animals (mean pressure increased by 40%), and the secretion rate of cortisol and of corticosterone rose in 6 out of 8 and in 8 out of 8 animals, respectively. The mean cortisol and corticosterone production increased by 120 and 157%, respectively; there was considerable variation in response from one animal to another, but the increase of the rate of corticosterone production is significant ($p < 0.02 > 0.01$) when expressed as a percentage change from the control

TABLE V

Dose-response data for angiotensin II in 17 hypophysectomized, nephrectomized dogs

Dog*	Dose	Aldosterone		17-Hydroxycorticosteroid		Corticosterone		Mean blood pressure		Blood flow	
		Before†	During‡	Before†	During‡	Before†	During‡	Before†	During‡	Before†	During‡
	μg/min	mμg/min		μg/min		mμg/min		mm Hg		ml/min	
6	30.0	12.5	30.2	1.22	2.50			105	165	4.4	6.6
7	30.0	7.3	35.2	2.26	6.04			102	135	5.1	5.7
1	17.0	9.2	33.9	1.16	3.51			60	126	5.5	6.7
2	17.0	0.0	17.8	0.10	6.64			60	135	2.1	6.1
3	17.0	4.0	16.7	0.33	1.41			90	135	4.0	5.4
4	17.0	4.6	11.2	0.31	1.90			78	143	3.0	4.5
5	17.0	7.3	42.3	0.15	5.20			117	150	4.7	6.8
Mean		6.4	26.8	0.79	3.88			87	141	4.2	6.0
				Cortisol, mμg/min							
9	3.0	1.7	8.5	106	224	94	193	63	126	3.9	0.9
10	3.0	6.7	16.6			141	1,117	72	174	2.6	1.8
11	3.0			1,693	2,960	623	1,020	81	150	4.3	5.3
12	3.0			82	1,534	44	85	60	135	2.3	3.7
8	1.7	10.6	12.0					75	141	3.9	2.5
Mean		6.0	12.4	627	1,573	225	604	70	145	3.4	2.8
16	0.50	6.2	11.8	220	797	135	444	60.0	123.0	2.8	2.7
15	0.30	4.6	7.5	21	20	41	43	72	108	2.7	2.9
11	0.30	14.6	14.6	29	31	38	74	66	111	4.4	4.8
9	0.30	1.8	16.4	128	167	98	124	90	108	5.3	5.0
12	0.30			11	22	21	33	54	84	2.1	3.2
10	0.27	8.9	20.0	486	1,020	225	790	105	168	3.0	2.0
8	0.17	10.6	12.0					99	108	4.2	2.9
13	0.17	5.3	6.5	35	34	4	16	129	150	4.2	3.9
14	0.17	7.9	13.3	54	67	73	104	96	111	4.1	3.3
Mean		7.5	12.8	123	270	79	203	85	119	3.5	3.4
12	0.03			27	69	75	64	90	90	3.3	3.2
9	0.03	0.5	1.9	126	138	139	115	108	114	5.6	5.4
11	0.03	16.0	22.4	46	85	87	90	105	111	5.2	5.0
15	0.03	4.5	6.3	55	64	34	67	108	111	3.3	3.2
10	0.03	8.7	13.4	759	1,090	318	347	114	135	4.0	3.6
13	0.03	4.2	4.7	19	39	8	6	141	138	4.6	4.6
14	0.03	14.1	15.1	71	63	72	72	99	108	6.0	5.1
16	.025	1.8	10.1	95	250	53	154	63	66	2.6	2.4
Mean		7.1	10.5	150	225	98	114	104	109	4.3	3.7
13	0.017	5.6	1.3	23	16	5	3	108	120	4.6	4.3
14	0.017	26.6	26.4	71	97	31	13	105	114	8.5	8.6
17	0.017	17.6	22.3	12	43	6	18	96	87	3.3	3.8
Mean		16.6	16.7	35	52	14	11	103	107	5.5	5.6

* Time since hypophysectomy: Dog 17, 24 hr.; dogs 10, 13, and 14, 7 to 8 days; and dogs 3 and 9, 14 and 35 days, respectively; other 11 dogs, 2 to 3 hours. In dogs receiving more than one infusion, 30 to 60 minutes were allowed between infusions.

