Stimulation of Aldosterone Secretion by Hemorrhage in Dogs after Nephrectomy and Decapitation


ABSTRACT

In eight intact, anesthetized dogs the aldosterone secretory rate averaged 13.8 ± 1.9 ng/min (mean ± se). One hour after nephrectomy and decapitation, the aldosterone secretory rate decreased to 2.3 ± 1.7 ng/min and failed to increase in response to hemorrhage. By 8 hours after surgery, the aldosterone secretory rate had returned to control levels. The serum potassium concentration gradually increased from 3.2 ± 0.5 mEq/liter in the control samples to 5.3 ± 0.5 mEq/liter in the 8-hour samples. Eight hours after nephrectomy and decapitation, the arterial blood pressure was lowered to 70 mm Hg by hemorrhage in six dogs. During the next 3 hours, the aldosterone secretory rate increased from 11.2 ± 2.1 ng/min to 27.3 ± 2.1 ng/min. Associated with the increase in the aldosterone secretory rate following hemorrhage was a further increase in the serum potassium concentration from 5.3 ± 0.5 mEq/liter to 6.8 ± 0.5 mEq/liter. The aldosterone secretory rate did not increase following hemorrhage when the rise in serum potassium concentration was prevented by hemodialysis. These data indicate that normal levels of aldosterone secretion can be maintained in the absence of the renin-angiotensin system and the pituitary secretion of adrenocorticotropic hormone. Also, hemorrhage will greatly enhance aldosterone secretion in the absence of the head and the kidneys but only if the serum potassium concentration is allowed to rise following the hemorrhage.

KEY WORDS

angiotensin II    adrenal cortex    radioimmunoassay    potassium
sodium renin arterial blood pressure    hemodialysis

Several investigators have demonstrated that the rate of aldosterone secretion is reduced markedly within 1 hour after bilateral nephrectomy of sodium-depleted, hypophysectomized dogs (1, 2). Furthermore, the rate of secretion remains low for at least 3 hours after surgery and does not increase in response to hemorrhage during this time. However, Davis et al. (3) have shown that the rate of aldosterone secretion can be restored to the level that existed before nephrectomy, even during this depression period, by infusion of a saline extract made from the animal’s two kidneys, by infusion of angiotensin II, or by infusion of potassium ions (4).

Recent studies from our laboratory indicate that the plasma aldosterone concentration in nephrectomized man is within normal physiological limits only a few hours after bilateral nephrectomy despite the lack of kidneys to produce renin. Furthermore, plasma aldosterone concentration increases twofold in nephrectomized patients during the course of hemodialysis, even though renin activities measured at the same time are always undetectable (5). These data suggest that adrenal production of aldosterone can be maintained independent of the renal renin-angiotensin system and can be increased in response to acute stimuli, with a resulting increase in plasma aldosterone concentration.

The present study was designed to evaluate the rate of aldosterone secretion in the dog for an extended period of time following bilateral nephrectomy and decapitation and thus to obtain a more thorough understanding of the factors which regulate the secretion of this hormone. The ability to maintain the nephrectomized, decapitated preparation under stable conditions for up to half a day after surgery enabled us to determine whether the aldosterone secretory rate could be maintained independent of both the renal renin-angiotensin system and the pituitary secretion of adrenocorticotropic hormone and whether it could be increased in response to hemorrhage in this preparation.
Methods

Thirty-three mongrel dogs (18–24 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv) to permit exposure of the kidneys, catheterization of blood vessels, administration of spinal anesthesia, and decapitation.

