Role of the Kidney and the Renin-Angiotensin System in the Response of Aldosterone Secretion to Hemorrhage

By Patrick J. Mulrow, M.D., and William F. Ganong, M.D.

Hemorrhage has been shown by several investigators to increase aldosterone secretion in the normal man and dog. We therefore selected hemorrhage as a stimulus and studied the mechanism by which it increased aldosterone secretion. The development of a highly sensitive and specific isotope method for measuring aldosterone in adrenal vein blood by Kliman and Peterson made these studies possible. The present report describes the effect of acute hemorrhage on adrenocortical secretion in normal, hypophysectomized, and hypophysectomized-nephrectomized dogs.

Method

The normal dogs had their right lumboadrenal veins cannulated on the day prior to the experiment to minimize the amount of trauma on the experimental day. On the experimental day, the dogs were anesthetized with nembutal. One hour later, 10- to 15-ml control samples of adrenal vein blood were collected. Each sample was immediately replaced with hypophysectomized dog bank blood to avoid the effects of intermittent small hemorrhages. The dogs were then hemorrhaged 15 ml of blood per Kg. body weight from the femoral artery. Ten minutes later, collection of the first posthemorrhage sample of adrenal vein blood was begun. One to 2 additional samples were collected at 20- to 30-minute intervals. The shed blood was then transfused, and 2 to 3 posttransfusion samples were collected. Following the last sample, 1 unit of ACTH was administered intravenously, and collection of adrenal venous blood was begun 10 minutes later.

The studies with the hypophysectomized dogs were carried out in the same manner, except they were hypophysectomized and had their right lumboadrenal vein cannulated on the experimental day. The studies with hypophysectomized-nephrectomized dogs were similar to the hypophysectomized dogs, except that the left kidney was removed approximately 1 to 3 weeks prior to the experimental day. On the experimental day, the right kidney was removed during the cannulation of the right lumboadrenal vein. This added little trauma to the surgery.

Blood samples were centrifuged immediately after collection, and the plasma was separated and frozen for subsequent analysis. Aldosterone and corticosterone were measured by the isotope derivative technic of Kliman and Peterson and 17-hydroxy corticoids (17-OH corticoids) by the Silber-Porter method.

Effect of Hemorrhage on Adrenocortical Secretion of Normal Dogs

In all 5 normal dogs, acute hemorrhage produced an increment in aldosterone secretion which persisted throughout the posthemorrhage period. Following transfusion of the shed blood, there was a decrement in aldosterone secretion. In all dogs, administration of 1 unit of ACTH intravenously resulted in an increment in aldosterone secretion which was greater than the preceding secretory rate and, in 4 dogs, was even greater than the highest posthemorrhage rate.

The changes in corticosterone and 17-OH corticoids showed 2 separate patterns. In 3 dogs with low control levels of corticosterone and 17-OH corticoids, hemorrhage produced a significant rise in their secretion rates, indicating an ACTH effect. Following transfusion of the shed blood, secretion rates of all steroids including aldosterone decreased. Then, administration of 1 unit of ACTH reproduced the pattern that occurred following hemorrhage. The results from 1 of these dogs are shown in figure 1. However, in 2 dogs, apparently the stress of the anesthesia and the manipulations resulted in high control secretion levels of corticosterone and 17-OH corticoids. Hemorrhage produced a marked rise in aldosterone secretion but very little change in corticosterone and 17-hydroxy corticoid secretion. After transfusion of the shed blood, aldosterone secretion diminished,

From the Department of Medicine, Yale University School of Medicine, New Haven, Connecticut; the Veterans Administration Hospital, West Haven, Connecticut, and the Department of Physiology, University of California Medical School, San Francisco, California.
but corticosterone and 17-OH corticoid secretion remained high. In this situation, when glucocorticoids were being maximally secreted, administration of 1 unit of ACTH increased aldosterone secretion only, as if it were a specific aldosterone-stimulating hormone. The results of such an experiment are shown in figure 2. This finding emphasized the importance of studying the regulation of aldosterone secretion in hypophysectomized animals.