† Blood sample collected immediately before the beginning of the angiotensin infusion.

‡ Blood sample collection started 3 to 21 minutes after the beginning of the angiotensin infusion.

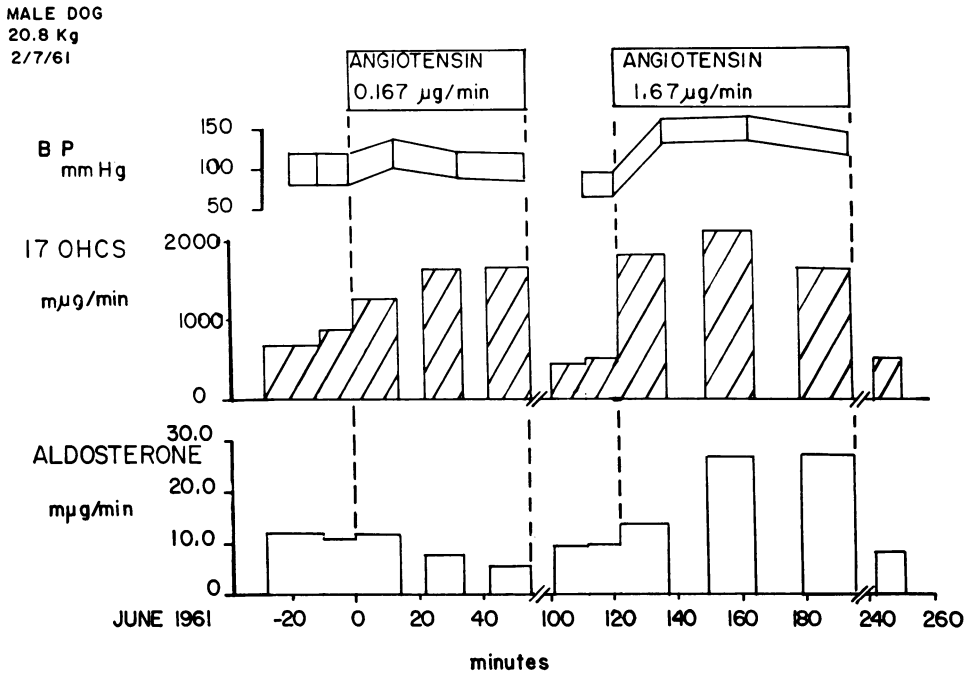

FIG. 7. COMPARISON OF A BARELY PRESSOR DOSE OF ANGIOTENSIN II WITH A CLEARLY PRESSOR DOSE IN A HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOG.

value. The cortisol figures are not significant by Student's *t* test, however expressed, owing to the large variability of response (see Methods).

Infusions at the rate of 0.025 to 0.030 μg per minute also increased aldosterone secretion significantly ($p < 0.05 > 0.02$), but to a smaller extent. The mean increase of aldosterone production was 48%. The blood pressure increased only slightly (mean pressure increased by 5%) at this low rate of infusion. Again the secretion rate of cortisol and corticosterone increased (cortisol by 50% and corticosterone by 16%), but the response was so variable that the changes are not significant statistically.

Three dogs were given infusions at the rate of 0.017 μg per minute. No effect on blood pressure, aldosterone, or corticosterone secretion could be detected, but there was probably some stimulation of cortisol production.

Thus, the smallest dose of angiotensin that is capable of stimulating aldosterone production in the hypophysectomized, nephrectomized dog probably lies between 0.017 and 0.030 μg per minute. The data suggest that the secretion rates of all three steroids (aldosterone, cortisol, and corticosterone) are stimulated to a comparable extent at all dose levels, with the possible exception of the response at .025 to .030 μg per minute. There is certainly no clear evidence of a specific effect of angiotensin II on the production of aldosterone. A definite though small stimulation of aldosterone production could be obtained with amounts of angiotensin that increased the blood pressure only very slightly.

