Biphasic Stimulation of Aldosterone Secretion during Hemorrhage in Dogs


ABSTRACT
The increase of aldosterone secretion in response to hemorrhage has been reevaluated with the aid of a newly developed micromethod using gas-liquid chromatography. Pentobarbital-anesthetized large male dogs were slowly exsanguinated through the lumboadrenal vein while sequential 15-minute samples of adrenal venous effluent were collected. Mean arterial blood pressure and blood loss were monitored. The response to hemorrhage was found to be biphasic: a significant increase in aldosterone secretion occurred before blood pressure changed significantly, the secretory rates then fell toward control, and rose again as the blood pressure fell to shock levels. Statistical evaluation of the data confirmed the quartic nature of the response and indicated a best-fit equation of $y = 169.14x - 800.22x^2 + 1258.46x^3 - 619.24x^4$.

These results demonstrate that control of aldosterone secretion is dependent on complex functions of blood volume and blood pressure. Volume receptor mechanisms as well as pressure receptor mechanisms would seem to be involved. Any investigation of the aldosterone response to hemorrhage, to be valid, must take these complex relationships into consideration.

ADDITIONAL KEY WORDS
aldosterone secretion
hemorrhage
arterial blood pressure
adrenal cortex

Loss of blood represents one of the most fundamental threats to life. Under conditions of blood loss, a series of homeostatic mechanisms comes into play to defend the adequacy of the circulation (1, 2). Among these is an increased secretion of aldosterone presumably to effect conservation of sodium. Farrell and coworkers (3) first demonstrated an increase in aldosterone secretory rates in hemorrhage. Other investigators have confirmed these findings (4-8). Many of these latter investigators measured one control aldosterone level and then determined the aldosterone secretory rates at one or two posthemorrhage sample times, on the assumption that a simple sustained level of response occurs (although there is no a priori reason to assume such to be the case). This inadequate sampling procedure does not permit the analysis of the dynamics of the response of aldosterone secretion to changes in blood pressure and volume, nor the study of possible receptor organs and effector mechanisms.

Recently a new method of aldosterone analysis has been developed in this laboratory based on electron-capture gas-liquid chromatography (9). This method is rapid, specific, and reproducible. Using this technique we have reinvestigated the aldosterone response to small cumulative changes in blood pressure and blood volume induced by slow hemorrhage.

Methods
Male mongrel dogs (25 to 30 kg) were anesthetized with pentobarbital sodium (30 mg/kg). A 4-mm polyethylene cannula freshly rinsed with heparin solution was inserted into the femoral artery and attached to a mercury manometer for blood pressure determinations. The left adrenal was exposed by making a 6-inch incision into the left flank caudal and parallel to the last rib. The lumboadrenal vein was dissected free, and collateral veins were ligated. A ligature was placed around the adrenal vein at its junction.
with the vena cava (10). A polyethylene (PE-240) cannula was installed in the lumboadrenal vein. The animals were heparinized (500 USP units/kg), and the ligature was tightened on the adrenal vein. Total left adrenal venous effluent was collected in serial 15-minute samples. Samples were continuously chilled during collection and subsequently frozen until analysis. Mean arterial blood pressure was recorded at 5-minute intervals, body temperature was noted at 15-minute intervals with a rectal thermometer, and blood loss was measured as the cumulative volume of the adrenal venous samples.

Steroid analysis was performed by the method of Fabre et al. (9). In brief, a small amount of C-14 aldosterone was added to each whole blood sample. The isolation procedure then involved: chloroform extraction, benzene : water partition, thin-layer chromatography to separate aldosterone from cortisol, periodic acid oxidation to form aldosterone γ-lactone, thin-layer chromatography to separate products from reaction mixture, and electron-capture gas chromatography. Steroid concentrations were determined by a function of the peak height of the gas chromatographic recorder and percent of the original sample placed on the gas chromatographic column as determined by scintillation counting. The results are expressed in μg aldosterone/100 kg body weight/hour. This method has been shown to allow 100% ± 6% calculated recovery of aldosterone added to femoral blood samples. Specificity has been proven by gas chromatography and mass spectroscopy, and by radioisotope monitoring of the gas chromatograph effluent (9).

