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Research Article

RENAL ORIGIN OF AN ALDOSTERONE-STIMULATING HORMONE IN DOGS WITH THORACIC CAVAL CONSTRICTION AND IN SODIUM-DEPLETED DOGS

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Recent studies (1–5) indicate that the immediate stimulus to aldosterone production is humoral. Evidence for secretion of an aldosterone-stimulating hormone (ASH) by the kidney in response to acute blood loss was presented at the Laurentian Hormone Conference in 1960 (4). The present study is concerned with the locus of secretion of an ASH in two other experimental situations: *1*) hyperaldosteronism secondary to chronic constriction of the thoracic inferior vena cava, and *2*) chronic Na depletion. The present data provide evidence for extension of our knowledge of an ASH to clinical states with edema; the dog with thoracic caval constriction is very similar in regard to electrolyte and water metabolism and hyperaldosteronism to the patient with cirrhosis of the liver and ascites. Studies of the origin of secretion of an ASH during Na depletion were made because changes in electrolyte intake constitute one of the most important physiological mechanisms in the regulation of aldosterone secretion. Evidence for secretion of an ASH by the kidney in these two experimental situations was obtained by study of the effects of acute nephrectomy on aldosterone secretion.

METHODS

In a preliminary study, the acute effects of nephrectomy on aldosterone and corticosterone secretion were observed in 6 dogs with thoracic caval constriction and an intact anterior pituitary. Following control observations, the kidneys were removed and steroid secretion was measured again 1 to 2 hours later; the effects of subsequent intravenous administration of crude saline extracts of each dog's kidneys were studied.

In Experiment I of the definitive study, 10 dogs with chronic caval constriction were subjected to hypophysectomy; the animals were given 75 to 100 mg of cortisone acetate intramuscularly on the day of hypophysectomy and daily for the remainder of the experiment. Two or three days after hypophysectomy the acute effects of bilateral nephrectomy were studied 1 to 2 hours after removal of the kidneys. In 3 of the 10 animals, crude saline extracts of each animal's kidneys were infused intravenously and the effects on steroid secretion observed. Because of a decline in venous pressure after nephrectomy in 5 of the first 6 animals, an additional ligature was placed around the thoracic inferior vena cava in the last 4 dogs and tightened to maintain a constant high venous pressure.

In Experiment II, Na depletion was produced in 8 normal dogs by means of a low Na diet (1 to 2 mEq per day of Na and 26 mEq per day of K) over a period of 10 to 14 days and by administration of 2 ml of Mercuhydrin (meralluride) intramuscularly on at least two occasions. The Na-depleted dogs were hypophysectomized 2 to 3 days before acute nephrectomy was performed and the animals received 75 to 100 mg of cortisone acetate daily as in Experiment I.

All animals were mongrel dogs weighing 15 to 20 kg. The animals were anesthetized lightly with Na pentobarbital for the acute observations. Adrenal vein blood was collected by methods described previously (6); all blood removed was replaced with blood from normal dogs. In some animals an infusion of *l*-norepinephrine was given to sustain adrenal blood flow at a fairly constant rate throughout the experiment. The concentrations of aldosterone and corticosterone in adrenal vein plasma were measured by the double isotope derivative assay of Kliman and Peterson (7). Porter-Silber chromogens were determined by a modification of the procedure of Peterson, Karrer and Guerra (8). The kidney extracts were prepared from each animal's own two kidneys. Two to 3 hours after removal of the kidneys, which remained at room temperature during this time, the 2 kidneys were homogenized in 300 ml of normal saline in a Waring blender for 3 minutes. The homogenate was filtered through cheesecloth and fluid from the pulp of the homogenate was squeezed through the cloth. This filtrate was centrifuged at 8,000 rpm in a Lourdes model AT centrifuge for 10 minutes. The supernatant was infused intravenously at a rate of 5 to 6 ml per minute by means of a constant infusion pump.

