Regulation of Aldosterone Biosynthesis During Sodium Deficiency
Evidence for an Essential Role of the Pituitary Gland

ROBERT E. MCCAA, PH.D., HERBERT G. LANGFORD, M.D., JOSE M. MONTALVO, M.D., ORLANDO J. ANDY, M.D., VIRGINIA H. READ, PH.D., AND CONNIE S. MCCAA, M.D., PH.D.

SUMMARY The aldosterone response to adrenocorticotropic hormone (ACTH) and angiotensin II (AH) was evaluated in patients with pituitary insufficiency before and after dietary sodium restriction (10 mEq Na+/day for 12 days). On normal sodium intake, plasma aldosterone concentration and plasma cortisol concentration failed to change from control levels in response to a single injection of ACTH or to a continuous 1-hour infusion of AH in patients with pituitary insufficiency. In response to dietary sodium restriction for 12 days, plasma renin activity (PRA) increased fivefold in patients with pituitary insufficiency, while plasma aldosterone concentration failed to increase significantly, averaging 11.0 ± 3.1 before and 123 ± 3.7 ng/dl (ns, p > 0.05) after sodium deficiency. Although aldosterone secretion failed to increase during sodium deficiency, the patients came into balance at 10 mEq without a significant change in arterial blood pressure (BP). In spite of this, aldosterone concentration increased from 12.9 ± 3.3 to 156 ± 17.3 ng/dl (p < 0.001) in response to ACTH after sodium deficiency. Although the adrenal glomerulosa cells were markedly sensitive to ACTH during sodium deficiency, they remained almost totally refractory to AH since aldosterone secretion failed to increase significantly in response to continuous infusion of a pressor dose of AH for 1 hour. Replacement therapy with ACTH gel for 3 months in patients with pituitary insufficiency failed to restore a normal aldosterone response to either ACTH or AH. These data demonstrate that some non-ACTH pituitary factors are essential for a normal aldosterone response to ACTH, AH, and sodium deficiency.

KEY WORDS • adrenocorticotropic hormone • angiotensin II • pituitary insufficiency • plasma aldosterone • plasma cortisol • sodium restriction

In 1954, Luetscher and Axelrad demonstrated a high aldosterone level in the urine of human beings when sodium intake was restricted, a normal level in patients with hypopituitarism, and an undetectable level in patients with Addison’s disease. In subsequent studies, Lieberman and Luetscher reported that patients with pituitary insufficiency secreted normal amounts of aldosterone under normal resting conditions, but that aldosterone failed to increase in response to ACTH administration or sodium deprivation. However, a normal aldosterone response to ACTH administration and sodium deprivation was observed in patients maintained on glucocorticoid replacement therapy to suppress endogenous pituitary secretion of ACTH.

Similar observations have been made in experimental animals. In chronically hypophysectomized dogs, neither angiotensin II (AH) nor ACTH had a marked stimulatory effect on aldosterone secretion, while the aldosterone response to AH and ACTH was normal in intact dogs treated with glucocorticoids to suppress endogenous ACTH secretion. Also, aldosterone secretion failed to increase in response to sodium depletion by hemodialysis in hypophysectomized-nephrectomized dogs maintained on background infusions of ACTH and AH, indicating that some undefined interrelationship between ACTH and AH was not essential for the normal aldosterone response to sodium depletion. In rats, hypophysectomy abolished the aldosterone response to sodium deprivation, while injections of extracts from whole pituitary glands restored the aldosterone response to sodium deficiency. Collectively, these
observations suggest that chronic deficiency of some non-ACTH pituitary secretory factor(s) inhibits the adrenal aldosterone response to AII, ACTH, and sodium deficiency, and indicate that some additional pituitary factor(s) is required to account fully for the aldosterone response to sodium deficiency.

The present study was designed to evaluate quantitatively the role of the pituitary gland in the regulation of aldosterone secretion during sodium deficiency in human beings. In this study, the aldosterone response to ACTH administration and continuous AII infusion was determined in healthy volunteer subjects before and after dietary sodium restriction (10 mEq Na+/day for 12 days) and compared with the aldosterone response observed in patients with pituitary insufficiency. Thus, the major purpose of this study was to determine whether the aldosterone response to sodium deficiency is mediated solely by the activity of the renin-angiotensin system or whether the aldosterone response is modulated, or mediated, by some additional unidentified pituitary factor(s).

