Nontraditional aspects of aldosterone physiology

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Ngarmukos, Chardpraorn, and Roger J. Grekin. Nontraditional aspects of aldosterone physiology. Am J Physiol Endocrinol Metab 281: E1122–E1127, 2001.—Aldosterone is the most important circulating mineralocorticoid. It is secreted by the zona glomerulosa of the adrenal gland and plays a major role in sodium and potassium metabolism by binding to epithelial mineralocorticoid receptors (MR) in the renal collecting duct, promoting sodium resorption and potassium excretion. The action of aldosterone on its classic target epithelia has been extensively studied, and many of the signaling events that mediate its effects have been described. Recently, there has been increased interest in aldosterone actions on the cardiovascular system, which are mediated through nonclassical actions. These include local tissue production, nongenomic actions, and effects on nonepithelial targets. In this review article, we focus on the effects of aldosterone in nonepithelial tissues that are mediated through MR, especially cardiovascular effects.

aldosterone; cardiac fibrosis; hypertension; blood pressure; central nervous system

ALDOSTERONE ACTIONS

Mineralocorticoid receptors. Most of the aldosterone actions that have been described in nonepithelial tissues appear to be mediated through the interaction of aldosterone and mineralocorticoid receptors (MR). Therefore, to explore these actions of aldosterone, one needs to understand the aldosterone-MR interaction and the localization of MR in target organs. MR belong to a superfamily of steroid/thyroid/retinoid/orphan receptors and operate as ligand-activated transcription factors to regulate gene expression. MR are most closely related to other steroid receptors, notably the glucocorticoid receptor (GR). Human MR are 984-amino acid proteins that have been cloned and sequenced (2); they share high homology with GR, 57% homology in the ligand binding domain and 94% in the DNA bonding domain (23). It has been demonstrated and confirmed that MR bind to both aldosterone and cortisol with almost indistinguishable high affinity in vitro (38, 44), whereas in vivo MR clearly have specificity for aldosterone, despite the fact that glucocorticoids are present at much higher concentrations in plasma. The factors that enhance specific binding of MR to aldosterone are 1) binding of plasma glucocorticoids to glucocorticoid-binding protein and albumin, allowing only a small amount (~10%) of the unbound hormone to freely cross cellular membranes (22); 2) presence in target cells of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), an enzyme that metabolizes active forms of glucocorticoids into inactive forms in aldosterone target tissues (24); 3) discrimination of aldosterone from glucocorticoid by the MR. The dissociation of aldosterone from MR is five times slower than that of glucocorticoids, despite their similar affinity constants (40). The role of 11βHSD2 has received the most attention; 11βHSD2 converts cortisol and corticosterone to inactive forms that cannot bind to MR. In contrast, aldosterone is not a substrate for 11βHSD2, because its 11-OH group is protected by cyclization. Structural or functional defects of 11βHSD2 are known to cause the syndrome of apparent mineralocorticoid excess (23), and inhibition of 11βHSD2 with glycyrretinic acid mimics the syndrome (58). Signs and symptoms of mineralocorticoid excess in these patients indicate that functioning 11βHSD2 is necessary to prevent glucocorticoid binding and activation of MR.

Target cells. MR have been identified in nonepithelial as well as epithelial cells. The characteristics and properties of MR in these tissues are similar (36, 38, 39); however, MR-mediated effects appear to be different in the different tissues. In epithelial cells, aldoste-
rone acts to enhance sodium reabsorption by activating apical epithelial Na⁺ channels (ENaC) and the basolateral Na⁺ pump. The earliest mineralocorticoid effect is an increase in the activity of ENaC; however, ENaC mRNA and protein levels do not increase. A recent report by Chen et al. (13) indicates that aldosterone induces expression of sgk, a serine-threonine kinase that in turn directly stimulates ENaC activity. These effects on the renal collecting duct mediate a major regulatory site for control of body sodium and water, as well as blood pressure regulation (see review in Ref. 4). In contrast, aldosterone effects in the heart and brain appear to be unrelated to epithelial sodium transport. In the heart, aldosterone promotes cardiac hypertrophy and fibrosis unrelated to hemodynamic effects (7). In the brain, aldosterone affects neural regulation of blood pressure, salt appetite, volume regulation, and sympathetic outflow (16).