The kidneys were removed through retroperitoneal flank incisions. A polyvinyl catheter was inserted into the abdominal aorta through the right femoral artery to monitor arterial blood pressure. Another catheter was inserted into the right femoral vein for continuous intravenous infusion of norepinephrine. Venous blood samples were drawn from a catheter inserted into the left femoral vein. Adrenolumbar venous blood samples were obtained through a cannula inserted into the right adrenolumbar vein, using the procedure described by Hume and Nelson (6). This technique consisted of cannulating the lateral portion of the right adrenolumbar vein with a polyvinyl catheter. The catheter was brought to the outside through the operative wound. A polyethylene choker was placed around the adrenal gland and the entrance of the adrenal vein into the vena cava and was brought to the outside through the operative wound so that it could be manipulated externally. When the choker was relaxed, adrenal secretions were released into the vena cava in a normal fashion. When the choker was tightened, the adrenal blood flowed to the outside through the cannula and was collected in a graduated tube over a measured period of time (usually 4 minutes). The quantity of blood flowing out during this time indicated the adrenal blood flow.

The spinal reflexes were blocked by the administration of procaine (12 ml) into the lumbar spinal canal. The carotid arteries were ligated, and artificial respiration was instituted. The neck was crushed rapidly using a 2-ton hydraulic truck jack to compress a large steel vise. The steel vise was completely closed within 12 seconds, and the head was removed distal to the vise without any bleeding. The arterial blood pressure was maintained at 100 mm Hg by carefully adjusting the rate of norepinephrine infusion from a Beckman solution-metering pump (model 746). The rate varied between 0.1 and 2.0 µg/kg min⁻¹, but no differences in the experimental results were observed at the different rates. The preparations generally required increasing amounts of norepinephrine for 20–40 minutes after decapitation to maintain the arterial blood pressure at 100 mm Hg. Following this initial period of adjustment, the preparations generally remained stable for 10–14 hours.

Hemodialysis Study

Five dogs were connected to a Mini-Cobe Dialyzer through a femoral artery and vein. To control sodium and potassium concentrations during the study, these dogs were dialyzed with a dialysate containing 144.0 mEq/liter of sodium and 3.0 mEq/liter of potassium.

Experimental Protocol

Control blood samples were collected from the adrenolumbar vein for determination of the aldosterone secretory rate or from the left femoral vein for determination of the plasma aldosterone concentration in intact, anesthetized dogs. Following bilateral nephrectomy and decapitation, 10 ml of blood were collected each hour for 8 hours. The volume of blood drawn each hour was replaced with an equal volume of dextran. Eight hours after surgery, the arterial blood pressure was lowered to 70 mm Hg by hemorrhage. Generally this procedure required the removal of about 100 ml of blood via the left femoral vein. Blood samples were drawn for an additional 4 hours following hemorrhage. All blood samples drawn for aldosterone assay were collected in tubes treated with ethylenediaminetetraacetic acid. These samples were centrifuged immediately after collection, and the plasma was frozen until assay for aldosterone could be performed.

Serum Electrolyte Determination

A portion of each of the blood samples drawn throughout the experiment for aldosterone determination was also used for the determination of serum sodium and potassium concentrations with an Instrumentation Laboratories flame photometer (model 143).

Aldosterone Determination

Aldosterone secretory rate and plasma aldosterone concentrations were determined using a rapid, sensitive radioimmunoassay procedure for aldosterone described by McCaa et al. (7).

Results

Aldosterone Secretory Rates in Nephrectomized, Decapitated Dogs

Mean values for the aldosterone secretory rate in six dogs before and for 3 hours after bilateral nephrectomy and decapitation are shown in Figure 1. Between 1 and 2 hours, approximately 250 ml of blood was removed from each dog to determine whether the aldosterone secretory rate could be
stimulated by hemorrhage. The control rate of aldosterone secretion in the intact, anesthetized dogs before nephrectomy and decapitation was 12.6 ± 1.4 ng/min (mean ± se). Within 1 hour after nephrectomy and decapitation, the rate of aldosterone secretion had fallen to an average of 1.9 ± 0.6 ng/min. Three hours after surgery, the aldosterone secretory rate was 2.6 ± 1.3 ng/min. The removal of 250 ml of blood did not elicit increased secretion of aldosterone during the time these dogs were studied. However, the aldosterone secretory rate increased slightly in each dog toward the end of the experiment, which was terminated 3 hours after surgery.