Methods

Experimental Protocol

Experimental procedures used in human beings in this study were approved by the Committee on Human Investigation of the University of Mississippi Medical Center and were performed in the Clinical Research Center at the University Medical Center. Informed consent was obtained from all of the human subjects, none of whom suffered adverse effects or complained of any unusual symptoms during the study.

The aldosterone and cortisol responses to corticotropin, σ 1-25 [Cortrosyn, 250 µg i.v. (Organon, Inc., West Orange, New Jersey)] were determined in 32 patients with pituitary insufficiency before and after dietary sodium restriction. The aldosterone response to AII (Hypertensin, Ciba) infusion for 1 hour at the rate of 6 ng/kg/min was determined in four patients with pituitary insufficiency before and after dietary sodium restriction. Plasma renin activity (PRA) was measured in all of the subjects before, during, and after dietary sodium restriction. Ten human volunteers between the ages of 16 and 27 years with no known disease served as intact controls for this study. Both male and female patients between the ages of 19 and 53 were used in this investigation, and all patients were clinically without anterior pituitary function.

The causes of hypopituitarism in these patients included postpartum pituitary necrosis, craniopharyngiomas, apparent spontaneous infarction of the pituitary, "empty sella syndrome" with demonstrated lack of pituitary function, and surgical removal of the pituitary gland usually for nonfunctioning or prolactin-secreting adenomas. None of the patients had ACTH-secreting tumors. Lack of thyrotropic and gonadotropic secretion was documented in all patients, and adrenal cortisol secretion was absent in all of the patients used in this investigation. All of the patients with pituitary insufficiency were maintained on replacement therapy consisting of thyroxine and either hydrocortisone or cortisone. All medication was stopped on the morning of testing with ACTH and AII. All of the patients remained on medication during the period of dietary sodium restriction.

The following experimental protocol was used for the ACTH study. Each of the subjects was admitted to the Clinical Research Center on the evening before the study. The ACTH stimulatory test was performed on the following morning at 8:00 a.m. after the patients had remained supine overnight and before breakfast and replacement medication. The patients were required to remain recumbent during the study. Control blood samples were collected for the determination of plasma aldosterone concentration, plasma cortisol concentration, and PRA. A single injection, 250 µg corticotropin, σ 1-24 i.v. (Cortrosyn) was administered. Blood samples were collected at 15-minute intervals for 1 hour after ACTH administration. On the following day, a similar procedure was used for the AII study, except continuous AII infusion was maintained for 1 hour at the rate of 6 ng/kg/min instead of a single injection of the vasopressor octapeptide. The patients were maintained on dietary sodium restriction (10 mEq Na+/day) for 12 days and replacement medication continued during this period. Total 24-hour urinary output was collected daily for determination of urinary sodium excretion. After 12 days of dietary sodium restriction, the ACTH and AII stimulatory tests were repeated in the same manner described above. Three patients with pituitary insufficiency were selected to replace hydrocortisone medication with ACTH gel (120 units per week in three divided doses) for 3 months. At the end of this time, these patients were evaluated again in the same manner as before.

Collection and Analysis of Blood Samples

Blood samples for the determination of plasma aldosterone concentration and plasma cortisol concentration were collected in disposable syringes. A portion of the blood was transferred to tubes pretreated with ethylenediamine tetra-acetate (EDTA) and centrifuged for 20 minutes at 4°C. A portion of each blood sample drawn for the control sample before and after dietary sodium restriction was used for the PRA determination. Plasma samples from each patient were frozen until the experiment was complete, and all samples from the same patient were analyzed during the same assay period. The PRA was determined by the radioimmunoassay method of Haber et al. Plasma cortisol concentration was determined by the competitive protein-binding procedure reported by Murphy. Plasma aldosterone concentration was determined using a previously described modification of the radioimmunoassay method of Mayes et al.

Statistical Method

Student's t test for paired observations was used to compare each experimental period with the average of the control period.
Results

Response of Plasma Aldosterone Concentration and Plasma Cortisol Concentration to ACTH Administration in Normal Subjects and in Patients with Pituitary Insufficiency Maintained on Normal Sodium Intake

