

THE ROLE OF THE ANTERIOR PITUITARY IN THE CONTROL OF ALDOSTERONE SECRETION IN EXPERIMENTAL SECONDARY HYPERALDOSTERONISM *

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Previous reports (1-9) have delegated a secondary role to anterior pituitary hormones in the control of aldosterone secretion.¹ Although hypophysectomy resulted in decreased aldosterone secretion (3) or reduced urinary aldosterone output (2), hyperaldosteronuria, marked sodium (Na) retention and ascites occurred in the absence of the adenohypophysis in dogs with thoracic inferior vena cava constriction (2). Also, in patients with hypopituitarism, Luetscher and Axelrad (6) and Hernando and associates (7) found that urinary aldosterone output was within normal limits in some patients on an unselected diet or a normal salt intake, and Liddle, Duncan and Bartter (8) reported hyperaldosteronuria in one patient with hypopituitarism on a low Na diet.

However, no conclusive evidence of hypersecretion¹ of aldosterone in the absence of anterior pituitary hormones has been reported. The critical pertinent data on the rate of aldosterone secretion during stimulation which produces hypersecretion in normal animals have not been reported for hypophysectomized dogs or patients. It is important that studies in man be conducted on hypophysectomized patients since patients with so-called panhypopituitarism may not have loss of all anterior pituitary function. Furthermore, our knowledge is incomplete on the importance of specific anterior pituitary hormones. Several studies (3, 7, 8-10) have demonstrated an increase in aldosterone secretion or urinary aldosterone excretion following administration of various corticotropin preparations, but the data are inadequate to establish the role of ACTH in secondary hyperaldosteronism.²

The question of the role of the anterior pituitary in the control of aldosterone secretion was reopened by the finding of a 76 to 97 per cent fall in adrenal vein aldosterone output following hypophysectomy of dogs with experimental secondary hyperaldosteronism (11). In the present report, data are presented on the efficacy of ACTH³ in preventing this fall in aldosterone secretion which follows hypophysectomy. Large doses of cortisone have been administered to inhibit ACTH secretion in dogs with hyperaldosteronism secondary to caval constriction; the resultant effects on aldosterone and corticosterone production were observed. Subsequently, the effects of hypophysectomy and ACTH were studied in these animals. Also, the effects of synthetic α -melanophore-stimulating hormone (MSH) and of highly purified preparations of natural α - and β -MSH have been studied. Attempts have been made to stimulate hypersecretion of aldosterone in simple hypophysectomized dogs by 1) a low Na diet and 2) acute constriction of the thoracic inferior vena cava.

² In this paper the trivial names of the steroids have been used: cortisone (17 α ,21-dihydroxy-4-pregnene-3,11,20-trione); corticosterone (11 β ,21-dihydroxy-4-pregnene-3,20-dione); 11-dehydrocorticosterone (21-hydroxy-4-pregnene-3,11,20-trione); aldosterone (11 β ,21-dihydroxy-18-aldo-4-pregnene-3,20-dione); cortisol (11 β ,17 α ,21-trihydroxy-4-pregnene-3,20-dione); and 2-methyl cortisol (2 α -methyl-11 β ,17 α ,21-trihydroxy-4-pregnene-3,20-dione).

³ Subsequent reference will be made to ACTH or to an ACTH preparation; it is recognized that contaminating substances with ACTH-like activity may be present.

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¹ The phrases "aldosterone secretion" or "hypersecretion of aldosterone" have been used to refer to actual measurements of the rate of secretion by the adrenal gland into the effluent plasma and are to be distinguished from urinary aldosterone excretion.

METHODS

The 26 animals used in this study were 15 to 20 kg mongrel dogs. Nine animals were dogs with chronic experimental hyperaldosteronism and ascites secondary to constriction of the thoracic inferior vena cava (12); urinary Na excretion was less than 5 mEq per day on a Na intake of 60 mEq per day. The other 17 animals were simple hypophysectomized dogs at the onset of the experiment. In Experiment 1, the efficacy of ACTH in blocking the usual striking fall in aldosterone and corticosterone secretion which follows hypophysectomy was studied. In Experiment 2, large daily intramuscular doses of cortisone acetate (100 mg to 4 dogs and 200 mg to 3 dogs) were given for 8 days except that in the 3 animals receiving 200 mg daily, 100 mg of the cortisone was given orally on the last day. On the last day of cortisone administration, adrenal vein aldosterone and corticosterone output was measured. Five of the 7 dogs receiving cortisone were hypophysectomized after the 3 initial observations on steroid secretion; subsequently, in 4 of these 5 dogs the effects of ACTH were evaluated. In Experiment 3, preparations of α - and β -MSH were assayed in 4 simple hypophysectomized dogs. In Experiment 4, 10 simple hypophysectomized dogs were placed on a low Na diet for 7 to 19 days; for 5 of the dogs, Na intake was 7 mEq per day, whereas the other 5 animals received 1 mEq per day of Na. Potassium intake was 40 mEq per day. After acute adrenal vein aldosterone and corticosterone measurements in these hypophysectomized dogs on a low Na intake, the effects of ACTH were studied in 4 animals. In 3 additional dogs, the anterior pituitary was removed and after 2 hours the thoracic inferior vena cava was constricted acutely in an attempt to stimulate the adrenal cortex.