Adrenal response to ACTH. Figure 9 shows the adrenocortical response to exogenous ACTH at four different dose levels.

Large doses of ACTH. At doses of 1.2 to 3.4 U per minute, the response of the adrenal cortex was probably maximal. The mean secretion rate of 17-OHCS in five normal dogs stressed by laparotomy (data not shown) was 6.78 μg per minute as compared with 5.23 μg per minute after these large doses of ACTH. These figures do not differ significantly ($p > 0.05$). The maximal effect of ACTH on aldosterone production is to increase it by about 140%. In contrast, large amounts of angiotensin increased aldosterone secretion by over 300%. The maximal effect of ACTH on 17-OHCS production is to produce a 500% increase. In contrast, large amounts of

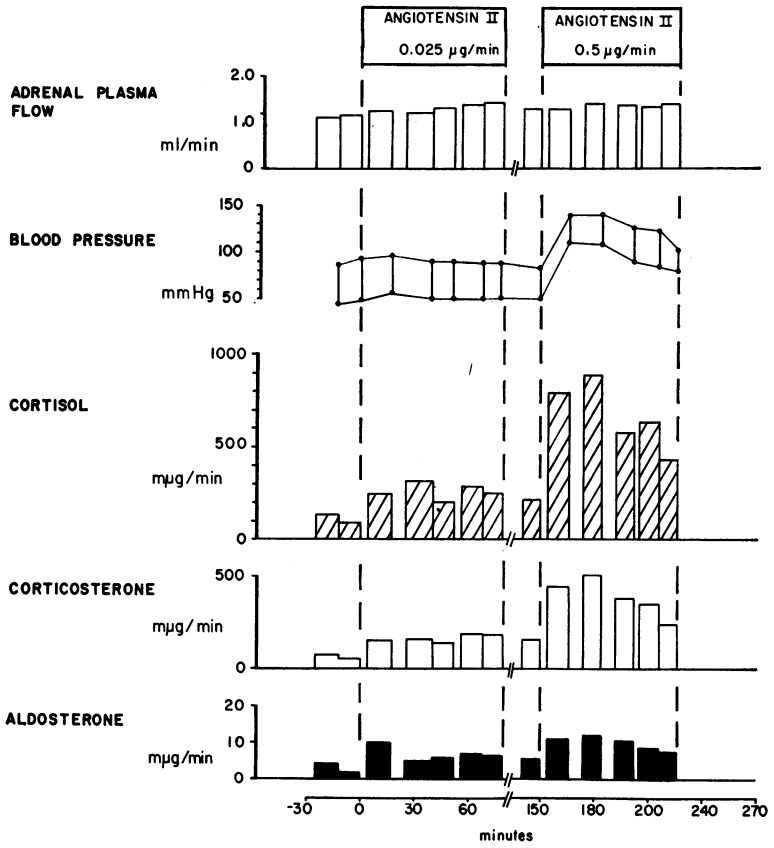

FIG. 8. COMPARISON OF THE ADRENAL EFFECTS OF A NONPRESSOR DOSE OF ANGIOTENSIN II WITH THOSE OF A PRESSOR DOSE IN A HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOG.