The response of plasma aldosterone concentration and plasma cortisol concentration to ACTH administration (250 µg, Cortrosyn, i.v.) in 10 normal human beings and in 32 patients with pituitary insufficiency maintained on normal sodium intake (150 mEq Na⁺/day) is illustrated in figure 1. In normal human subjects, plasma cortisol concentration increased from 12.6 ± 3.4 to 32.4 ± 4.8 µg/dl (p < 0.001) within 1 hour after ACTH administration, while plasma aldosterone concentration increased from 7.8 ± 2.7 to 34.5 ± 5.7 ng/dl (p < 0.001) within 1 hour after ACTH administration. In contrast, plasma cortisol concentration and plasma aldosterone concentration failed to change significantly from control levels in response to ACTH administration in patients with pituitary insufficiency. In these patients, plasma cortisol concentration averaged 9.0 ± 2.6 µg/dl before and 10.3 ± 3.2 µg/dl (ns, p > 0.05) after ACTH, while plasma aldosterone concentration averaged 10.8 ± 2.3 ng/dl before and 12.6 ± 3.6 ng/dl (ns, p > 0.05) after ACTH administration.

Response of Plasma Aldosterone Concentration to Continuous Angiotensin II Infusion in Normal Subjects and in Patients with Pituitary Insufficiency Maintained on Normal Sodium Intake

The response of plasma aldosterone concentration to continuous AII infusion at the rate of 6 ng/kg/min for 1 hour in normal human beings and in patients with pituitary insufficiency maintained on normal sodium intake is illustrated in figure 2. In normal subjects, plasma aldosterone concentration increased from 8.0 ± 3.4 ng/dl to a steady-state level of 42.8 ± 6.7 ng/dl (p < 0.001) during continuous AII infusion for 1 hour. In sharp contrast, plasma aldosterone concentration failed to change significantly in patients with pituitary insufficiency, averaging 11.8 ± 2.7 ng/dl before and 14.5 ± 4.0 ng/dl (ns, p > 0.05) after 60 minutes of continuous AII infusion.

Response of Arterial Blood Pressure, Urinary Sodium Excretion, Plasma Renin Activity, and Plasma Aldosterone Concentration to Dietary Sodium Restriction in Patients with Pituitary Insufficiency

In 10 normal human beings maintained on dietary sodium restriction (10 mEq Na⁺/day) for 12 days, PRA increased from 0.68 ± 0.21 to 3.36 ± 0.37 ng/ml/hr (p < 0.001), plasma aldosterone concentration increased from 7.2 ± 2.3 to 34.3 ± 6.4 ng/dl (p < 0.001), while arterial BP failed to change.
significantly from control levels. The response of arterial BP, urinary sodium excretion, PRA, and plasma aldosterone concentration to dietary sodium restriction (10 mEq Na+/day) for 12 days in 32 patients with pituitary insufficiency is illustrated in figure 3. In patients with pituitary insufficiency, PRA increased from 0.65 ± 2.7 to 3.43 ± 0.53 (p < 0.001) ng/ml/hr while plasma aldosterone concentration failed to change significantly from control levels, averaging 10.8 ± 3.2 before and 12.3 ± 3.9 ng/dl (ns, p > 0.05) after sodium deficiency. Although aldosterone secretion failed to increase during sodium deficiency, these patients came into sodium balance at 10 mEq without a significant change in arterial BP.

Response of Plasma Aldosterone Concentration and Plasma Cortisol Concentration to ACTH Administration in Normal Subjects and in Patients with Pituitary Insufficiency After Dietary Sodium Restriction

The response of plasma aldosterone concentration and plasma cortisol concentration to ACTH administration in normal human subjects and in patients with pituitary insufficiency after dietary sodium restriction (10 mEq Na+/day) for 12 days is illustrated in figure 4. In normal human subjects, plasma cortisol concentration increased from 15.1 ± 3.2 to 38.3 ± 5.7 Mg/dl (p < 0.001), while plasma aldosterone concentration increased from 37.8 ± 7.3 to 163.2 ± 13.9 ng/dl (p < 0.001) within 1 hour after ACTH administration. In patients with pituitary insufficiency, plasma cortisol concentration failed to change significantly in response to ACTH during sodium deficiency, averaging 10.2 ± 2.3 µg/dl before and 11.2 ± 2.6 µg/dl (ns, p > 0.05) after ACTH administration. Although plasma aldosterone concentration failed to increase in patients with pituitary insufficiency in response to dietary sodium restriction, plasma aldosterone concentration increased markedly from 12.9 ± 3.5 to 156.8 ± 17.3 ng/dl (p < 0.001) in response to ACTH administration during sodium deficiency.