The same dose and preparation of ACTH were used in all experiments; 0.075 unit per minute of an Upjohn preparation was given intravenously in 5 per cent glucose at a rate of 0.62 ml per minute of solution with a constant infusion pump into the left subclavian vein. Synthetic α -MSH was prepared and supplied to us by Dr. Klaus Hofmann. This synthetic preparation is like

the natural hormone except that glutamic acid is in the form of glutamine, and lysine is in the form of ϵ -formyl-lysine. The synthetic preparation was assayed by Dr. Aaron B. Lerner and found to have 6.6×10^6 units of MSH activity per mg. Highly purified preparations of α - and β -MSH from hog anterior pituitary were provided by Doctors Aaron B. Lerner and Teh H. Lee. The MSH potency of the natural α -MSH preparation was 2.8×10^6 units per mg, whereas the β -MSH preparation had 2.6×10^6 units of activity per mg. The MSH preparations were dissolved in 5 per cent glucose and infused at the rate of 0.62 ml per minute over a 30 minute period.

Observations on aldosterone and corticosterone secretion in adrenal vein plasma were made at 30 to 60 minute intervals in animals under light Nembutal anesthesia. The dogs received 100 per cent oxygen through an endotracheal tube attached to a pneophore at a pressure of 10 to 15 cm water. The right adrenolumbar vein was cannulated and measurements of adrenal blood flow were made by techniques described previously (13). Ten or 20 ml of adrenal vein blood and 10 ml of peripheral blood were removed for each group of analyses; normal donor blood was injected immediately to replace the blood removed for analysis. Urinary Na was measured by flame photometry. Cardiovascular pressures were measured in the abdominal aorta and inferior vena cava with Statham strain gages and a Sanborn recording system. An infusion of norepinephrine (100 μ g per ml) was given to 3 of the hypophysectomized dogs on a low Na diet (Dogs 4, 6 and 8) during the latter part of the experiment to maintain arterial pressure and adrenal blood flow.

The concentrations of aldosterone and corticosterone in adrenal vein plasma were measured by the double isotope derivative procedure as described previously (14), with one exception; a third chromatography was used to purify corticosterone. Corticosterone monoacetate was oxidized with chromic trioxide for 5 minutes and converted to 11-dehydrocorticosterone acetate. This compound was chromatographed for 14 to 16 hours in a cyclohexane: benzene: methanol: water (100: 40:

TABLE I
Evidence of specificity of the double isotope derivative assay procedure for aldosterone

No. of sample	Source of adrenal vein plasma	H^3/C^{14} ratios after each chromatography			
		1st	2nd	3rd	4th
1	Chronic caval constriction	20.3	1.44	0.47	0.74
2	Chronic caval constriction	54.4	9.48	7.93	8.26
3	Chronic caval constriction	44.7	16.38	15.67	17.20
4	Chronic caval constriction	4.8	0.80	0.52	0.68
5	Normal	14.6	0.54		0.68
6	Chronic caval constriction and cortisone	27.2	7.08	7.22	6.84
7	Chronic caval constriction and cortisone	20.0	2.77	1.90	1.62
8	Chronic caval constriction and cortisone	14.4	1.81	0.68	0.59
9	Chronic caval constriction and cortisone	12.1	0.76	0.46	0.44
10	Chronic caval constriction after hypophysectomy and during ACTH	27.3	10.53	9.46	10.00

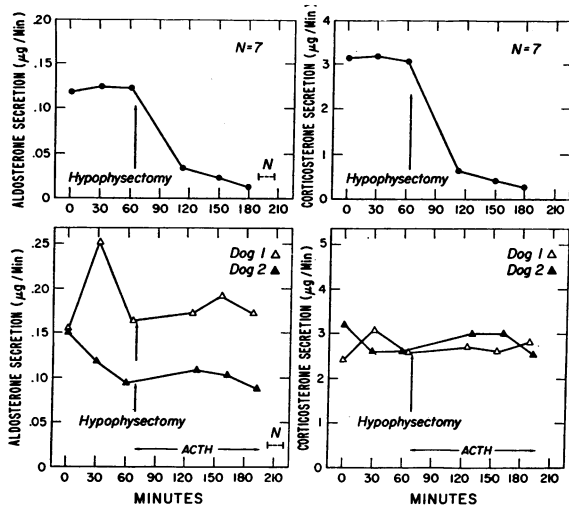


FIG. 1. EFFECTS OF HYPOPHYSECTOMY (UPPER FIGURES) AND THE BLOCKING EFFECTS OF ACTH (0.075 UNIT PER MINUTE) FOLLOWING HYPOPHYSECTOMY (LOWER FIGURES) IN DOGS WITH THORACIC CAVAL CONSTRICTION AND HYPERALDOSTERONISM. The average value of 0.024 μ g per minute for aldosterone secretion in 10 normal dogs is represented by the dotted line with the N above it in the lower right corners of the figures on the left.