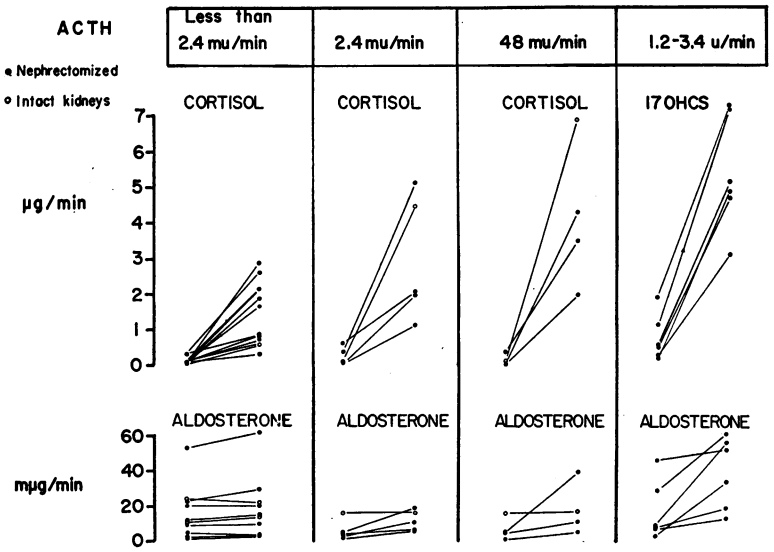

FIG. 9. ADRENOCORTICAL RESPONSE TO ACTH AT FOUR DIFFERENT DOSE LEVELS IN HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOGS.

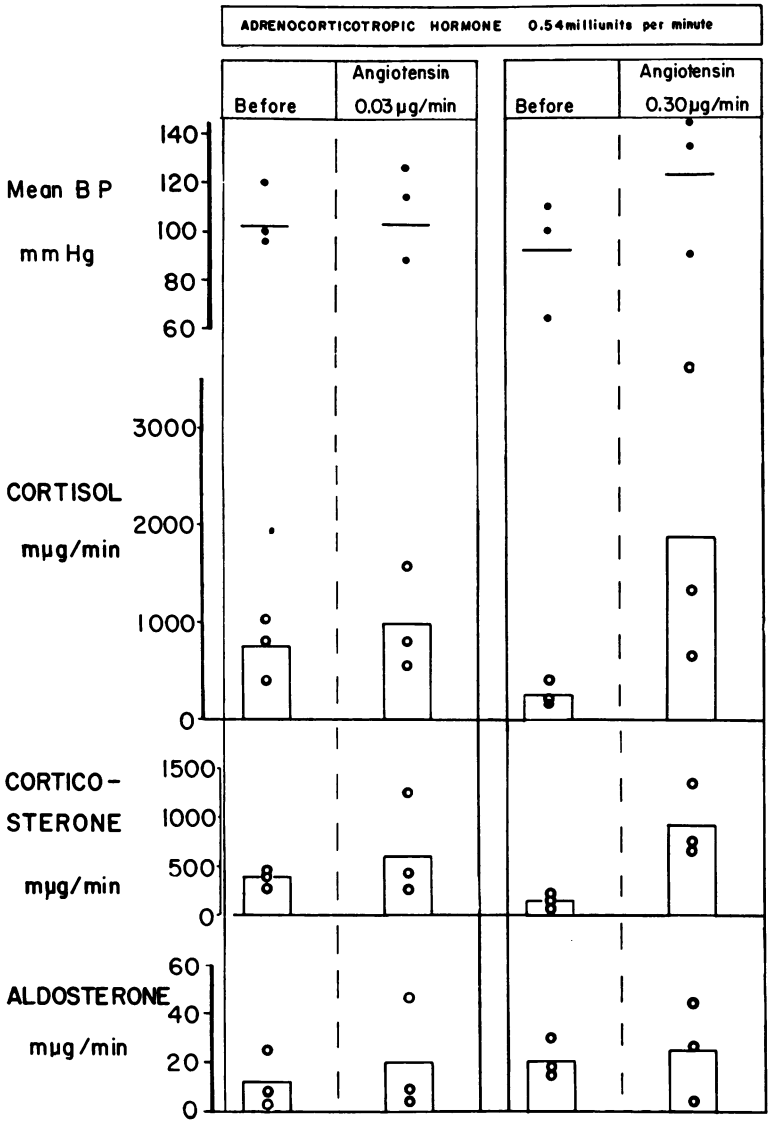

FIG. 10. ADRENAL AND PRESSOR EFFECTS OF ANGIOTENSIN AT TWO DOSE LEVELS IN THREE HYPOPHYSECTOMIZED, NEPHRECTOMIZED DOGS RECEIVING ACTH IN SUBMAXIMAL AMOUNTS.

angiotensin increased 17-OHCS secretion by about 400%.