Response of Plasma Aldosterone Concentration to Continuous Angiotensin II Infusion in Normal Subjects and in Patients with Pituitary Insufficiency after Dietary Sodium Restriction

The response of plasma aldosterone concentration to continuous AII infusion at the rate of 6 ng/kg/min in normal human subjects and in patients with pituitary insufficiency after dietary sodium restriction is illustrated in figure 5. In normal human subjects, plasma aldosterone concentration increased from 36.3 ± 7.4 ng/dl to a steady-state level of 93.8 ± 13.6 ng/dl (p < 0.001) during continuous AII infusion. In striking contrast to the marked increase in aldosterone
secretion in response to ACTH in patients with pituitary insufficiency during sodium deficiency, plasma aldosterone concentration failed to increase significantly in response to continuous All infusion for 1 hour, averaging 13.2 ± 3.6 ng/dl before and 17.2 ± 4.3 ng/dl (ns, p > 0.05) after continuous All infusion.

Response of Plasma Aldosterone Concentration and Plasma Cortisol Concentration to ACTH Administration in Three Patients with Pituitary Insufficiency Maintained on Long-Term Replacement Therapy with ACTH Gel

Three patients with pituitary insufficiency were maintained on ACTH gel replacement therapy for 3 months to determine whether ACTH is essential for a normal aldosterone response to ACTH and All. The response of plasma cortisol concentration and plasma aldosterone concentration to ACTH administration in normal subjects and in three patients with pituitary insufficiency maintained on replacement therapy with ACTH gel is illustrated in figure 6. In normal subjects, plasma cortisol concentration increased from 15.2 ± 4.3 to 33.0 ± 5.4 µg/dl (p < 0.001), while plasma aldosterone concentration increased from 7.9 ± 2.4 to 35.0 ± 5.7 ng/dl (p < 0.001) within 1 hour after ACTH administration. In response to ACTH administration in three patients with pituitary insufficiency maintained on replacement therapy with ACTH gel, plasma cortisol concentration increased from 12.9 ± 3.1 to 27.8 ± 4.7 µg/dl (p < 0.001) while plasma aldosterone concentration averaged 11.2 ± 2.5 ng/dl before and 13.6 ± 3.2 ng/dl (ns, p > 0.05) after ACTH administration. Although replacement therapy with ACTH gel restored the adrenal cortisol response to ACTH, plasma aldosterone concentration failed to increase significantly from control levels in patients with pituitary insufficiency after replacement therapy with ACTH gel for 3 months.

Discussion

The aldosterone response to dietary sodium restriction, or removal of sodium from the body by any of several means, is well established. Numerous investigators have demonstrated parallel increases in activity of the renin-angiotensin system and aldosterone secretion in experimental animals and man during sodium deficiency. In contrast, in experimental animals maintained on normal sodium intake, continuous All infusion at a rate that increases blood All concentration three times higher than that observed in sodium-deficient animals increases aldosterone secretion to levels less than one-third of those observed in sodium-deficient animals. These quantitative discrepancies between aldosterone secretion during...
sodium deficiency and aldosterone secretion in response to AII infusion have been the subject of intense debate for nearly 20 years. There have been two concepts advanced to explain the differences in aldosterone secretion during sodium deficiency and in response to infusion of AII. First, some investigators[5, 14] believe that the adrenal glomerulosa sensitivity to AII may be increased during sodium deficiency so that smaller amounts of AII produce larger rates of aldosterone secretion. Second, other investigators[6, 7, 8] believe that some additional unidentified factor(s), or undefined mechanism(s), may be involved in the regulation of aldosterone secretion during sodium deficiency. In the present study, we have evaluated the aldosterone response to AII, AII, and sodium restriction in human beings with pituitary insufficiency to determine whether the pituitary gland is necessary for the control of aldosterone secretion during sodium deficiency. Our data provide experimental evidence that some unidentified pituitary hormone(s) is essential for a normal aldosterone response to sodium deficiency.

The present study demonstrates that under normal conditions neither ACTH nor AII has a marked stimulatory effect on aldosterone secretion in human beings in the absence of normal pituitary function. Although the importance of the role of the renin-angiotensin system in mediating the aldosterone response to sodium deficiency is well recognized,[16, 17] aldosterone secretion failed to increase during sodium deficiency in human beings without pituitary glands despite a normal increase in PRA. Yet, these patients without normal pituitary function came into sodium balance at 10 mEq without a significant change in arterial BP or aldosterone secretion. Other investigators[5, 4] have demonstrated a considerable delay in the time required to achieve sodium balance in patients with pituitary insufficiency. It is of interest to note that Lohmeier et al.[18] maintained adrenalectomized dogs on continuous fixed infusions of aldosterone and cortisol to produce sodium-replete blood levels of aldosterone and cortisol. Severe sodium restriction in these animals resulted in a normal rise in PRA with only an 8 mm Hg decrease in arterial BP. Therefore, while it is well established that both mineralocorticoid and glucocorticoid activity are essential for the maintenance of normal sodium balance, renal function, and arterial BP during sodium deficiency, the actual required blood level of these hormones is less certain.