Since the total time involved for each animal was variable, each parameter was linearly transformed to unit length (11) for each dog to allow for the pooling of data of several dogs. A quartic equation was fitted using a least-squares technique (12) to blood pressure, accumulative blood volume, and aldosterone with time as a base. In fitting the polynomial equation, the dispersion matrix was inverted to permit estimation of the fiducial limits of each coefficient and its confidence interval. All computations were carried out on an IBM 7094 Model II digital computer. The least-squares procedure and matrix-inversion routine were adapted from the IBM System/360 Scientific Subroutine Package (13).

Blood pressure and aldosterone secretory rates up to the maximum of the first peak of aldosterone secretion were intercorrelated using the Pearson product-moment method (11).

**Results**

Figures 1 to 5 represent plots of all aldosterone secretory rates, recordings of
Figure 2
Legend as in Figure 1.

Figure 3
Legend as in Figure 1.
FIGURE 4

Legend as in Figure 1.

FIGURE 5

Legend as in Figure 1.
mean arterial blood pressure, and measurements of cumulative blood loss for animals employed in this study. It may be seen that the animals demonstrate a similar pattern with regard to aldosterone secretion: secretion rates begin rather low, increase approximately fivefold before mean blood pressure changes significantly, fall toward normal with increasing blood loss, rise again as the blood pressure drops to between 70 and 50 mm Hg, and then fall terminally. Seventeen animals have been studied in this manner and all have demonstrated the biphasic response.

While all animals exhibit the biphasic M-shaped aldosterone response to hemorrhage, they lose blood at varying rates. The total time of exsanguination for each animal therefore varies. If the data are transformed as indicated above, maxima and minima in the aldosterone curves become essentially coincident for the several animals.

The composite curves generated by the fitted polynomial equations are shown in Figure 6. The biphasic response of aldosterone to hemorrhage over time is evident and may be expressed by the relationship:

\[ y = 169.14x - 800.22x^2 + 1258.46x^3 - 619.24x^4. \]

The M-shaped form of the curve is statistically significant in that all coefficients vary reliably from zero \((P < 0.01)\). The form for the positive acceleration of blood loss is \(y = 899.11x + 1175.50x^2 - 1620.62x^3 + 360.12x + 0.1470\); while the relationship for mean arterial blood pressure is given as \(y = 94.16x - 415.60x^2 + 385.13x^3 - 131.16x^4 + 115.10\).

The intercorrelation between blood pressure and aldosterone secretory rates over the period up to the maximum of the first peak of aldosterone secretion was not significant \((r = 0.0359; P > 0.10)\).

**Discussion**

The finding that aldosterone secretion is biphasic in response to hemorrhage is an extension and refinement of previous investigations in this field (3-8). The average initial aldosterone secretory rate of 2.6 \(\mu g/100\) kg/hour (8.9 ng/min) and the average peak level during hemorrhage of 13.25 \(\mu g/100\) kg/hour (44.1 ng/min) are within the range of values reported by other investigators using different methods of aldosterone analysis.
It is interesting that early investigators approached differently the study of aldosterone secretion in response to hemorrhage. Farrell and coworkers (3) bled animals slowly through the lumboadrenal vein, collected sequential samples of adrenal effluent, and pooled corresponding samples of many dogs to obtain results. A significant rise of aldosterone in response to small amounts of blood loss was found, and the aldosterone secretory rates fell to nearly control levels as the blood pressure approached 80 mm Hg, the end point of their experiments. Davis and coworkers (8) and others (6, 7) have studied hemorrhage by taking a control sample of adrenal venous effluent, bleeding the dog acutely to an arterial pressure of 70 mm Hg, and then taking one or two posthemorrhage samples. With this design, a significant rise in aldosterone secretory rates was also observed. From the results presented here demonstrating the biphasic response of aldosterone secretion to hemorrhage, however, one wonders whether both groups were measuring the same response. Perhaps Farrell's group (3) was measuring the first phase of the response, while Davis et al. (8) and Ganong and Mulrow (6, 7) were measuring the second. Since both groups proceeded to use the aldosterone response to hemorrhage in the study of mechanisms of aldosterone control, this may explain somewhat the difference in the results subsequently obtained (7, 8, 15).