Figure 2 illustrates the aldosterone secretory rates in six dogs when the observation period was extended to 10 hours after nephrectomy and decapitation. Aldosterone secretory rate averaged 10.6 ± 2.1 ng/min in the control dogs and decreased to less than 2.0 ng/min 1 hour after nephrectomy. By 5 hours after decapitation, the spinal reflexes had returned in all of the dogs. Seven hours after surgery, the aldosterone secretory rate was 6.7 ± 1.8 ng/min, and, by 8 hours, the rate of aldosterone secretion had returned essentially to control levels, 9.4 ± 1.6 ng/min.

PLASMA ALDOSTERONE CONCENTRATIONS IN NEPHRECTOMIZED, DECAPITATED DOGS

Sequential determinations of plasma aldosterone, serum sodium, and serum potassium concentrations were made in eight bilaterally nephrectomized, decapitated dogs (Fig. 3). Plasma aldosterone concentration averaged 15.0 ± 1.8 ng/100 ml plasma in the intact, anesthetized dogs. One hour after nephrectomy and decapitation, plasma aldosterone levels decreased to 2.2 ± 1.0 ng/100 ml plasma and failed to increase in response to hemorrhage. Plasma aldosterone concentration remained at low levels for 6 hours after surgery but then returned to control levels during the next 2 hours. The spinal reflexes returned, in general, 1–3 hours ahead of the return of the aldosterone concentrations to normal levels. By 8 hours after surgery, plasma aldosterone concentration averaged 16.5 ± 2.5 ng/100 ml plasma and remained approximately at this level for the next 4 hours.

There were parallel increases in plasma aldosterone concentration and serum potassium concentration following nephrectomy. Serum potassium concentration averaged 3.2 ± 0.5 mEq/liter in the control samples and increased to 5.6 ± 0.5 mEq/liter by 8 hours after surgery. During the next 4 hours, plasma aldosterone concentration and serum potassium concentration remained stable. Serum sodium concentration did not change significantly from the average control value of 146 mEq/liter.

CHANGES IN PLASMA ALDOSTERONE CONCENTRATION IN RESPONSE TO HEMORRHAGE IN THE NEPHRECTOMIZED, DECAPITATED DOG

Figure 4 shows the results of changes in plasma aldosterone concentration in response to hemorrhage in four nephrectomized, decapitated dogs. Eight hours after surgery when the plasma
aldosterone concentration had returned to the level that existed before nephrectomy, the arterial blood pressure was lowered to 70 mm Hg by hemorrhage. This procedure required the removal of about 100 ml of blood. During the next 3 hours, plasma aldosterone concentration increased from 16.5 ± 2.1 ng/100 ml plasma to 35.3 ± 2.7 ng/100 ml plasma. Associated with the increase in plasma aldosterone concentration after hemorrhage was a further increase in serum potassium concentration from 5.4 ± 0.5 mEq/liter to 7.1 ± 0.5 mEq/liter. There was no significant change in serum sodium concentration throughout the experiment.

CHANGES IN ALDOSTERONE SECRETORY RATE IN RESPONSE TO HEMORRHAGE IN THE NEPHRECTOMIZED, DECAPITATED DOG

To determine whether the increase in plasma aldosterone concentration in response to hemorrhage as shown in Figure 4 in the nephrectomized, decapitated preparation was due to an increase in the rate of aldosterone secretion or to a reduction in the rate of metabolic clearance of aldosterone, the rate of aldosterone secretion was studied in an additional four dogs. Following nephrectomy and decapitation, the aldosterone secretory rate was allowed to return to control levels (Fig. 5). In previous studies, this procedure required 6-8 hours. The arterial blood pressure was then decreased to 70 mm Hg by hemorrhage. The rate of aldosterone secretion increased from 11.2 ± 2.1 ng/min to 27.3 ± 2.1 ng/min during the 3 hours after hemorrhage. Associated with the increase in aldosterone secretory rate was an increase in serum potassium concentration from 5.5 ± 0.5 mEq/liter at the beginning of hemorrhage to 7.2 ± 0.5 mEq/liter 3 hours after hemorrhage. Serum sodium concentration did not change significantly.