**Effect of Hemorrhage on Adrenocortical Secretions of Hypophysectomized Dogs**

In all 10 hypophysectomized dogs, there was an increase in aldosterone secretion. In 9 of 10 dogs, this increase occurred within 20 minutes after hemorrhage. In general, the posthemorrhage secretion rate was not so high as that seen in normal dogs. In contrast to aldosterone, 17-OH corticoid secretion was not increased by hemorrhage. In the 4 dogs in which corticosterone secretion was measured, there was a slight increase. This increment, however, was not significant statistically.

Administration of 1 unit of ACTH to 8 of the hypophysectomized dogs resulted in a marked increase in aldosterone, corticosterone, and 17-OH corticoid secretions. Figure 3 illustrates the results of hemorrhage in 1 hypophysectomized dog. These results in hypophysectomized dogs demonstrated that hemorrhage could increase aldosterone secretion by a mechanism independent of the pituitary.

The next phase of this investigation, therefore, was designed to study the mechanism by which hemorrhage increased aldosterone secretion in hypophysectomized dogs.

Several lines of evidence suggested that the renin-angiotensin system could influence aldosterone secretion. These have been well summarized by Tobian in an excellent review. Of particular importance were the findings of Deane and Masson in 1951. They reported that encapsulation of 1 kidney or injection of renin extracts into rats caused hypertrophy of the zona glomerulosa of the adrenal gland, the site of aldosterone formation. Also, Hart-
The effect of hemorrhage on adrenocortical secretions of a hypophysectomized dog. The relationship between the arrow indicating the hemorrhage and the blood pressure are slightly out of line. The blood pressure did not begin to fall until after the hemorrhage.

The effect of nephrectomy on the adrenocortical response to hemorrhage in the hypophysectomized dog. Kidney extract denotes the injection of a crude saline extract of a normal dog’s kidney.

Circulation, Volume XXV, January 1962
not increase aldosterone or 17-OH corticoid secretion in the hypophysectomized-nephrectomized dog. Transfusion of the shed blood had no effect on steroid secretion despite a rise in blood pressure. Intravenous administration of a crude saline extract from a normal dog kidney produced a marked rise in aldosterone secretion and increased 17-OH corticoid secretion to a lesser extent. The administration of 1 unit of ACTH produced a slightly different pattern. The 17-OH corticoid secretion was increased to a higher level than with the kidney extract, while aldosterone secretion was not increased so much.

Figure 5 summarizes the results of hemorrhage in 10 hypophysectomized dogs and 10 hypophysectomized dogs without kidneys. P values were calculated by the paired t-test. Each bar represents the mean of 20 to 30 measurements; that is, the several pre- and posthemorrhage measurements in each dog were averaged separately. Hemorrhage increased aldosterone secretion in all 10 hypophysectomized dogs with kidneys. No significant change occurred in corticosterone or 17-OH corticoid secretion. In contrast, hemorrhage failed to increase aldosterone secretion in 8 of 10 hypophysectomized-nephrectomized dogs. In addition, nephrectomy lowered the control rate of aldosterone secretion. The adrenal responsiveness to ACTH was equal in the 2 groups.

Intravenous administration of crude saline extracts of normal dog kidneys increased aldosterone secretion markedly and 17-OH corticoid and corticosterone secretion submaximally.7 A marked pressor response was noted. Davis and coworkers8 have shown similar results with hemorrhage and nephrectomy and kidney extracts.

Renin extracts from kidneys of normal dogs and dogs hypophysectomized 4 hours pre-
RESPONSE OF ALDOSTERONE SECRETION TO HEMORRHAGE

HYPOX. NEPHRECTOMIZED DOGS

Figure 6
A summary of the effects of renin extracts and small doses of ACTH on adrenocortical secretions. The dose of renin extract indicates what portion of the renin extract from a whole kidney was actually injected. The numbers in parentheses indicate the number of injections.