100:20) system. Because the present data on aldosterone secretion were obtained under different experimental conditions from those reported previously (14), additional evidence on specificity has been provided (Table I). H^3/C^{14} ratios were determined for aldosterone diacetate as described elsewhere (14), after each of 4 chromatographies on adrenal vein plasma from 4 dogs with chronic thoracic caval constriction, one normal dog, 4 of the dogs with chronic thoracic caval constriction given cortisone, and one dog receiving ACTH. For the fourth chromatography, the second chromatographic system employed for routine aldosterone analysis was used and 2-methyl cortisol was added to provide a visible marker. The chromatogram was developed for 18 hours. The H^3/C^{14} ratios were not significantly reduced after the third chromatography with the possible exception of sample 7 (Table I). This finding indicates that no H^3 -labeled impurities were present after the third chromatography.

Hypophysectomy was performed by the oral approach. The pituitary was exposed so that it was visible through the dura mater before the initial prehypophysectomy observations were made. After these observations, the pituitary was removed within a 2 to 3 minute period. In Experiment 3, the base of the pituitary stalk was cauterized to destroy any remnant of the pars tuberalis in the first 6 of the 10 dogs; in the remaining 4 dogs, hypophysectomy alone was performed. In Experiment 1, tissue remaining in the sella turcica and the hypothalamus was examined for remnants of adenohypophysis.

RESULTS

Experiment 1. Effects of ACTH following hypophysectomy of dogs with thoracic caval constriction

It has been shown previously (11) that hypophysectomy of dogs with thoracic caval constriction results in a striking fall in the secretion of both aldosterone and corticosterone. In the present experiment, an intravenous infusion of ACTH was given after hypophysectomy of two dogs

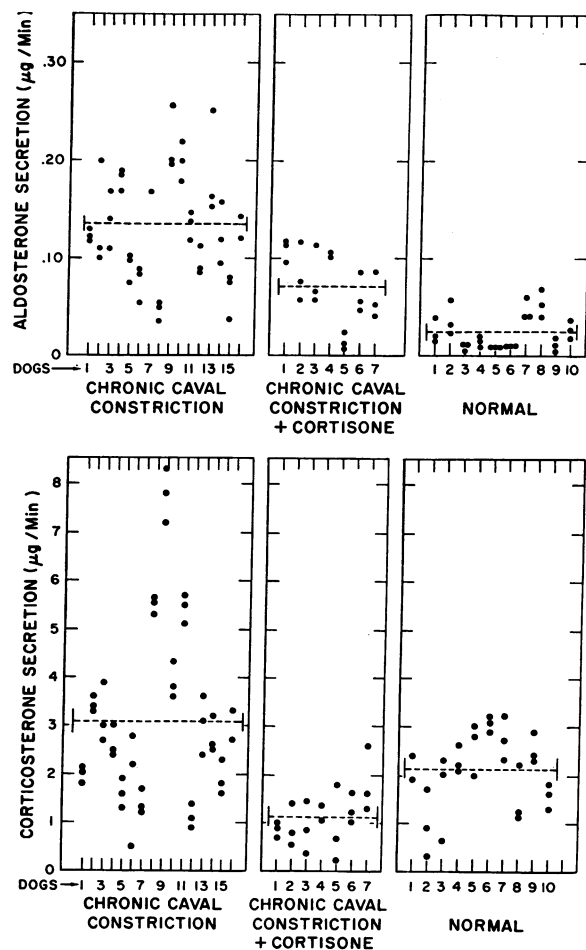


FIG. 2. EFFECT OF CORTISONE (100 MG PER DAY TO DOGS 1 THROUGH 4 AND 200 MG PER DAY TO DOGS 5 THROUGH 7) ON ALDOSTERONE AND CORTICOSTERONE SECRETION IN DOGS WITH CHRONIC THORACIC INFERIOR VENA CAVA CONSTRICTION (CENTER SECTIONS). For comparison, steroid secretion rates in 16 dogs with chronic caval constriction (left sections) and 10 normal dogs (right sections) are presented. The dotted lines show the mean values.