Although aldosterone secretion failed to increase in response to ACTH and AII in patients with pituitary insufficiency on normal sodium intake, aldosterone secretion increased markedly in response to ACTH during sodium deficiency. During sodium deficiency, the aldosterone response to ACTH in patients without normal pituitary function was quantitatively similar to the response observed in normal human subjects, indicating that in the absence of the pituitary gland, the adrenal glomerulosa retains the capacity to secrete normal amounts of aldosterone and can respond to some stimuli with marked increases in aldosterone secretion. In addition, the marked increase in aldosterone secretion in response to ACTH from near sodium-replete levels to levels quantitatively similar to those observed in sodium deficient normal subjects demonstrates the capacity of the adrenal glomerulosa to alter its sensitivity during sodium deficiency even in the absence of normal pituitary function. In sharp contrast to the marked increase in aldosterone secretion induced by ACTH administration during sodium deficiency, the adrenal glomerulosa remained almost totally refractory to AII since continuous AII infusion for 1 hour failed to stimulate aldosterone secretion.

In three patients with pituitary insufficiency, ACTH gel was substituted for hydrocortisone replacement therapy to determine whether ACTH was essential for a normal aldosterone response to sodium deficiency or ACTH. After 3 months of replacement therapy with ACTH gel, the aldosterone and cortisol responses to ACTH and dietary sodium restriction was evaluated in these patients. Although plasma cortisol concentration returned to normal levels in these patients, and a normal cortisol response to ACTH administration was observed, aldosterone secretion failed to increase in response to ACTH, AII, or sodium deficiency in patients maintained on ACTH gel replacement therapy. These observations with ACTH replacement in the present study, and earlier studies in patients with isolated growth hormone deficiency,[19] demonstrate that the essential pituitary hormone is neither corticotropin nor somatotropin.

Recently, Matsuoka et al.[20] reported that β-lipotropin, an anterior pituitary peptide, produced a potent aldosterone-stimulating effect in collagenase-dispersed rat adrenal capsular cells, and suggested that β-lipotropin may be involved in the regulation of aldosterone secretion. Also, a glycoprotein has been extracted from human urine and been found to stimulate aldosterone production by capsular cells in vitro and produce hypertension and hyperaldosteronism when administered to rats in vivo.[21] It is of interest to note that this substance cannot be found in urine from hypophysectomized man.[22] Thus, these data and the clinical observations in the present study provide provocative experimental evidence that some unidentified pituitary hormone(s) is essential for a normal aldosterone response to sodium deficiency.

In summary, there are several aspects of this study that we believe will be significant in our final understanding of aldosterone regulation. First, although the adrenal glomerulosa retains the capacity to secrete normal amounts of aldosterone under normal resting conditions in the absence of the pituitary gland, aldosterone secretion fails to increase in response to ACTH, AII, or sodium deficiency. Second, in the absence of normal pituitary function, aldosterone secretion failed to increase in response to sodium deficiency. Yet, the adrenal glomerulosa sensitivity was markedly increased in response to ACTH during sodium deficiency since plasma aldosterone concentration increased from sodium-replete levels to extremely high levels after ACTH administration. Thus, the adrenal glomerulosa sensitivity can be altered dur-
ing sodium deficiency even in the absence of the pituitary gland. Third, while the adrenal glomerulosa sensitivity to ACTH was markedly increased during sodium deficiency, the adrenal glomerulosa remained almost totally refractory to All since plasma aldosterone concentration failed to increase in response to continuous infusion of a pressor dose of All for 1 hour. Fourth, ACTH replacement therapy for several months in patients with pituitary insufficiency failed to restore a normal aldosterone response to ACTH, All, or sodium deficiency. Finally, these data provide experimental evidence that some non-ACTH pituitary hormone(s) is essential for the aldosterone response to sodium deficiency. We conclude from these clinical data that this unknown factor(s) modulates, or mediates, the action of All on the adrenal glomerulosa cell.

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