Small doses of ACTH. At 2.4 mU per minute or less, the difference between the pattern of adrenocortical hormone secretion produced by ACTH and that produced by angiotensin is clearly demonstrable (Figure 9). At this rate of infusion, the rate of cortisol production attained is lower than the rate produced by 48 mU per minute, so that 2.4 mU per minute or less provides a submaximal stimulus. Nevertheless, cortisol production increased nearly 20 times, and corticosterone production, nearly 7 times. In contrast, aldosterone production increased insignificantly ($p < 0.1 > 0.05$). This is obviously different from the effect of angiotensin on the adrenal cortex and clearly indicates that ACTH increases production of cortisol and corticosterone to a much greater extent than that of aldosterone.

Combined effects of ACTH and angiotensin. Two experiments were performed to give an indication of whether ACTH can produce a fur-

TABLE VI

Effect of large quantities of angiotensin II (3.0 μg/min) on the rate of secretion of cortisol and corticosterone (in mμg/min) and the effect of adding large quantities of ACTH (2.4 U/min) to the perfusion fluid in two nephrectomized, acutely hypophysectomized dogs

Dog	Steroid*	Control	Angiotensin	Angiotensin + ACTH
1	Cortisol	27	2,900	5,940
	Corticosterone	191	1,020	2,100
2	Cortisol	24	1,543	2,203
	Corticosterone	82	852	1,718

* Blood samples were collected between 4 and 12 minutes after the beginning of the angiotensin infusion, ACTH was then added, and the last sample collected between 4 and 9 minutes after the beginning of the infusions of both substances.

ther stimulation of adrenocortical hormone secretion when large amounts of angiotensin are infused (Table VI). The rate of production of cortisol and corticosterone, although markedly increased by this dose of angiotensin, was further increased by large amounts of ACTH.

In three dogs, angiotensin at 0.03 and 0.30 μg per minute was infused while the animal was receiving a continuous infusion of ACTH at 0.5 mU per minute to raise the rate of corticosterone and cortisol production to levels nearer those seen in normal, unstressed animals. The results are shown in Figure 10. At the higher rate of angiotensin infusion, cortisol and corticosterone production increased sharply in all three animals, despite insignificant increases of aldosterone production and relatively small increase of arterial blood pressure. At the lower rate of infusion, corticosterone and cortisol secretion rose sharply in one of the three animals. Dog 10 of Table V was incompletely hypophysectomized, yet angiotensin at both .3 and .03 μg per minute stimulated cortisol secretion.

DISCUSSION

Our results on the effect of hypophysectomy confirm the conclusions of others that the rate of aldosterone secretion may be relatively independent of the anterior pituitary. They suggest, indeed, that the anterior pituitary may have an even less important role in maintaining the basal rate of secretion of aldosterone than that suggested by Davis and his associates, who found that the rate of aldosterone secretion falls profoundly on the day of hypophysectomy in anesthetized animals (5). Our results indicate that aldosterone values may be essentially unchanged the day after hypophysectomy. Davis and his colleagues did indeed find low rates of aldosterone secretion in dogs with hypophysectomy of long standing which had been deprived of sodium after hypophysectomy (7). In another experiment however, in which dogs were hypophysectomized after sodium depletion had been produced (by dietary sodium deprivation and injections of Mercuhydrin), the rate of aldosterone secretion was high (32). Perhaps the difference may be related to the fact that cortisone was given only to the latter group after hypophysectomy.