TABLE II
Aldosterone and corticosterone secretion in dogs with secondary hyperaldosteronism treated with cortisone: effects of subsequent hypophysectomy† and ACTH†*

	Initial observations			After hypophysectomy			During ACTH infusion	
	1	2	3	4	5	6	7	8
Dog 1								
Time, min	0	26	54	117	150	134	220	227
Aldosterone, $\mu\text{g}/\text{min}$	0.097	0.114	0.120	0.058	0.039	0.030	0.254	0.248
Corticosterone, $\mu\text{g}/\text{min}$	0.72	1.00	0.90	0.37	0.15	0.13	1.3	1.2
Adrenal blood flow, ml/min	3.88	3.88	3.47	2.40	2.18	2.21	2.90	3.18
Dog 2								
Time, min	0	27	53	123	163	191	251	
Aldosterone, $\mu\text{g}/\text{min}$	0.117	0.057	0.076	0.011	0.011	0.013	0.128	
Corticosterone, $\mu\text{g}/\text{min}$	1.4	0.60	0.80	0.09	0.05	0.06	0.90	
Adrenal blood flow, ml/min	5.27	4.68	3.80	1.65	1.26	1.09	1.42	
Dog 3								
Time, min	0	30	59	136	166	197	260	
Aldosterone, $\mu\text{g}/\text{min}$	0.114	0.065	0.056	0.012	0.013	0.008	0.157	
Corticosterone, $\mu\text{g}/\text{min}$	1.43	0.84	0.37	0.029	0.021	0.017	1.38	
Adrenal blood flow, ml/min	2.92	3.21	2.72	1.92	1.90	1.76	2.58	
Dog 4								
Time, min	0		54		162	196	263	271
Aldosterone, $\mu\text{g}/\text{min}$	0.104		0.106		0.039	0.043	0.102	0.108
Corticosterone, $\mu\text{g}/\text{min}$	1.36		1.07		0.12	0.11	0.94	1.06
Adrenal blood flow, ml/min	3.41		1.75		1.02	1.04	1.30	1.38
Dog 5								
Time, min	0	31	60					
Aldosterone, $\mu\text{g}/\text{min}$	0.012	0.023	0.007					
Corticosterone, $\mu\text{g}/\text{min}$	0.67	1.79	0.22					
Adrenal blood flow, ml/min	4.06	5.87	5.47					
Dog 6								
Time, min	0	29	52	116	144	168		
Aldosterone, $\mu\text{g}/\text{min}$	0.047	0.084	0.055	0.004	0.004	0.005		
Corticosterone, $\mu\text{g}/\text{min}$	1.00	1.61	1.20	0.05	0.02	0.01		
Adrenal blood flow, ml/min	3.09	3.21	2.40	2.34	2.29	2.16		
Dog 7								
Time, min	0	0	25					
Aldosterone, $\mu\text{g}/\text{min}$	0.052	0.042	0.087					
Corticosterone, $\mu\text{g}/\text{min}$	1.63	1.29	2.58					
Adrenal blood flow, ml/min	2.31	2.30	2.84					

* Dogs 1 through 4 received 100 mg/day of cortisone intramuscularly (i.m.) for 8 days. Dogs 5 through 7 were given 200 mg/day of cortisone i.m. for 7 days; on the eighth day only 100 mg of cortisone was injected i.m. and 100 mg of cortisone was given orally 3 to 5 hours before collection of adrenal vein blood.

† Hypophysectomy was performed immediately after the third period and the ACTH infusion (0.075 unit/min) was begun after Period 6.

with chronic caval constriction; aldosterone and corticosterone secretion was unaltered. The results are presented in Figure 1 and are compared with the previous finding of decreased steroid secretion following hypophysectomy alone. Histological studies of tissue removed at postmortem examination showed no remaining adenohipophysis in either dog.

Experiment 2. Effects of the chronic daily administration of cortisone on aldosterone and corticosterone secretion in dogs with chronic thoracic caval constriction: subsequent acute effects of hypophysectomy and ACTH

The average value for adrenal vein aldosterone output was 47.3 per cent lower in the seven dogs receiving cortisone (100 to 200 mg per day) than

in untreated dogs with caval constriction ($p < 0.01$, Figure 2). In one of the animals treated with cortisone (Dog 5, Figure 2), all three determinations of aldosterone were at the normal level. The present data are compared with previous results in 16 dogs with chronic caval constriction. Dogs 5 through 7 received 200 mg per day of cortisone and the average value for aldosterone secretion for these animals was $0.045 \mu\text{g}$ per minute; this value was significantly lower than the average value of $0.135 \mu\text{g}$ per minute for dogs with caval constriction ($p < 0.02$). The rate of aldosterone secretion for the entire group of seven dogs receiving cortisone was significantly greater than the normal level ($p < 0.01$), whereas aldosterone secretion in the three dogs given 200 mg per day of cortisone was not significantly different from normal ($p > 0.1$).