CHANGES IN PLASMA ALDOSTERONE CONCENTRATION IN RESPONSE TO HEMORRHAGE IN THE NEPHRECTOMIZED, DECAPITATED DOG DURING HEMODIALYSIS

Figure 6 illustrates the changes in plasma aldosterone concentration in response to hemorrhage in two nephrectomized, decapitated dogs that underwent hemodialysis for 4 hours to bring their serum potassium concentrations back within normal limits. The kidneys were removed 2 days before the experiment to allow the use of heparin during the dialysis period. Serum potassium concentration increased from 3.4 mEq/liter to 5.6 mEq/liter during the 2 days the dogs were without kidneys; however, the serum potassium concentration was reduced to 3.6 mEq/liter after 3 hours of dialysis. Plasma aldosterone concentration averaged 10.0 ± 2.0 ng/100 ml plasma in the anesthetized dogs before nephrectomy. Two days after nephrectomy, plasma aldosterone concentration had increased to 23.0 ± 4.8 ng/100 ml plasma. After
Effect of hemorrhage on plasma aldosterone, potassium, and sodium concentration in nephrectomized-decapitated dogs. Plasma potassium concentration was lowered to the level that existed before nephrectomy by hemodialysis for 4 hours. Hemodialysis was stopped at the time of hemorrhage. Vertical lines indicate ± SE. N indicates the number of dogs studied.

decapitation and 4 hours of hemodialysis, plasma aldosterone concentration averaged 6.9 ± 1.8 ng/100 ml plasma and plasma potassium concentration averaged 4.1 mEq/liter. At this time the dialyzer was turned off and the arterial blood pressure was lowered to 70 mm Hg by hemorrhage. During the next 2 hours there was a gradual increase in plasma aldosterone concentration to 21.0 ± 2.4 ng/100 ml plasma. There was also an increase in serum potassium concentration from 4.1 mEq/liter to 6.0 mEq/liter during this 2-hour interval. Figure 7 illustrates the changes in plasma aldosterone concentration in three nephrectomized, decapitated dogs when the arterial blood pressure was lowered to 70 mm Hg by hemorrhage with the dialyzer working to prevent the rise in serum potassium concentration. Under these conditions, in which the serum potassium concentration was not allowed to change, plasma aldosterone concentration did not increase in response to hemorrhage.

Discussion

Recent studies showing that the rate of aldosterone secretion is maintained within physiological limits in nephrectomized man despite the lack of kidneys to produce renin (7) and that aldosterone secretion can be stimulated by volume depletion, sodium depletion (8), or increased plasma potassium concentrations (9) lead to the conclusion that the known mechanisms involved in the regulation of aldosterone secretion are far from completely understood. The concept that both volume and sodium depletion stimulate aldosterone secretion primarily through the renal renin-angiotensin system is based on the fact that sodium deficiency induces increased renin content of the kidneys (10) and elevated levels of plasma angiotensin II (11). Yet, Blair-West et al. (12) reported that, in the sheep, rapid correction of sodium depletion resulted in complete dissociation between plasma angiotensin II concentration and the rate of aldosterone secretion.