The presence of MR in the cardiovascular system has been confirmed at the mRNA and protein levels in animal models and in humans. Lombes et al. (41) showed that, in rabbit, MR are present in myocytes, endocardium, vascular endothelium, and vascular smooth muscle cells (SMC) of large vessels. The study also showed that these MR have identical characteristics to the MR of renal origin. Human cardiac tissue biopsy also demonstrated MR expression in myocardium and endocardium by use of in situ hybridization and immunohistochemistry (39). As we have noted, 11βHSD2 enhances selectivity of MR to mineralocorticoids. Therefore, its presence or absence in target tissues needs to be included in the consideration of MR-mediated mineralocorticoid effects on each tissue. Coexpression of MR and 11βHSD2 has been documented in both the heart and vascular smooth muscle cells, suggesting that MR activity is aldosterone specific in these tissues (30, 39, 55, 64). In the brain, although there is evidence of 11βHSD2 expression, its activity has been reported to be negligible (26).

Nongenomic effects. Recent reports have indicated that some effects of aldosterone are not mediated through binding to MR (15, 63, 68, 69). These actions have been termed “nongenomic” to distinguish them from the “genomic” effects that occur as a result of gene transcription after activation of nuclear receptors. In many cell lines, aldosterone induces a rapid (<10-min) increase of inositol triphosphate and calcium, repression of protein kinase C activity, and activation of the Na⁺-K⁺ pump. These actions have been observed in human mononuclear leukocytes, vascular SMC, and rat cardiomyocytes. Specific antagonists of MR and inhibition of transcription or protein synthesis did not block these actions (14, review in 15, 67). A double-blind placebo-controlled randomized study in humans showed that, after intravenous administration of a large dose of aldosterone, there were significant changes in systemic vascular resistance, cardiac output, and cardiac index compared with the placebo group. The effects appeared at 3 min and dissipated within 10 min, a time course that is too rapid to be explained by genomic effects (53, 69). The effects of mineralocorticoid antagonists on these actions have not been studied in humans.

In summary, most of the known actions of aldosterone in both epithelial and nonepithelial cells are mediated by activation of MR, which in turn regulate gene expression and transcription. MR have high homology with other steroid nuclear receptors, and the selectivity of MR for aldosterone is enhanced by several mechanisms, the most important of which is the presence of 11βHSD2. Some actions of aldosterone do not require binding to MR for their effect.

DIRECT ALDOSTERONE ACTIONS ON THE CARDIOVASCULAR SYSTEM

Hypertrophy and fibrosis. Aldosterone has been shown to induce ventricular hypertrophy and fibrosis in cardiac interstitial and perivascular tissues. Myocardial fibrosis does not occur in cardiac hypertrophy unless there is concurrent activation of the renin-angiotensin-aldosterone system. Thus chronic anemia, arteriovenous fistula, and atrial septal defect lead to hypertrophy without fibrosis. This observation led Brilla et al. (7) to explore the role of aldosterone in the genesis of cardiac fibrosis. They compared different experimental hypertensive rat models: elevated angiotensin II and aldosterone induced by a renovascular lesion, normal angiotensin II and aldosterone in infrarenal aorta binding, isolated elevated aldosterone by minipumps plus high salt intake, and control. The results showed ventricular hypertrophy with myocardial fibrosis in the models with elevated aldosterone levels and with high, normal, and suppressed angiotensin II levels, suggesting that elevation in circulating angiotensin II alone was not the decisive mediator of the fibrous tissue response. They concluded that elevated aldosterone was important in the regulation of collagen synthesis within the cardiac interstitium and in the adventitia of intramyocardial coronary arteries (7). Subsequently, the same laboratory demonstrated that administration of an aldosterone antagonist, spironolactone, at a dose that had no antihypertensive effect, could prevent cardiac fibrosis (6). Recently, Schlaich et al. (52) confirmed by use of M-mode echocardiography a correlation between plasma levels of aldosterone and cardiac left ventricular mass in human subjects. This correlation was not related to differences in blood pressure. A recent prospective clinical trial has shown that treatment with spironolactone significantly reduced morbidity and mortality among patients with severe heart failure. Blood pressure was not affected by spironolactone treatment, suggesting that endogenous aldosterone has direct deleterious effects on the cardiovascular system in these patients (46).

The cellular mechanisms by which aldosterone induces fibrosis are still under investigation. Initial studies from Weber’s laboratory reported that aldosterone stimulated transcription of collagen I mRNA and increased levels of collagen I synthesis in rat cardiac fibroblasts (5, 11). Robert et al. (48) also demonstrated...
an increase in cardiac type I and type III collagen mRNA in rats made hypertensive with aldosterone and salt treatment. However, other laboratories (21, 37) could not confirm these findings. They proposed that aldosterone does not influence fibroblast collagen synthesis directly but increases endothelin receptor number, which leads to increased collagen synthesis (21). These studies are difficult to interpret, because the effect of aldosterone to increase cardiac fibrosis occurs over a long period of time and may not be detectable in an in vitro culture system. This topic is reviewed in depth by Slight et al. (56).