Corticosterone secretion was 63.4 per cent lower (Figure 2) in dogs receiving cortisone than in dogs with caval constriction alone ($p < 0.01$). Although the response for corticosterone appeared greater than that for aldosterone, the difference was not significant ($p = 0.2$ for the corticosterone:aldosterone ratio). Also, corticosterone output was lower (46.5 per cent) in dogs receiving cortisone than in normal dogs studied under the same experimental conditions ($p < 0.01$). The values for corticosterone secretion in both the untreated dogs with caval constriction and normal dogs are probably high and reflect an increased output of ACTH resulting from the "stress" of

adrenal vein cannulation. The rates of adrenal blood flow, inferior vena caval pressures and arterial pressures were essentially the same in the dogs with caval constriction in the absence of or during cortisone administration.

Daily Na balance studies were conducted throughout the period of cortisone administration. Sodium retention continued and Na excretion was less than 5 mEq per day on the day before sacrifice for adrenal vein blood studies in all but one animal (Dog 7); in this animal, Na excretion was 17 mEq per day.

Immediately following the initial observations on the cortisone-treated dogs, hypophysectomy was performed in five of the seven dogs and studies were continued for another two hours. A further marked drop in both aldosterone and corticosterone secretion occurred (Table II). Adrenal blood flow was reduced in four of the five dogs. After the post-hypophysectomy observations, an infusion of ACTH was given to four of the five animals. Adrenal vein aldosterone output increased 8-, 10-, 20- and 2.4-fold with respect to the post-hypophysectomy level in Dogs 1 through 4, respectively, while corticosterone secretion increased 10-, 15-, 81- and 8-fold during the same periods.

Experiment 3. Effects of α - and β -MSH on aldosterone and corticosterone secretion

Synthetic α -MSH (4.75 mg) was assayed in a simple hypophysectomized dog; aldosterone and

TABLE III
Failure of synthetic α -MSH and naturally occurring β -MSH to increase aldosterone and corticosterone secretion in hypophysectomized dogs

	Time	Aldosterone secretion	Corticosterone secretion
	<i>min</i>	<i>$\mu\text{g}/\text{min}$</i>	<i>$\mu\text{g}/\text{min}$</i>
Control	0	0.010	0.39
	30	0.015	0.09
Infusion of 4.75 mg of synthetic α -MSH	30-60		
After α -MSH	60	<0.005	0.15
	90	<0.005	0.03
Control	0	<0.005	0.01
	30	<0.005	0.01
Infusion of 7 mg of natural β -MSH	30-60		
After β -MSH	60	<0.005	0.02
	90	<0.005	0.01

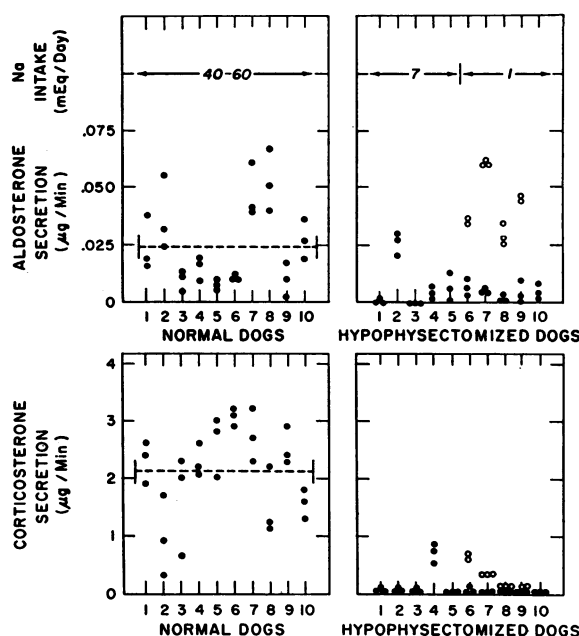


FIG. 3. EFFECTS OF A LOW Na DIET ON ALDOSTERONE AND CORTICOSTERONE SECRETION IN TEN SIMPLE HYPOPHYSECTOMIZED DOGS (SOLID SYMBOLS). The open symbols represent values obtained after 30 to 60 minutes of infusion of ACTH (0.075 unit per minute).

corticosterone secretion was unaffected. Highly purified natural hormone preparations of α - and β -MSH (2.18 mg of α -MSH and 2.0 and 7.0 mg of β -MSH) were without effect on aldosterone

and corticosterone secretion in hypophysectomized dogs. The results of the assays of the two largest quantities of MSH are presented in Table III.