The results of infusing crude saline extracts of the kidneys suggest that the aldosterone-stimulating factor present in these extracts is not ACTH, that it is associated with pressor activity and with the presence of granules in the juxtaglomerular cells, and that it can be made to disappear by loading the animal with salt and treating it with desoxycorticosterone. Low rates of extract infusion were without effect on the adrenal cortex or blood pressure, but with rates of over 2% of whole kidney per minute, the extracts increased the rates of cortisol and aldosterone secretion to comparable degrees. As we have shown, these are the properties of renin and angiotensin. In accord with the findings of Gross and Sulser in the rat, the effect of our extracts on the arterial blood pressure also depended upon the sodium intake of the animals from whose kidneys the extracts were made (25).

On indirect evidence, Deane and Masson (8) suggested that extracts of the kidney are capable of affecting adrenocortical function. They showed that crude renin preparations caused both enlargement of the zona glomerulosa and changes, which they thought resembled those caused by ACTH, in the histological appearance of the zona fasciculata. The animals lost weight (and hence presumably salt and water), so that the hypertrophy of the zona glomerulosa may have been secondary to the well-known diuretic effect of renin preparations (33). Our results and those of others (see below) suggest a more direct effect, not due to ACTH, of renal extracts on the production of adrenocortical steroid hormones. The histological changes described by Deane and Masson support our findings that secretion of cortisol and corticosterone as well as aldosterone is stimulated.

Recently, Mulrow and Ganong (34) have obtained similar results. Extracts of normal kidneys given as a single injection to hypophysectomized, nephrectomized dogs produced sharp rises in aldosterone secretion. The arterial blood pressure also rose, and there were considerable increases of Porter-Silber chromogen secretion. Extracts from the kidneys of animals hypophysectomized 4 hours previously were less effective in stimulating aldosterone secretion, but the rise of arterial blood pressure was correspondingly smaller (19). Porter-Silber chromogen secretion did not change, but as noted above, this is a poor index of cortisol secretion in hypophysectomized animals, especially when only small changes are expected (see Methods). It is not clear why the renal extracts prepared from hypophysectomized animals were less active than those from normal animals, but they do not mention the dietary regimen of their animals. Mulrow and associates (19) report that renin concentrates of kidneys taken from hypophysectomized dogs increase the rate of secretion of both aldosterone and corticosterone and raise the blood pressure.

Results differing from our own and from those of Mulrow and Ganong were obtained by Davis, Carpenter, Ayers, Holman, and Bahn (20). They made aqueous extracts of kidneys taken from normally fed animals hypophysectomized about 1 hour previously. These extracts, when infused into hypophysectomized, nephrectomized dogs 112 minutes after an acute hemorrhage, were without effect on the arterial blood pressure. Aldosterone secretion rose considerably, however, while corticosterone secretion was unaffected. Unless the pattern of response of the adrenal cortex to renin is greatly influenced by other factors, this suggests that the aldosterone-stimulating factor in these renal extracts was not renin. The rate of aldosterone and corticosterone secretion was also studied under the same experimental conditions in animals infused with aqueous extracts of liver. Aldosterone secretion rose in 4 out of 7 animals, without a change of corticosterone secretion. Thus, it is possible that the increases of aldosterone secretion during infusions of renal extract having no effect on blood pressure into these nephrectomized animals depended not upon renin but upon some other factor that may be related to the previous hemorrhage.

If small amounts of renin or angiotensin could increase aldosterone secretion specifically without raising the arterial blood pressure or increasing corticosterone or cortisol secretion, the properties of angiotensin would be those of a specific aldosterone-stimulating agent. Our dose-response studies and those of others (see below) indicate that this is not the case in hypophysectomized dogs. At the smallest dose of angiotensin that quite definitely increased aldosterone secretion (mean dose, 0.27 μg per minute per dog or approximately 0.013 μg per minute per kg), the rise of aldosterone secretion was small. At this dose level, the mean arterial blood pressure was clearly raised (from an average of 86 to an average of 120 mm Hg), and the secretion of both cortisol and corticosterone showed an increase comparable to that of aldosterone. This was so despite the fact that the responsiveness of the zona fasciculata was probably diminished, because in only half the animals used for these experiments was the pituitary gland removed on the day of the experiment. The sensitivity of the zona fasciculata to angiotensin is emphasized by the results in which the adrenal effects of a small dose of angiotensin are compared with those of a large one in the same animal.