Regulation of electrolyte and water balance in relation to hemorrhage has received considerable attention in recent years. A number of receptors have been described which respond to hemorrhage, invoking control systems operating to maintain circulatory volume and pressure. Among these are carotid and aortic baroreceptors, carotid chemoreceptors, and renal juxtaglomerular apparatus and atrial volume receptors. With regard to antidiuretic hormone, Share and coworkers (16) have recently shown that increased secretion of ADH occurs before any change in arterial pressure parameters. In a nonhypotensive hemorrhage of 10% to 20% of blood volume, Gupta et al. (17) have shown that no change in impulse traffic from the aortic baroreceptors occurs, while an 80% decrease in firing rate of atrial receptors is produced. They believe, therefore, that in modest hemorrhage (10% to 20% of blood volume) the only receptors involved are atrial volume receptors. The data presented here reveal alterations in aldosterone secretion prior to any significant change in mean arterial pressure.

Considerable data have involved the juxtaglomerular apparatus in the control of aldosterone secretion by means of the renin-angiotensin mechanism (6-8, 14). The juxtaglomerular apparatus, however, responds primarily to changes in the high pressure system (18). Volume receptors monitoring parameters of the low pressure system would seem to be involved in the first phase of the aldosterone response described above.

The existence of volume receptors in controlling mechanisms for aldosterone secretion have been postulated by several investigators (18-21). Anderson and coworkers (20) have in fact described right atrial volume receptors influencing aldosterone secretion and presumably involving neurohumoral effector mechanisms. Baisset et al. (21) have confirmed these findings and added that posterior diencephalic lesions prevented the response. Several studies have indicated that plasma renin concentrations are elevated in hemorrhage before blood pressure is significantly altered (22-24). Recently it has been postulated that renin release is regulated in part by the sympathetic nervous system, or by hormonal factors, or both (25-27). Perhaps the first phase of the aldosterone stimulation to hemorrhage is governed by a control system having receptors in the low pressure system and an efferent limb operating to increase renin secretion. As the arterial blood pressure falls, other receptor organs are undoubtedly stimulated; these may participate in the second phase of aldosterone stimulation in response to hemorrhage. A temporally spaced hierarchy of activation of receptor mechanisms may therefore be involved in the biphasic response.

While the relationships between aldosterone...
secretory rates and blood pressure and blood volume are important considerations in hemorrhage, blood loss alters other parameters which may affect adrenal secretory rates. Alterations in plasma electrolytes have been purported to affect secretion of aldosterone (28), and the hemodilution coincident to blood loss results in such alterations. Farrell and coworkers investigated this phenomenon (see Fig. 3 of ref. 3). They found that during slow hemorrhage via the lumbar adrenal vein, plasma potassium did not change significantly, and plasma sodium, if anything, tended toward a slight increase. No changes were found in these experiments, implicating electrolyte changes as mediators of the increased aldosterone secretion found in hemorrhage. These experiments have been repeated recently in this laboratory with identical results.

Hemorrhage also results in decreased adrenal blood flow. This is evident from data on blood loss presented in Figures 1 through 6. Adrenal secretory rates are reported to be independent of adrenal blood flow over a large range (3, 10); the terminal downward trend in aldosterone secretory rates, however, may reflect terminal reduction of adrenal blood flow prior to death.

The first decrease in adrenal secretory rates occurring with progressive hemorrhage was a surprising finding. In retrospect, however, this phenomenon occurs with ADH as well (29). In the case of vasopressin, the stored pool is heterogenous and comprised of a quickly releasable component and a slowly releasable component (30). This is analogous to the mechanism operable in neuronal secretion of neurotransmitter substances (30). The biphasic response may therefore simply reflect alterations in the release or biosynthetic rate of aldosterone: transient depletion of adrenal stores, followed by recovery, and then by agonal collapse.

In conclusion, the data presented describe a biphasic stimulation of aldosterone secretory rates during hemorrhage, the first phase of stimulation occurring prior to a decrease in blood pressure. This effect may be due to one or more of three possibilities: (1) participation of two or more stimulatory hormones, (2) regulation by control systems involving a hierarchy of receptor mechanisms, or (3) depletion of adrenal stores of aldosterone and precursors. The data do not permit a distinction among these possibilities, and further studies are indicated.

References


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