Experiment 4. Failure to produce hypersecretion of aldosterone in simple hypophysectomized dogs

A. With a low salt intake. Of the five dogs on a Na intake of 7 mEq per day, only one animal showed a secretion rate similar to the average normal value (Figure 3); all other values were low. A further reduction in Na intake to 1 mEq per day also failed to produce a secretion rate comparable to that for normal dogs ($p < 0.01$; $n = 5$). Adrenal blood flow was essentially the same in the present hypophysectomized dogs on a low Na intake as in the normal control animals ($p > 0.2$). Although aldosterone secretion was lower than normal in hypophysectomized dogs on a low salt intake, the secretion rates for some of these dogs were greater than those in two of the three simple hypophysectomized dogs in which aldosterone was undetectable (compare data in Figure 3 and Table IV). The ability of the adrenal cortex to respond to ACTH in hypophysectomized dogs during Na deprivation was demonstrated by giving ACTH after the initial observations were complete (Figure 3). The rate of aldosterone secretion during infusion of ACTH

TABLE IV
Effects of acute thoracic caval constriction in simple hypophysectomized dogs

	Time	Aldosterone secretion	Corticosterone secretion	Adrenal blood flow	Inferior vena caval pressure	Arterial pressure
	min	$\mu\text{g}/\text{min}$	$\mu\text{g}/\text{min}$	cc/min	mm H ₂ O	mm Hg
Dog 1						
Control	0	< 0.005	0.036	2.92	62	100
	32	< 0.005	0.056	2.58	55	104
Caval constriction	37					
After constriction	69	< 0.005	0.14	2.02	148	86
	100	< 0.005*	0.15*	2.07*	188*	94*
Dog 2						
Control	0	< 0.005	0.14	3.53	44	121
	27	< 0.005	0.13	2.61	44	131
Caval constriction	36					
After constriction	96	< 0.005	0.29	2.50	155	100
	106	< 0.005	0.25	1.70	155	96
Dog 3						
Control	0	0.008	0.026	2.00	90	100
	27	0.014	0.029	1.98	90	118
Caval constriction	32					
After constriction	57	0.009	0.111	2.81	185	84

* A norpinephrine infusion (100 $\mu\text{g}/\text{cc}$) was given before and during this collection of adrenal vein blood.

increased markedly. The initial rate of corticosterone secretion was very low in all animals and the response in corticosterone output to ACTH was negligible.

B. By acute thoracic inferior vena caval constriction. Two hours after hypophysectomy of three normal dogs, the thoracic inferior vena cava was constricted. The degree of constriction and the resultant changes in venous and arterial pressures were similar to those reported (15) for normal dogs following acute caval constriction. Aldosterone and corticosterone output remained unchanged at the low post-hypophysectomy level (Table IV).

DISCUSSION

Our knowledge of the role of the anterior pituitary in the control of aldosterone secretion and electrolyte metabolism is incomplete. Before aldosterone was discovered, it was recognized that hypophysectomized dogs (16, 17) and rats (18) remain in apparently normal electrolyte balance. The experiments of Lane and de Bodo (16) and of Rolf, Surtshin and White (17) demonstrated Na conservation in hypophysectomized dogs even on a very low Na intake. Also, hypophysectomized dogs with thoracic caval constriction retained Na and formed ascites in the presence of a sufficiently elevated venous pressure (1). The finding of Farrell, Rauschkolb and Royce (3) of only a moderately reduced rate of aldosterone secretion following hypophysectomy in the dog was in agreement with earlier studies on electrolyte metabolism.

Other workers, however, have reported a striking fall in aldosterone output following hypophysectomy. In rats, Singer and Stack-Dunne (19) reported an 83 per cent reduction in aldosterone secretion after hypophysectomy. Ganong and associates (20) also found an 83 per cent lower value for aldosterone production in simple hypophysectomized dogs than in normal animals. In experimental secondary hyperaldosteronism in the dog, hypophysectomy resulted in an average reduction of 90 per cent in aldosterone and corticosterone secretion (11). The fall in aldosterone secretion following hypophysectomy of normal anesthetized animals may be exaggerated because of the initial high output which results from the

stress of adrenal vein cannulation. However, in unanesthetized dogs with chronic thoracic caval constriction, aldosterone secretion occurs at the same high rate as in anesthetized dogs with caval constriction which are stressed by adrenal vein cannulation (personal observations). The striking drop in aldosterone secretion following hypophysectomy of dogs with thoracic caval constriction emphasizes the important role of the anterior pituitary in the production of aldosterone. Maintenance of a normal electrolyte balance in hypophysectomized dogs in the presence of a low aldosterone output may be the result of the markedly reduced rate of glomerular filtration (21, 22).

In contrast to the situation in the dog, man appears to be fragile. There are several well documented cases of hypopituitarism with salt loss and the resultant clinical features of adrenocortical insufficiency (23). Hernando and associates (7) reported studies of several hypophysectomized patients or patients with hypopituitarism with urinary aldosterone outputs at the lower limit of normal. In one of these patients, a low Na intake failed to increase urinary aldosterone excretion on two occasions. The experiments of Llauro (24) have demonstrated that the increase in urinary aldosterone excretion and the reduction in the urinary Na/K ratio were consistently greater after nonendocrinological operations than following total hypophysectomy. These data indicate that the response in aldosterone production to surgery is less following hypophysectomy, a finding which suggests the necessity of the anterior pituitary for a normal response in aldosterone secretion to surgery.