RESULTS

In the preliminary studies, aldosterone secretion fell after nephrectomy in only two of the six ani-

TABLE I

Effects of nephrectomy on steroid secretion in dogs with chronic thoracic caval constriction

Dog	Aldosterone secretion* μg/min			Corticosterone secretion μg/min		
	Control	After nephrectomy	After kidney extract	Control	After nephrectomy	After kidney extract
1	0.071	0.012	0.052	2.42	2.19	0.82
2	0.196	0.208	0.183	8.80	7.22	7.97
3	0.061	0.047	0.053	5.60	4.80	3.48
4	0.078	0.086	0.031	3.66	3.88	2.49
5	0.091	0.046	0.148	4.60	3.40	4.91
6	0.138	0.118	0.107	3.64	2.70	2.62
Dogs with thoracic caval constriction stressed by laparotomy	0.135 ±0.056 (N = 16)			3.11 ±1.78 (N = 16)		

* Each value for aldosterone secretion represents the average of 2 to 4 determinations on separate collections of adrenal vein plasma.

mals with thoracic caval constriction and an intact anterior pituitary (Dogs 1 and 5 of Table I). The values for aldosterone secretion during each of the three periods of Table I represent the averages of two to four determinations on separate collections of adrenal vein plasma. Corticosterone secretion was very high during the control period in all but Dog 1 (average value of 4.79 μg per min-

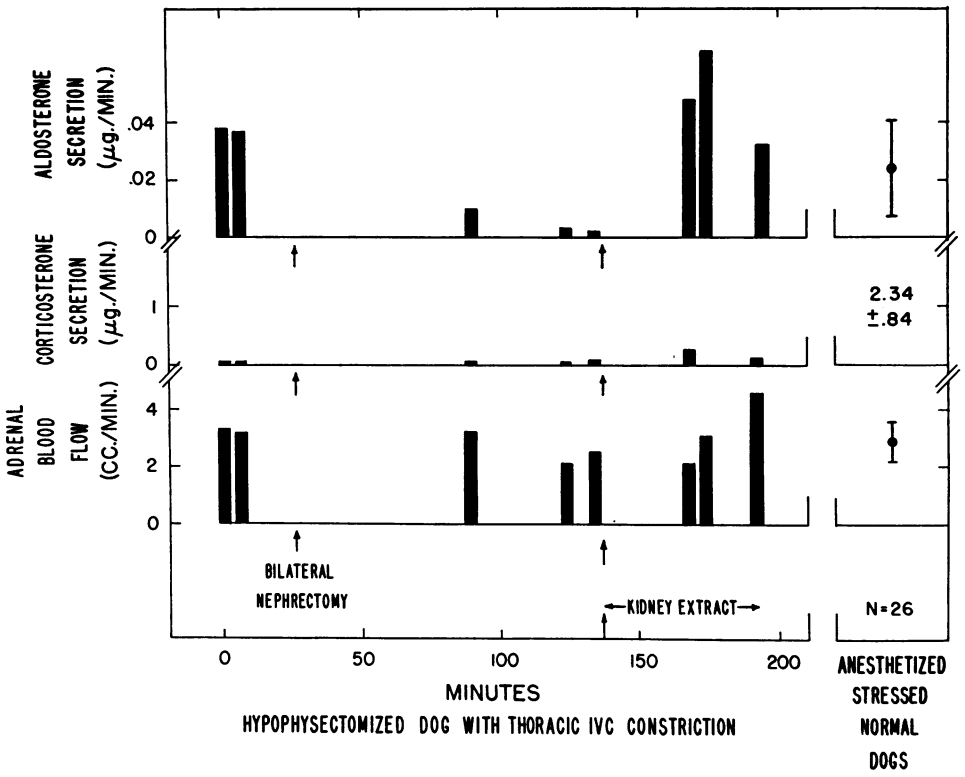

FIG. 1. EFFECTS OF NEPHRECTOMY AND INTRAVENOUS INJECTION OF A KIDNEY EXTRACT ON STEROID SECRETION AND ADRENAL BLOOD FLOW IN A HYPOPHYSECTOMIZED DOG WITH THORACIC INFERIOR VENA CAVAL (IVC) CONSTRICTION. Comparative data for anesthetized stressed normal dogs are presented in the right section.