Therefore, the present study was designed to evaluate some aspects of the regulation of aldosterone secretion and the control of plasma aldosterone concentration in the absence of any factors from the head and the kidneys. We were assisted in these experiments by two recent technical innovations. Decapitation was accomplished with a rapid (few seconds) and bloodless vise method rather than by the traumatic procedure of ligation of blood vessels and surgical removal of the head. Also, a sensitive, rapid radioimmunoassay procedure for aldosterone allowed us to analyze both peripheral plasma and adrenal venous plasma samples at numerous sequential times. In the present study, the rate of aldosterone secretion fell to 17% of control levels within 1 hour after bilateral nephrectomy and decapitation. Hemorrhage did not cause an increase
in the rate of aldosterone secretion during the first 3 hours after surgery. These results agree with those reported by other investigators (1, 2). However, we were able to maintain these dogs under stable conditions for half a day after surgery and to follow the aldosterone secretory rate and plasma aldosterone concentration for a much longer time after nephrectomy and decapitation than has been reported by other investigators. By 5 hours after surgery the spinal reflexes had returned in all of the dogs. The spinal reflexes returned 1 or more hours before the return of normal aldosterone secretion, which may have been incidental although a causal relationship has not been ruled out. By 6 hours after surgery the aldosterone secretory rate began to increase, and by 8-10 hours the rate of aldosterone secretion and the plasma aldosterone concentration had returned to basal levels. Associated with the return of the aldosterone secretory rate and plasma aldosterone concentration to basal levels was an increase in plasma potassium concentration of 2.5 mEq/liter.

Although hemorrhage failed to increase the rate of aldosterone secretion during the first few hours after nephrectomy and decapitation, both the rate of aldosterone secretion and the plasma aldosterone concentration increased in response to hemorrhage provided the aldosterone secretory rate was first allowed to return to basal levels, which required about 8 hours after surgery. Also, there was always a further increase in plasma potassium concentration associated with the increase in aldosterone secretion in response to hemorrhage, which suggested that the increase in aldosterone secretion might have been caused by the increased potassium.

Further evidence that the increased aldosterone secretory rate was caused by the increased serum potassium concentration is provided by an additional study using hemodialysis to prevent the rise in serum potassium following hemorrhage. Dogs that had undergone bilateral nephrectomy 2 days before the study were dialyzed for 4 hours to lower the serum potassium to the level that existed before nephrectomy. When the dialyzer was turned off immediately before the arterial blood pressure was lowered to 70 mm Hg, the serum potassium concentration increased from 4.0 mEq/liter to 6.0 mEq/liter, and plasma aldosterone concentration increased from 7.0 ng/100 ml plasma to 21.0 ng/100 ml plasma. However, when the dialyzer remained on and the serum potassium concentration was first lowered and then held constant by hemodialysis, hemorrhage failed to cause an increase in the plasma aldosterone concentration.

These studies indicate that hemorrhage can cause an increase in the aldosterone secretory rate and the plasma aldosterone concentration in the absence of kidney and head factors. It appears that this effect of hemorrhage is mediated by increased plasma potassium concentration, although it is not clear why plasma potassium concentration increased following hemorrhage in the nephrectomized, decapitated preparation. Laragh and Stoerk (13) first noted the stimulatory effect of increased plasma potassium concentrations in man, and infusion of potassium ions has been shown to stimulate directly the adrenal gland to produce aldosterone in the dog (4), the sheep (14), and in vitro preparations (15). Although there has been some controversy as to whether plasma potassium concentration varies enough with sodium depletion or potassium loading to act as a major determinant of aldosterone secretion, Boyd and Mulrow (16) reported that the variations in plasma potassium concentration in the rat could act to control aldosterone secretion. Also, Baumber et al. (17) observed that intra-adrenal potassium is increased following angiotensin II infusion and sodium depletion in dogs.

In conclusion, this study demonstrates three facts that will probably be significant in finally understanding regulation of aldosterone secretion. First, aldosterone can be secreted in normal and even supranormal amounts without immediate stimulatory factors from either the kidneys or the head. Second, aldosterone secretion is markedly increased following hemorrhage even in the absence of the kidneys and the head. And, third, the high rates of secretion in the nephrectomized, decapitated dog seem to correlate positively with high plasma concentrations of potassium ions. These experiments do not in any way suggest that kidney and head factors are unimportant in the control of aldosterone secretion. They merely point out that aldosterone secretion can go on without these factors and that in some situations—hemorrhage, for instance—at least a moderate degree of acute control of aldosterone secretion still persists.

References


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