What anterior pituitary hormones are important in the control of aldosterone secretion? The response to different corticotropin preparations (3, 7, 8-10) suggests that ACTH influences aldosterone production. The present data show that the 90 per cent drop in aldosterone and corticosterone secretion which follows hypophysectomy was blocked completely by an ACTH preparation. Since the possibility of a contaminating substance in corticotropin preparations cannot be excluded, the effect of inhibition of ACTH secretion on adrenal steroid production was studied.

In dogs with secondary hyperaldosteronism treated with cortisone, a decrease in both aldosterone and corticosterone secretions occurred in the absence of a detectable change in cardiovascular function. The ACTH content of the adenohypophysis has been demonstrated to be markedly decreased during treatment of normal dogs with similar doses of cortisone and cortisol (25). It appears likely, therefore, that the drop in aldosterone and corticosterone production was secondary to decreased output of ACTH. The data provide more conclusive evidence than those from administration of corticotropin preparations that ACTH plays an important role in aldosterone production. Also, following hypophysectomy and a resultant fall in aldosterone secretion in these ACTH-suppressed dogs, administration of ACTH restored aldosterone and corticosterone output to the very high levels characteristic of dogs with caval constriction. In earlier chronic studies (2), failure of ACTH to prevent the drop in urinary aldosterone excretion following hypophysectomy of dogs with caval constriction seems explicable on the basis of an inadequate level of venous pressure; reconstriction of the thoracic inferior vena cava in these dogs and a resultant further rise in venous pressure was followed by hyperaldosteronuria.

The values for corticosterone secretion in dogs with caval constriction and in normal dogs (Figure 2) are markedly elevated in comparison with the basal rate of corticosterone secretion in trained unanesthetized dogs (personal observations). These high rates of corticosterone secretion reflect the "stress" secondary to adrenal vein cannulation. Hilton and associates (26) reported near maximal output of cortisol in anesthetized dogs during adrenal vein cannulation. Also of interest is the finding of higher values for corticosterone secretion in normal dogs than in the cortisone-treated dogs with caval constriction, while the reverse situation was observed for aldosterone output. These data suggest that the stimulus which increased corticosterone secretion (presumably increased ACTH output) in normal dogs did not similarly influence aldosterone production. The present observations favor the view that ACTH provides important support for steroidogenesis by the adrenal cortex and does not necessarily play an initiative regulatory role in

the control of aldosterone secretion. This view is in agreement with the finding of Liddle, Duncan and Bartter (8) that patients with secondary hyperaldosteronism excrete normal amounts of 17-hydroxycorticoids.

In the experiments of Farrell, Banks and Kolesky (25), cortisone and cortisol, 100 mg per day, markedly depressed the ACTH content of the anterior pituitary in normal dogs, and the rates of secretion of cortisol and corticosterone were very low. In contrast, aldosterone secretion was essentially the same in their steroid-treated dogs as in their normal control animals. Their dogs were bled from the adrenolumbar vein over a four hour period. Since bleeding stimulates aldosterone secretion (27, 28), it is possible that a fall in aldosterone output was obscured by the effect of bleeding. In the present experiments only 20 to 30 cc of blood was removed for each group of analyses and this was replaced immediately.

The further decrease in aldosterone secretion following hypophysectomy of the cortisone-treated, ACTH-suppressed dogs of the present study raises the question of the influence of anterior pituitary hormones other than ACTH on aldosterone production. There is, however, the possibility that ACTH secretion was not completely suppressed by cortisone. The present α - and β -MSH preparations had no effect on aldosterone and corticosterone secretion. During chronic balance studies, thyrotropin and growth hormone preparations resulted in marked Na retention in hypophysectomized dogs with thoracic caval constriction, and discontinuation of both hormones was followed by increased Na excretion (1). It was suggested (1) that the effects of both thyrotropin and growth hormone were at least partially indirect and mediated through an increase in venous pressure which occurred concurrently with the Na retention. Canter and co-workers (29) have recently reported that thyroidectomy resulted in a Na diuresis in dogs with thoracic caval constriction and ascites. Conclusive evidence for a direct effect of thyrotropin and growth hormone is lacking and there is evidence to the contrary for growth hormone (5). Finally, it should be pointed out that another highly active ACTH-like anterior pituitary hormone with potent effects on aldosterone secretion is a possibility.