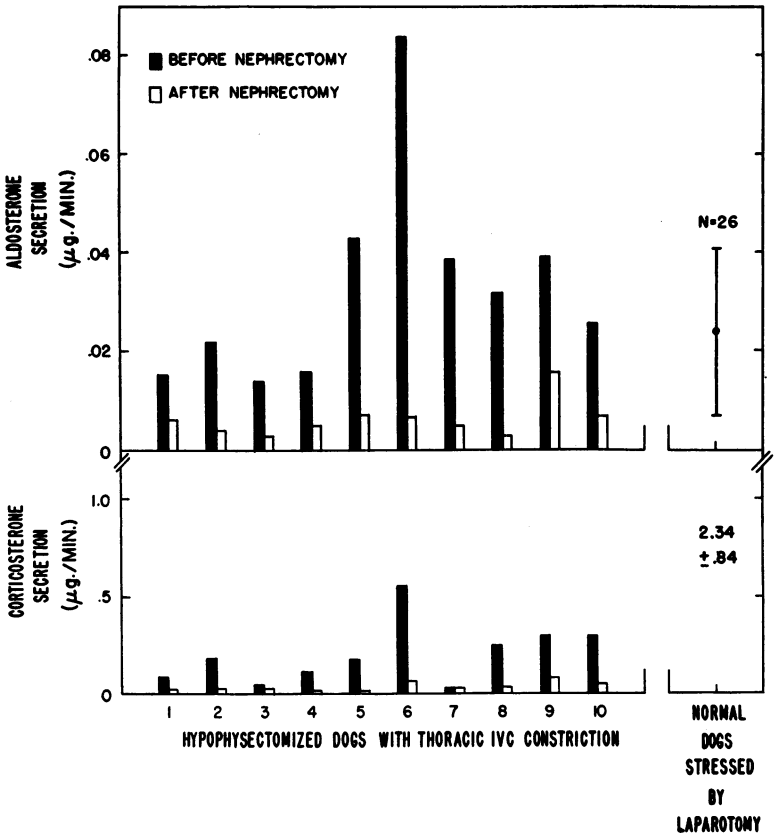

FIG. 2. EFFECTS OF ACUTE NEPHRECTOMY ON ALDOSTERONE AND CORTICOSTERONE SECRETION IN 10 HYPOPHYSECTOMIZED DOGS WITH THORACIC CAVAL CONSTRICTION. Each bar represents the average of 2 or 3 analyses from separate collections of adrenal vein blood. Comparative data from 26 normal dogs stressed by laparotomy are presented on the right. Dogs 4–9 received an infusion of *l*-norepinephrine to maintain arterial pressure and adrenal flow at the control level.

ute in comparison with 3.11 μg per minute for another series of dogs with thoracic caval constriction) and remained high after nephrectomy. Venous pressure fell slightly in all six dogs during the course of the acute experiment. A marked increase in aldosterone production occurred following the infusion of the kidney extract only in the two animals (Dogs 1 and 5) in which aldosterone secretion fell after nephrectomy. Corticosterone secretion was apparently not influenced by the kidney extracts.

Experiment I: Effects of nephrectomy and subsequent administration of kidney extracts on steroid secretion in hypophysectomized dogs with chronic thoracic caval constriction. Because of the high secretion rates for corticosterone and presumably increased ACTH release in the preliminary experiment, the anterior pituitary was removed 2 to 3 days before nephrectomy was performed in the definitive study. The experimental design and the results of a typical experiment are presented in Figure 1 and the findings for all ten dogs are plotted in Figure 2. As a result of hypophysectomy, the average control value of 0.033 μg per minute for aldosterone secretion was considerably lower than the average value of 0.135 μg per minute obtained in dogs with chronic caval constriction and an intact pituitary ($p < 0.01$). However, in several of the animals (Dogs 5–9), the control rates of aldosterone secretion were higher than the average value for normal dogs stressed by laparotomy (right section of Figure 2). After nephrectomy, aldosterone production fell in every animal and, in most instances, the

final rate of aldosterone secretion was very low; this decline in aldosterone secretion was frequently maximal at the end of 1 hour. Aldosterone output decreased from the mean control value of 0.033 μg per minute to an average value after nephrectomy of 0.006 μg per minute ($p < 0.01$), an 82 per cent fall. Although the initial values for corticosterone secretion were low as a result of hypophysectomy, a further decline from 0.20 to 0.04 μg per minute ($p < 0.01$) was demonstrable after nephrectomy. Porter-Silber chromogen output was reduced from an average value of 0.22 to 0.13 μg per minute ($p > 0.05$); although the difference for the group was not statistically significant, the degree of response was sufficiently great in some animals to indicate a definite decrease. Inferior vena caval pressure fell in five of the first six animals. In the last four animals an additional ligature was placed around the thoracic inferior vena cava and tightened during the acute study; venous pressure was maintained at the high control level in all four animals. Adrenal blood flow was unchanged ($p > 0.05$). In three dogs, saline extracts of each animal's two kidneys produced an increase in aldosterone production, but corticosterone and Porter-Silber chromogen secretion increased in only one of the three animals; this animal (Figure 1) showed a 25-fold increase in aldosterone output, which was the greatest response for the group.