Plasminogen activator inhibitor-1. Aldosterone may have a role in the regulation of plasminogen activator inhibitor-1 (PAI-1) (8, 9). Although aldosterone had no direct effect on PAI-1 activity in vitro, it did enhance the stimulatory effect of angiotensin II on PAI-1 activity. In a rat model of radiation-induced glomerulosclerosis, spironolactone decreased the degree of sclerosis and the level of PAI-1 mRNA expression (10).

Effects on vascular tone. Aldosterone also modulates vascular tone, although the exact mechanisms remain to be elucidated. Possible mechanisms include increased vasoconstrictive effects of catecholamines through decreased tissue catecholamine uptake (65), impaired vasodilatation in response to acetylcholine (60), upregulation of β-adrenergic (34) and angiotensin II (50, 51) receptors, and a direct aldosterone effect, possibly through nongenomic mechanisms. There is evidence that aldosterone also affects vascular SMC via MR, causing transmembrane influx of sodium and potentiating angiotensin II-induced leucine incorporation into SMC in vitro (31). These mechanisms may be involved in hypertrophy of SMC. Aldosterone administration, in nonpressor doses, augmented neointimal thickening in the rabbit iliac artery after balloon-induced vascular injury. Conversely, spironolactone administration reduced the thickening compared with both aldosterone and control groups (61). Wang et al. (65) demonstrated that perfusion of the carotid sinus with aldosterone reduced baroreceptor discharge in the dog, and a similar finding has been reported in humans (59).

Cerebrovascular effects. Studies in stroke-prone spontaneously hypertensive rats have indicated a possible role for aldosterone in vascular remodeling and stroke. Spironolactone treatment in these animals did not reduce blood pressure but did prevent spontaneous cerebral infarction (49). Captopril, an angiotensin-converting enzyme inhibitor, also prevented cerebral infarction, but coadministration of aldosterone reversed the captopril effect (42, 57). Dorrance et al. (19) reported that spironolactone treatment reduced the size of cerebral infarcts induced by middle cerebral artery ligation. These authors also found that mineralocorticoid treatment increased vascular expression of receptors for epidermal growth factor (EGF). They proposed that aldosterone increases vascular remodeling by increasing vascular EGF receptor activity (19).

Local biosynthesis. In addition to effects of circulating mineralocorticoids on the heart, local biosynthesis of aldosterone has recently been reported and may have physiological or pathophysiological importance. In the isolated rat heart, Silvestre et al. (54) used quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis to document mRNA expression of both aldosterone synthase (CYP11B2) and 11β-hydroxylase (CYP11B1), crucial enzymes in the pathway of mineralocorticoid synthesis. They used celiite column chromatography coupled to radioimmunoassay to measure tissue levels of aldosterone. The cardiac aldosterone synthesis pathway is responsive to a low-sodium/high-potassium diet and to angiotensin II in a manner similar to that of the adrenal cortex. Silvestre et al. demonstrated that cardiac aldosterone levels are many times higher than those in plasma, suggesting that locally synthesized aldosterone within the heart may be important for local paracrine or autocrine function. The physiological role of this local system remains to be explored.

In summary, aldosterone appears to play an important role in the pathogenesis of cardiovascular disease. Possible mechanisms of these effects include promotion of fibrosis, modulation of vascular tone and vascular remodeling, and enhancement of angiotensin II-mediated PAI-1 stimulation.