The marked effects of hypophysectomy and the importance of ACTH in aldosterone production raise the question of the capacity of the adrenal cortex to secrete increased amounts of aldosterone in the absence of adequate anterior pituitary function. The secretion rates for aldosterone in the present chronic simple hypophysectomized dogs on a low Na intake were below the normal level. The duration of the low Na intake was greater than that required for a response in normals to altered electrolyte intake (8, 30, 31). Several of these low values for aldosterone secretion in hypophysectomized dogs on a low Na intake were slightly higher, however, than aldosterone secretion in the two of the three dogs which were on a normal Na intake and aldosterone output was measured two hours after hypophysectomy (Table IV). This finding may indicate some adrenocortical stimulation by the low Na diet in the simple hypophysectomized dogs. Corticosterone output was extremely low and apparently unaffected by the low Na diet. These observations are in agreement with the earlier reports of Singer and Stack-Dunne (19) and Eisenstein and Hartroft (32) that a low Na diet increased aldosterone production in normal rats while corticosterone output was unaltered or declined. Also, Rosnagle and Farrell (33) found no influence of a low Na diet on cortisol output in normal dogs whereas aldosterone secretion was doubled.

Following acute thoracic caval constriction of simple hypophysectomized dogs, aldosterone secretion failed to increase with essentially the same alterations in cardiovascular function as those produced previously in normal dogs (15). In normal dogs, caval constriction consistently increased aldosterone secretion (15). The data agree with the finding (11) that the acute effect of hypophysectomy in dogs with hyperaldosteronism is a marked fall in aldosterone secretion. In previous chronic experiments (2) it was found, however, that increased aldosterone output in urine and presumably an elevated level of circulating aldosterone occurred frequently in association with marked Na retention and ascites in hypophysectomized dogs with caval constriction. More recently, chronic studies of conscious hypophysectomized dogs with caval constriction (personal observations) have demonstrated that

aldosterone secretion, although markedly reduced, remains sufficiently elevated to explain the associated hyperaldosteronuria and Na retention. These data, then, provide evidence of hypersecretion of aldosterone in the absence of the anterior pituitary and, thereby suggest an extra-pituitary origin for the aldosterone stimulating hormone, demonstrated previously by cross circulation experiments (14).

The present results are consistent with certain studies and proposed schema for the biogenesis of aldosterone *in vitro* from glomerulosa slices (34). Ayres and associates (34) have proposed that corticosterone is the immediate precursor to aldosterone in the major pathway of aldosterone synthesis; under certain experimental circumstances 50 to 92 per cent of aldosterone produced *in vitro* was derived from corticosterone. In the present study, there was a striking similarity in the changes in aldosterone secretion and corticosterone secretion following hypophysectomy and during ACTH administration to hypophysectomized dogs with caval constriction. During cortisone suppression of ACTH secretion, both aldosterone and corticosterone production fell in dogs with caval constriction. During ACTH administration to hypophysectomized dogs on a low Na intake, corticosterone output failed to increase while aldosterone production reached the upper limit of the normal range. These results may reflect inadequate substrate (precursors) for corticosterone production due to the chronic effects of hypophysectomy and atrophy of the zona fasciculata and reticularis. Sufficient corticosterone (if it is the precursor for aldosterone) was present for conversion to aldosterone and this presumably occurred in the zona glomerulosa. Although corticosterone secretion was markedly reduced in the present hypophysectomized dogs on a low Na diet the concentration of corticosterone in adrenal vein plasma was several times higher than that of aldosterone so that increased aldosterone production by ACTH might have occurred by conversion of corticosterone to aldosterone.

SUMMARY AND CONCLUSIONS

An ACTH preparation given following hypophysectomy of two dogs with thoracic caval con-

striction and hyperaldosteronism prevented the usual fall in aldosterone and corticosterone secretion which follows anterior pituitary ablation. To inhibit ACTH secretion, 100 to 200 mg per day of cortisone was given for eight days to seven dogs with experimental secondary hyperaldosteronism. Aldosterone and corticosterone production was significantly lower than in dogs with chronic ascites without cortisone therapy, and in one of the animals aldosterone output was depressed to normal. Subsequent hypophysectomy of five of these seven ACTH-suppressed dogs resulted in a further decline in aldosterone and corticosterone output. In four of these five animals subjected to hypophysectomy, the ACTH preparation increased aldosterone and corticosterone secretion to or above the control levels. In simple hypophysectomized dogs, α - and β -MSH were without effect on aldosterone and corticosterone secretion. Attempts to produce hypersecretion in simple hypophysectomized dogs failed: 1) adrenal vein aldosterone output was significantly lower than normal in ten simple hypophysectomized dogs during maintenance on a low Na diet; 2) in three simple hypophysectomized dogs, acute thoracic caval constriction failed to produce an increase in aldosterone secretion. Although aldosterone output was lower in the hypophysectomized dogs on a low Na intake than in normal dogs on normal electrolyte intake, the data suggest some adrenocortical stimulation in the absence of the anterior pituitary. The response in aldosterone secretion to the ACTH preparation in simple hypophysectomized dogs on a low Na diet was striking, whereas the increase in corticosterone production was slight. It is concluded that the anterior pituitary plays an important role in the increased production of aldosterone in experimental secondary hyperaldosteronism. It seems likely that ACTH supports steroidogenesis at a very high level.

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