Experiment II: Effects of acute bilateral nephrectomy on steroid secretion in Na-depleted hypophysectomized dogs. All eight animals lost weight during the period of low Na intake. After the first injection of meralluride, 70 to 100 mEq of Na was excreted, whereas the second injection resulted in a considerably smaller loss of Na (8 to 40 mEq). The experimental design and the results of a typical acute experiment are presented in Figure 3. The initial control values for aldosterone secretion for these Na-depleted hypophysectomized dogs before nephrectomy were high. The mean control value for the group was 0.054 μg per minute (Figure 4) in comparison with a value of 0.008 μg per minute for simple hypophysectomized dogs (10) ($p < 0.001$) and a value of 0.024 μg per minute for normal dogs stressed by laparotomy ($p < 0.02$).

After nephrectomy, aldosterone secretion fell in every animal; the response was maximal or nearly maximal at the end of the first hour. The average

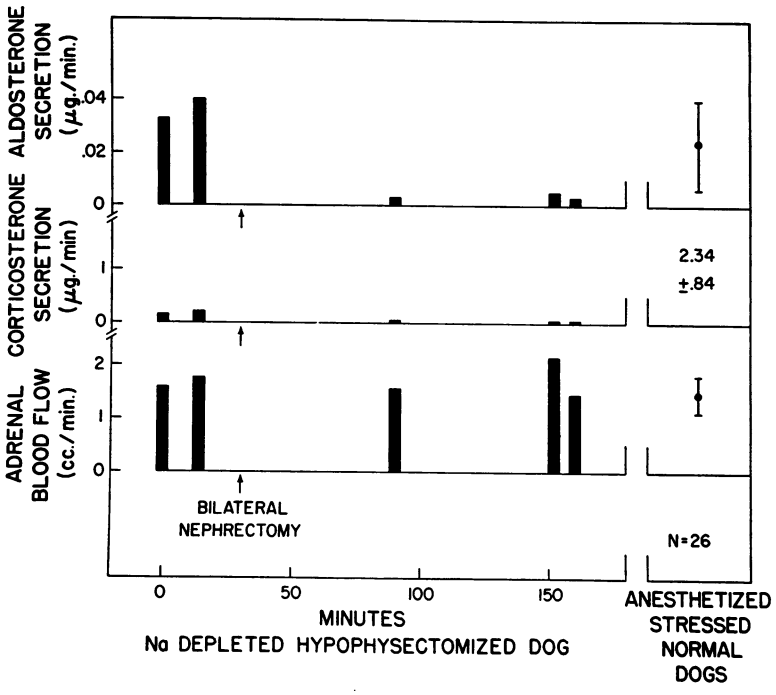

FIG. 3. TYPICAL EFFECTS OF NEPHRECTOMY ON ALDOSTERONE AND CORTICOSTERONE SECRETION AND ADRENAL BLOOD FLOW IN A NA-DEPLETED HYPOPHYSECTOMIZED DOG.