Previously were prepared by the technic of Haas and Goldblatt. By this method, a water-soluble extract of ground kidney is purified by selective acid denaturation, ammonium sulfate precipitation, and 24-hour dialysis against distilled water in the cold. The extracts were assayed in hypophysectomized-nephrectomized dogs. Semipurified renin extracts from normal and hypophysectomized dog kidneys produced similar results. The results, therefore, were pooled.

Figure 6 shows the secretion rates immediately before and during a 10-minute period starting 4 minutes after the injection. The extract was administered intravenously over 1 minute. Most dogs received more than 1 injection. The steroid secretion rates prior to injection of the extract are not true basal levels since the effect of previous injections of renin extract often persisted despite a 1-hour delay between injections. Large doses of renin extract from one-third to whole dog kidneys markedly increased aldosterone, corticosterone, and 17-OH corticoid secretion and produced a marked rise in blood pressure. These results were similar to the results following the crude saline extracts. However, much smaller doses of renin extracts, like hemorrhage in hypophysectomized dogs with intact kidneys, stimulated mainly aldosterone secretion. Two to 10 milliunits of ACTH stimulated corticosterone and 17-OH corticoid secretion as much as the large doses of renin extract but did not increase aldosterone se-
creatin significantly. Injections of control tissue extracts and infusions of norepinephrine had no effect.

A similar type of dose response was found with angiotensin.* Figure 7 shows the adrenocortical secretions immediately before and during infusion of different doses of angiotensin II. Large doses increased aldosterone, 17-OH corticoid, and corticosterone secretion. Small doses increased primarily aldosterone secretion.9, 10

To determine whether angiotensin stimulates the adrenal directly or through release of some other humoral substance from a distal site, the left adrenal of 4 hypophysectomized-nephrectomized dogs was isolated in vivo and directly perfused with angiotensin II. In all 4 experiments, adrenal perfusion caused a marked increase in steroid production. Figure 8 demonstrates the results of 1 experiment. The control rates of all steroids were low. Infusion of 12.5 µg./min. of norepinephrine into the jugular vein produced a marked rise in systemic blood pressure but did not change steroid secretion rates. Infusion of angiotensin into the jugular vein markedly increased systemic blood pressure and aldosterone secretion. Corticosterone and 17-OH corticoïds were also increased. Infusion of the same dose of angiotensin into the isolated adrenal caused a marked rise in aldosterone, corticosterone, and 17-OH corticoid secretion which persisted after cessation of the infusion. This persistence of effect on aldosterone secretion is commonly seen after cessation of a periph-

*Supplied as Hypertensin CIBA.

Figure 7
A summary of the effects of different doses of angiotensin II on adrenocortical secretions. The actual doses administered have been rounded off. The numbers in parentheses indicate the number of infusions of that particular dose.
eral infusion of a large dose. Also, the very marked increases in corticosterone and 17-OH corticoid secretion were similar to the findings following systemic infusion of a large dose of angiotensin. Obviously, any dose of angiotensin administered directly into the adrenal gland is many times larger with respect to the amount reaching the adrenal than an equal peripheral dose. The failure of the direct adrenal perfusion of angiotensin to increase aldosterone secretion more than a peripheral dose would have may be due to the fact that in this dog the peripheral dose produced a maximum response. Other experiments with smaller doses of angiotensin have shown a greater aldosterone-stimulating effect following direct adrenal perfusion than after an equal peripheral dose.

Therefore, angiotensin probably stimulates the adrenal directly rather than through a release of a factor from a distal site.

Summary

In summary, these data show that in hypophysectomized dogs:

1. Hemorrhage stimulates mainly aldosterone secretion.
2. Nephrectomy prevents stimulation of aldosterone secretion by hemorrhage.
3. Nephrectomy lowers basal secretion of aldosterone.
4. Crude saline extracts of dog kidneys, semipurified renin extracts of dog kidneys, and synthetic angiotensin can stimulate aldosterone secretion.

These findings suggest that in hypophysec-
tomized dogs, the kidney, via the renin-angiotensin system, is an important mechanism by which hemorrhage stimulates aldosterone secretion.

References