At lower rates of angiotensin infusion (0.030 μg per minute, or 0.0017 μg per minute per kg), there was an insignificant rise of arterial blood pressure, but mean aldosterone secretion did increase significantly, although only from a mean value of 7.1 to a mean value of 10.5 mμg per minute. The secretion rate of cortisol and corticosterone also increased in six and in four of the eight animals, respectively. Here again, the responsiveness of the zona fasciculata was probably diminished because only four of the eight animals had had their pituitary glands removed on the day of the experiment.

These conclusions are very similar to those of Carpenter, Davis, and Ayers (21), who obtained similar results in studies concurrent with, and independent of, our own. Only amounts of angiotensin that clearly increased the blood pressure were capable of increasing aldosterone secretion (by increments of from 5 to 13 mμg per minute), and at these dose levels, corticosterone secretion also increased comparably (by increments of from 12 to 29 mμg per minute). The rate at which angiotensin was infused to produce this effect (1.0

μg per minute per 20-kg dog, or 0.050 μg per minute per kg) was nearly 4 times that which we find to produce comparable effects. Carpenter and his colleagues used acutely hypophysectomized dogs with intact kidneys, whereas all our animals were both nephrectomized and hypophysectomized. Nephrectomy is known to enhance the effect of angiotensin on the blood pressure in the rat (35), and the difference between our results and those of Carpenter, Davis, and Ayers suggests that in hypophysectomized dogs, nephrectomy increases the effect of angiotensin on both the arterial blood pressure and the rate of release of adrenocortical hormones.

This conclusion is supported by the findings of Mulrow and associates (19), who found that the minimal effective dose of angiotensin in nephrectomized, hypophysectomized dogs was the same as that reported here. They also observed increases in corticosterone secretion comparable in magnitude (and in statistical significance) to the increases in aldosterone secretion. The significance of the increases in the secretion of corticosterone and cortisol with angiotensin in the dog is minimized by Carpenter and his associates because the levels attained are often lower than those seen in normal, unstressed animals. They consider these "small in terms of physiological significance" and conclude that secondary aldosteronism may result via the renin-angiotensin mechanism from stimuli such as sodium depletion, which are not known to be associated with increases of corticosterone or cortisol secretion. The increased secretion of corticosterone and cortisol with angiotensin does appear to be relevant, however. If angiotensin produces measurable increases in corticosterone and cortisol secretion at any dosage at which it produces increases in aldosterone secretion, increases in secretion of all these steroids should be detectable after any stimulus to aldosterone that requires the mediation of angiotensin, unless, of course, other, as yet unknown factors can radically alter the pattern of the adrenocortical response to angiotensin. At present, there is no evidence that secretion of cortisol or of corticosterone is stimulated by sodium depletion.

Mulrow and associates (19) stress the fact that the adrenal response to angiotensin is clearly different from that to ACTH. We fully confirm their findings that low doses of ACTH stimulate cortisol and corticosterone production exclusively, whereas angiotensin at all dose levels stimulates the production of aldosterone as well. This fact, however, does not appear to be relevant to the question of whether angiotensin plays a role in the physiological control of aldosterone secretion. Injections of angiotensin II into the artery supplying the isolated, transplanted adrenal gland in five conscious sheep (36, 37) with intact pituitary glands consistently increased the rate of aldosterone secretion by a mean of about 10 times, whereas the effect on the rate of cortisol and corticosterone secretion was variable. Although this difference may depend on the species difference between sheep and the dog, transplanted glands prepared this way may show a greatly reduced ability to increase cortisol secretion with ACTH (36, 37). In fact, of the five animals concerned (37), only one could be said to respond well to ACTH. In this animal (TP9), angiotensin II infused locally by arterial injection increased corticosterone secretion on the first and cortisol secretion on the first two of six occasions when it produced an increase of aldosterone secretion. When infused intravenously, however, angiotensin II increased secretion of all three steroids comparably.