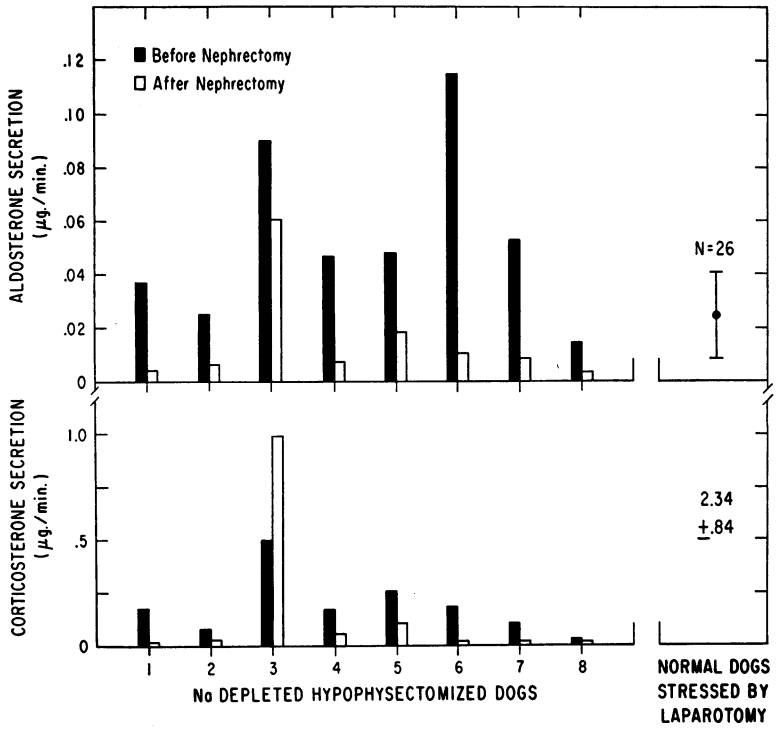

FIG. 4. EFFECTS OF NEPHRECTOMY ON STEROID SECRETION IN 8 NA-DEPLETED HYPOPHYSECTOMIZED DOGS. Each bar represents the average of 2 values from separate collections of adrenal vein blood. Same comparative data as in Figure 2. Only one animal (Dog 3) received an infusion of *l*-norepinephrine to maintain arterial pressure and adrenal blood flow at the control level.

decrease for the group was from 0.054 to 0.015 μg per minute, a 72 per cent fall (Figure 4). In Dog 3, hypophysectomy was apparently incomplete and only a slight fall in aldosterone secretion occurred; a further decrease in aldosterone output was probably blocked by release of ACTH which was reflected by increases in corticosterone and Porter-Silber chromogen output. All other dogs showed a drop in corticosterone secretion after nephrectomy.

DISCUSSION

The immediate stimulus to aldosterone production is mediated via a humoral mechanism (1–5). Indirect evidence for this mechanism was provided by stimulation of hypersecretion of aldosterone by the transplanted and completely denervated adrenal in response to chronic Na depletion (3), chronic thoracic caval constriction (9), and acute blood loss (9). Direct evidence that a humoral agent, an aldosterone-stimulating hormone, promotes increased aldosterone secretion in experimental secondary hyperaldosteronism was obtained by cross circulation of blood from donor dogs with chronic thoracic caval constriction through normal isolated adrenals (1); hypersecretion of aldosterone occurred in the isolated adrenals. Control experiments which involved cross circulation of blood from normal donor dogs through normal isolated adrenals failed to show an increase in aldosterone secretion.

In dogs with thoracic caval constriction and secondary hyperaldosteronism, this aldosterone-stimulating factor is not ACTH. In conscious unstressed dogs with thoracic caval constriction and maximal or near maximal rates of aldosterone secretion, the circulating level of ACTH was at a low basal rate as reflected by extremely low rates of corticosterone and Porter-Silber steroid secretion (5). These experiments clearly demonstrate that ACTH is not the primary humoral factor leading to increased aldosterone production in the dog with caval constriction.

The striking fall in aldosterone secretion which follows hypophysectomy of dogs with thoracic caval constriction and the complete blocking effects of this response by ACTH, indicate that ACTH is another important humoral agent in the control of aldosterone secretion. The marked effects of hypophysectomy have been observed within 1 to 2 hours after pituitary ablation in anesthetized dogs with caval constriction and throughout the entire post-hypophysectomy period of 1 week in conscious dogs with caval constriction (5). However, as indicated previously, it is clear that only a basal output of ACTH is required for maximal aldosterone secretion; an 8- to 30-fold increase in aldosterone secretion occurred following thoracic caval constriction in conscious dogs, with no appreciable increase in corticosterone or Porter-Silber steroid secretion (5). These findings have led to the suggestion that ACTH supports the biogenesis of aldosterone at a very high level, whereas ASH provides the immediate stimulus to aldosterone secretion in dogs with thoracic caval constriction (4).