CENTRALLY MEDIATED ALDOSTERONE ACTIONS

The classic GR is widely distributed in brain in both neurons and glial cells, with a high density in the hippocampus and other parts of the limbic system (25, 62). MR are less prevalent in the central nervous system (CNS), and their location is predominantly limited to neurons of the hippocampus and lateral septum (47). Unlike MR targets in epithelial tissue or the cardiovascular system, the brain lacks significant 11βHSD2 activity; therefore, brain MR are not protected from glucocorticoid binding, and MR can bind to both mineralocorticoids and glucocorticoids. Thus, it appears likely that glucocorticoids are the primary ligand for most brain MR. Reul and de Kloet (47) showed that >80% of MR in the brain are occupied by endogenous hormones under basal resting conditions. In contrast, brain GR are significantly occupied only when circulating glucocorticoid levels are high. De Kloet and colleagues (16, 17) postulated that MR mediate tonic actions of corticosteroids involved in maintenance of homeostasis. In contrast, the GR is likely to be important in dynamic responses and the restoration of homeostasis. Corticosteroids in brain appear to be involved in the regulation of several physiological systems: learning and memory (16), neuroendocrine hypothalmic-pituitary-adrenal axis regulation (17), autonomic function, control of sodium homeostasis, and blood pressure regulation. Most of these effects are mediated through activation of MR, possibly by interaction between MR and either glucocorticoids or mineralocorticoids. In this review, we will focus on the role of MR-mediated effects in the CNS on blood pressure control.
Despite almost negligible levels of 11βHSD2 activity in the brain, there is evidence that certain areas contain MR that bind preferentially to mineralocorticoids. This has been demonstrated in the anterior hypothalamus, hippocampus, anterior pituitary, and some nuclei in the brain stem (1, 3, 18, 20). The MR in the hypothalamus and circumventricular organs are involved in the regulation of blood pressure and sodium homeostasis.

Aldosterone acts directly in the CNS to increase blood pressure. This action is separate from its systemic effects and is not associated with changes in fluid and electrolyte balance, salt appetite, or vascular reactivity. Gomez-Sanchez and colleagues (27–29) have extensively explored this issue in a series of experiments comparing the effects of subcutaneous and intracerebroventricular administration of corticosteroid agonists and antagonists in rats. Continuous intracerebroventricular infusion of aldosterone produced a significant increase in blood pressure that was blocked by concomitant intracerebroventricular infusion of MR antagonists, either prorenone (27) or RU-28318 (28). The hypertension induced by the continuous intracerebroventricular infusion of aldosterone was dose responsive and independent of changes in renal sodium handling (45); blood pressure increased in sodium-deplete as well as sodium-replete dogs and rats (12, 35). The doses of steroids given intracerebroventricularly were small and did not cause changes in blood pressure when given subcutaneously. Similar findings were also reported in dogs receiving centrally administered aldosterone (35). Gomez-Sanchez et al. (29) also demonstrated that corticosterone antagonized the pressor effect of centrally administered aldosterone in a dose-dependent fashion if it was coinfused intracerebroventricularly with aldosterone. Bilateral adrenalectomy in rats prevented the pressor effect of intracerebroventricular aldosterone, and the effect was restored by administration of physiological replacement doses of corticosterone (29). Central infusion of a selective MR antagonist, RU-28318, diminished the hypertensive effect of subcutaneous aldosterone infusion (28, 32), and this amount of intracerebroventricular RU-28318 was less than that required to inhibit the increased appetite for saline associated with excess systemic mineralocorticoids (27, 29, 43).

Deoxy cortisolone acetate (DOCA) in combination with dietary salt is commonly used to induce mineralocorticoid hypertension. In this experimental model, attenuation of the baroreflex response can usually be detected before elevation of blood pressure occurs. In DOCA-salt-treated rats, intracerebroventricular infusion of RU-28318 normalized the baroreflex, reduced sympathetic tone, and prevented hypertension (33). The hypertensive stage of intracerebroventricular aldosterone-induced hypertension, in contrast to that of systemic mineralocorticoid excess states, is not associated with a decrease in baroreceptor reactivity, nor is there an increase in vascular reactivity to the intravenous infusion of angiotensin II, norepinephrine, or arginine vasopressin (32, 33).

These results, taken together, indicate that direct CNS effects of aldosterone play a significant role in the pressor effects that occur when steroids are administered systemically.

A recent study by Gomez-Sanchez et al. (26) suggested that the brain has the capability to produce aldosterone. RT-PCR/Southern blot hybridization demonstrated aldosterone synthase in the hypothalamus, hippocampus, amygdala, cerebrum, and cerebellum of rat brain. Incubation of [3H]DOCA, a substrate for aldosterone synthase, with minced brain from hippocampus, hypothalamus, and cerebellum, yielded [3H]aldosterone. In contrast, incubation of minced brain with aldosterone synthesis inhibitors, either cortisol or metyrapone, inhibited aldosterone production.

In conclusion, it is clear that aldosterone has important physiological and pathophysiological effects on nonepithelial tissues. In the cardiovascular system it promotes ventricular hypertrophy and interstitial cardiac and perivascular fibrosis. It may also increase PAI-1 activity. It probably plays an important role in the pathophysiology of heart failure. In the CNS, aldosterone acts to increase blood pressure and sympathetic activity. The importance of locally produced aldosterone in these tissues and the role of nongenomic actions of aldosterone remain to be determined.

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