Biron, Koiw, Nowaczynski, Brouillet, and Genest (10), first showed that iv infusions of angiotensin II increased the urinary excretion of aldosterone in man. In five normal volunteers, given six infusions of angiotensin at a mean rate of 1.92 mg in 10 hours, they found that aldosterone excretion in the urine increased nearly 4 times. In all four subjects studied, the urinary excretion of free cortisol increased comparably (from 272 to 821 μg per day).

The weight of evidence therefore suggests that angiotensin increases the secretion of aldosterone, cortisol, and corticosterone to roughly the same extent. If angiotensin plays an important role in the physiological control of aldosterone secretion, stimuli known to change aldosterone secretion should produce comparable effects in the secretion of the other two steroids. In hypophysectomized rats maintained on ACTH, Singer and Stack-Dunne (3) have shown that the rate of aldosterone secretion falls considerably after treatment with desoxycorticosterone acetate, whereas the rate of corticosterone secretion remains un-

changed. Similarly, Davis, Carpenter, Ayers, and Bahn (6) have shown in conscious dogs that with constriction of the thoracic inferior vena cava the rate of secretion of aldosterone is greatly elevated, whereas that of corticosterone remains at or near control levels. Unfortunately, there are few comparable data on the rates of adrenocortical steroid hormone secretion in man under conditions known to change aldosterone secretion. Certainly the production of cortisol, as reflected in the rate of urinary excretion of Porter-Silber chromogens, ketogenic steroids, and the like, does not change appreciably in the secondary aldosteronism of cirrhosis of the liver, the nephrotic syndrome, simple dietary salt restriction (2), or the syndrome of aldosteronism with hypertrophy of the juxtaglomerular apparatus (15). If angiotensin is responsible for the increase in aldosterone secretion, and if our reasoning is correct, there should be a comparable increase of cortisol secretion, unless other secondary factors such as suppression of ACTH output come into play. The conclusion cannot be firmly established, however, until comparable data are available for normal man. The findings reported here were obtained in hypophysectomized, nephrectomized dogs, and can be applied to either normal dogs or other species only with considerable caution.

SUMMARY

Experiments were performed to measure the effects on the rate of aldosterone, corticosterone, and cortisol secretion of *1*) hypophysectomy in sodium-deprived dogs and *2*) intravenous infusions of crude kidney extracts, of renin concentrates, of valine-5 asparaginyl angiotensin II, and of commercial ACTH in hypophysectomized, nephrectomized dogs.

In conscious, unstressed dogs, hypophysectomy did not change the rate of aldosterone secretion when measured not less than 18 hours later.

Crude kidney extracts from hypophysectomized, sodium-deprived dogs stimulated the rate of cortisol and aldosterone secretion and increased the arterial blood pressure when infused at a rate of over 2% of the whole kidney per minute. Extracts from sodium-loaded dogs were inactive at all rates of infusion.

Dose-response studies with angiotensin showed that 0.17 to 0.30 μg per minute was the lowest rate that clearly increased aldosterone secretion. At these dosages, the arterial blood pressure and the rate of corticosterone and cortisol secretion also increased considerably, even when the rate of corticosterone and cortisol secretion had been raised to levels seen in conscious, unstressed animals by infusion of ACTH at 0.5 mU per minute. Renin infused at 0.64 and 0.16 Goldblatt U per minute produced comparable changes. As compared to ACTH, angiotensin produces a greater increase in the rate of aldosterone secretion for a given increase in the rate of cortisol secretion.

The results suggest that the aldosterone-stimulating factor in kidney extracts is renin and that the renin-angiotensin system stimulates secretion of aldosterone, corticosterone, and cortisol to a comparable extent.

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