In a previous study (10), it was demonstrated that an ASH is secreted by the kidney in response to acute blood loss. Also, extensive ablation experiments indicated that this aldosterone-stimulating factor is not secreted by the anterior pituitary, the pineal gland, the brain or the liver. The present data provide evidence for the renal origin of an ASH in response to chronic thoracic caval constriction and to chronic Na depletion. Removal of the kidney from hypophysectomized dogs with thoracic caval constriction resulted consistently in a marked drop in aldosterone secretion. Failure of aldosterone secretion to fall after nephrectomy in four of the six dogs in which the anterior pituitary was present, probably reflects the large amount of ACTH release secondary to the stress of laparotomy for adrenolumbar vein cannulation; corticosterone secretion, which reflects the level of circulating ACTH, was very high. That circulating ACTH was effecting essentially a maximal rate of aldosterone production in these four dogs is also indicated by failure of aldosterone secretion to increase during infusion of the kidney extracts, whereas a striking response to the kidney extracts occurred in the other two dogs in which aldosterone production fell after nephrectomy. Similar results were obtained by Denton, Goding and Wright (3) after nephrectomy in sheep with the anterior pituitary intact. In one of the present Na-depleted animals (Dog 3, Figure 4) in which hypophysectomy was incomplete, the data clearly indicate that the occurrence of a slight reduction in aldosterone secretion rather than the usual marked fall was due to ACTH release; corticosterone production and Porter-Silber chromogen secretion were elevated after removal of the kidneys. In the definitive study in which the anterior pituitary was removed before nephrectomy of dogs with caval constriction, the drop in both aldosterone and corticosterone secretion was consistent and marked. Indeed, the 82 per cent fall in the secretion of aldosterone is the same degree of response observed following hypophysectomy of dogs with thoracic caval constriction (11). In the Na-depleted animals in which hypophysectomy was complete, a similar marked drop in both aldosterone and corticosterone output occurred after nephrectomy. It appears that loss of ACTH and subsequently of ASH leaves the adrenal cortex with a very low rate of aldosterone and corticosterone production which is dependent on a basic intrinsic mechanism of steroidogenesis.

No control experiments on sham-operated animals were performed. This seemed unnecessary since acute nephrectomy was performed on animals which were laparotomized 1 to 2 hours earlier for adrenal vein cannulation and no additional surgery was required for nephrectomy except ligation of the renal vessels and extirpation of the kidneys. It appears unlikely that the metabolic changes which occur after nephrectomy could have influenced aldosterone secretion appreciably, since the fall in aldosterone secretion was maximal or near maximal within 1 hour after removal of the kidneys.

The initial control values for aldosterone secretion in both the hypophysectomized caval and the hypophysectomized Na-depleted dogs were consistently greater than the rates of aldosterone secretion observed in simple hypophysectomized dogs and frequently higher than aldosterone secretion in normal dogs stressed by laparotomy. This finding indicates that hypersecretion of aldosterone occurred in the absence of the anterior pituitary both in the Na-depleted animals and in the dogs with caval constriction. This continued

hypersecretion of aldosterone in the absence of anterior pituitary hormones is presumably secondary to increased release of ASH. The data are in agreement with earlier evidence (5) that increased secretion of aldosterone occurs in response to caval constriction in the conscious hypophysectomized dog. The high initial values for aldosterone secretion in some of the Na-depleted hypophysectomized dogs indicate that chronic Na-depletion in the dog is a much more effective stimulus to aldosterone production than had heretofore been found; Rosnagle and Farrell (12) observed only a twofold elevation in Na-depleted dogs with the pituitary present.

The present experimental conditions and results are in contrast to an earlier experiment (11) in which normal dogs were hypophysectomized and subjected to a low Na diet for 7 to 19 days but were not given meralluride to reduce total body Na. In this earlier study, aldosterone secretion was apparently increased in some of these simple hypophysectomized dogs by Na depletion but hypersecretion was not achieved. In the present experiments, Na depletion was carried out in normal dogs for 10 to 14 days, which was probably adequate time for hypertrophy of the zona glomerulosa to occur, and measurements of aldosterone secretion were made 2 to 3 days after hypophysectomy in Na-depleted dogs supported with cortisone to maintain cardiovascular function and adrenal blood flow; the response in aldosterone production to Na depletion was evident after hypophysectomy. Failure of hypersecretion of aldosterone to occur after acute constriction of the thoracic inferior vena cava in anesthetized hypophysectomized dogs (11) is probably attributable to the inability of the animals to withstand the marked acute alterations in cardiovascular function in the absence of anterior pituitary hormones. It is frequently necessary to support the anesthetized hypophysectomized dog with *l*-norepinephrine or cortisone; the latter was used consistently in the present study to support cardiovascular function. The rates of aldosterone secretion in the present cortisone-treated hypophysectomized dogs with caval constriction are higher than those observed previously in such animals within 1 to 2 hours after hypophysectomy. It appears that the sudden loss of the anterior pituitary hormones has a marked influence on steroid secretion and that compensation is partially achieved within 2 to 3 days. This increase in aldosterone production during the first 2 to 3 days after hypophysectomy of dogs with caval constriction has been reported previously (5), and rates of aldosterone production from six to ten times normal were achieved.

The finding that an ASH is secreted by the kidney in three different experimental conditions may indicate the presence of an ASH common to all three situations. Secretion of an ASH by the kidney during Na depletion suggests that ASH is an important physiological regulator of aldosterone secretion since alterations in electrolyte intake constitute one of the most important mechanisms in the normal daily regulation of aldosterone secretion.

The crude kidney extracts from one of the three hypophysectomized dogs with caval constriction increased corticosterone and Porter-Silber chromogen secretion; this result occurred in the animal with the greatest increase in aldosterone production (a 25-fold increase). Also, in another hypophysectomized dog with caval constriction, a crude kidney extract increased aldosterone secretion tenfold and both corticosterone production and Porter-Silber chromogen output were augmented (personal observations). In contrast, kidney extracts from normal material increased aldosterone secretion consistently but failed to augment corticosterone and Porter-Silber chromogen output (10). These results may reflect a greater amount of the active agent in the kidneys of dogs with caval constriction than that present in normal dog kidneys. This aldosterone-stimulating factor is clearly not ACTH which is bound to the kidney (13), since the pattern of steroid response of the kidney extracts is distinctly different from that of ACTH. The primary effect of the kidney extracts was on aldosterone production, whereas ACTH produced a greater response in cortisol and corticosterone secretion than in aldosterone output. It is of interest in this connection that "renin" [1] and angiotensin (hypertensin II) effected a marked increase in aldosterone and corticosterone production in the nephrectomized, hypo-

[1] "Renin" has been placed in quotation marks to emphasize the lack of purity of preparations referred to here and cited throughout the literature as renin.

physectomized dog, whereas the Porter-Silber chromogen response was relatively less (14 and personal observations). Although crude extracts from normal kidneys have failed to show corticosterone- and cortisol-stimulating activity (10), fractionation of such extracts has consistently yielded aldosterone-, corticosterone- and cortisol-stimulating activity in the "renin" fractions which were precipitated with 1.7 and 2.5 M ammonium sulfate (15); pressor activity was always associated with aldosterone-stimulating activity. This finding, the decreases in aldosterone, corticosterone and Porter-Silber chromogen output after nephrectomy of dogs with caval constriction, and the effects of 50 per cent of the kidney extracts from these animals on the secretion of all three steroids, are consistent with the identity of ASH and "renin" or a renin-like substance. It appears that ASH has a biosynthetic action either at an early stage of the steroidogenic process or on multiple loci in biosynthesis, since not only aldosterone but corticosterone and cortisol synthesis are influenced.

What is the stimulus to ASH release and where are the effector cells located in the kidney? After acute blood loss, it is easy to visualize an acute decrease in pressure or volume leading to ASH release. Also, during chronic Na depletion the same stimulus could be operative. A plausible location for the effector cells is suggested by hypergranulation of the juxtaglomerular cells during Na depletion (16). Also, recent studies (17) have demonstrated hypergranulation and occasional hyperplasia of the juxtaglomular cells in dogs with hyperaldosteronism secondary to thoracic caval constriction. Since the juxtaglomerular cells are located in the media of the renal afferent arterioles, the possibility must be strongly considered that the afferent arterioles constitute the receptor site.

SUMMARY AND CONCLUSIONS

The effects of nephrectomy on aldosterone and corticosterone secretion have been studied in 16 dogs with chronic thoracic caval constriction and in 8 chronic Na-depleted animals. In the preliminary study with the anterior pituitary intact, aldosterone secretion failed to decrease consistently after nephrectomy, apparently because of the high circulating level of ACTH. Consequently, for the definitive study, hypophysectomy was performed before nephrectomy. In the hypophysectomized dogs with thoracic caval constriction, nephrectomy resulted in a fall in aldosterone secretion in every animal from an average control value of 0.033 to 0.006 μg per minute, an 82 per cent fall, and corticosterone output decreased from a very low rate of secretion of 0.20 to 0.04 μg per minute, an 80 per cent fall. A similar striking drop in both aldosterone and corticosterone secretion followed nephrectomy in the Na-depleted hypophysectomized dogs. The high initial control values for aldosterone secretion before nephrectomy of both hypophysectomized caval and hypophysectomized Na-depleted dogs demonstrated hypersecretion of aldosterone in the absence of the anterior pituitary. The findings provide evidence for increased secretion of an aldosterone-stimulating hormone by the kidney in response to chronic thoracic caval constriction and to chronic Na depletion.

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