Central Mineralocorticoid Receptors and Cardiovascular Disease

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Key Words

Mineralocorticoid receptor • Cardiovascular disease • Addison disease, rat model

Abstract

The mineralocorticoid receptor (MR) is expressed in many cell types throughout the body, including specific neurons, and mediates diverse functions, many of which are just now being appreciated. MR that pertain to the central modulation of cardiovascular function and health are addressed herein.

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Historical Perspective

Addison described patients with adrenal cortical destruction as having ‘asthenic’ hearts over 150 years ago. A century later deoxycorticosterone became the first effective treatment of Addison disease [1]; however, it was soon recognized that overzealous replacement led to hypertension and renal damage [2] and that it increased vascular responses to epinephrine and norepinephrine in healthy people [3]. Within a few years aldosterone, the primary mineralocorticoid, was isolated [4], and primary aldosteronism (Conn’s syndrome) was described as associated with refractory hypertension and hypokalemia leading to heart and kidney failure [5]. Notwithstanding early evidence of cardiovascular effects [3, 6, 7] occurring before the appearance of hypertension, the retention of

sodium and water by the kidneys [8] became commonly accepted as solely responsible for the hypertension produced by mineralocorticoid + salt excess.

Mineralocorticoid Receptors in the Brain Mediate Hemodynamic Effects

The demonstration of specific binding of aldosterone in select areas of the brain, as well as the heart and vessels [9], and the finding that the ablation of the anteroventral area of the third ventricle abrogated mineralocorticoid + salt, renovascular and Dahl salt-sensitive (SS) rat hypertension [for a review, see 10] kindled interest in the central hemodynamic effects of mineralocorticoids. Selective infusions of mineralocorticoid receptor (MR) agonists and antagonists in various animal models of hypertension confirmed that MR of the circumventricular organs were crucial for the development of several models of hypertension and that activation of MR in the amygdala increased salt appetite [for a review, see 11].

Mineralocorticoid Excess Mediates Inflammation and Structural Changes in the Heart, Vessels and Kidneys

Patients with primary aldosteronism have cardiac hypertrophy compared to patients with essential hypertension who have similar levels of hypertension for the same duration [12, 13]. Studies in experimental animals sug-

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gested that the inflammation leading to fibrosis and hypertrophy of the heart, vessels and kidneys associated with systemic mineralocorticoid + salt excess were mediated by MR in these tissues independently of significant increases in blood pressure [14–17]. The assumption that ‘end organ’ pathology is totally independent of hypertension has been contested [18]. MR are prominently expressed in the macrophages, important components of the inflammatory response that migrate into tissue and produce inflammatory cytokines soon after injury, as well as before any other pathology is noted when an animal is exposed to mineralocorticoid + salt excess [17, 19–21]. MR in the paraventricular nuclei are also involved in the increased proinflammatory cytokines in the blood and heart associated with cardiac ischemia and heart failure in the rat, as well as in the augmented neuronal activity in the paraventricular nuclei leading to increased sympathetic drive to the heart [22–24]. MR in the forebrain are crucial to survival of the acute phase of cardiac ischemia induced by coronary ligation in the rat; however, their continued, excessive and/or inappropriate activation exacerbates and accelerates the progression of heart failure due to inappropriate sympathetic nerve activity, volume retention and inflammation despite peripheral blockade of the renin-angiotensin-aldosterone system [25].

Ligand Specificity of the MR

The MR is a member of the steroid nuclear receptor superfamily, ligand-activated transcription factors that include the glucocorticoid receptors (GR) with which the MR shares some homology. In addition to promoting the transcription of cell-specific genes, the MR also initiates nongenomic effects through second-messenger pathways. MR bind aldosterone, corticosterone and cortisol with similar affinity [26], yet aldosterone activates the MR in target cells such as transport epithelia of the distal nephron and colon, despite basal circulating levels of glucocorticoids that are 100–1,000 times greater than normal values for aldosterone. There is only one MR gene; the splice variants so far described do not account for tissue-specific differences in MR ligand activation specificity [27, 28]. MR specificity for aldosterone in epithelial mineralocorticoid target tissues is conferred by the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) that inactivates cortisol and corticosterone, thus allowing access to aldosterone [29]. In the absence of 11 β -HSD2, MR in the kidney tubular epithelia are activated

by the more abundant glucocorticoids, producing the same effects as activation by an excess of aldosterone: inappropriate Na⁺ and water retention, K⁺ and H⁺ excretion, and hypertension [30]. Expression of 11 β -HSD2 in the brain is limited and restricted to a few circumventricular nuclei and select neurons of the nucleus tractus solitarius [31–34]. The highest concentrations of MR in the hippocampus, where they are not coexpressed with 11 β -HSD2, thus are bound primarily by glucocorticoids [35–37].

Notwithstanding limited 11 β -HSD2 expression in the brain, *in vitro* incubation of rat brain minces with tritiated corticosterone indicates that it is efficiently converted to the inactive steroid 11-dehydrocorticosterone [38, 39]. Moreover, intracerebroventricular infusions of 11 β -HSD inhibitors produces hypertension, and the intracerebroventricular infusion of an MR antagonist abrogates the hypertension produced by the systemic administration of these inhibitors [11, 40, 41]. Though there is evidence for another hydroxysteroid dehydrogenase, none has been isolated or cloned [42–44].

11 β -HSD inhibitors block both 11 β -HSD types 1 and 2. 11 β -HSD1 is a hydroxysteroid dehydrogenase like 11 β -HSD2 in the absence of hexose-6-phosphate dehydrogenase to regenerate NADPH, its obligate cofactor for reductase activity [45, 46]. Few neurons were found to express 11 β -HSD1, and most of these, with the exception of small neurons adjacent to Purkinje cells and a few neurons in brainstem nuclei, also expressed hexose-6-phosphate dehydrogenase [47].

Is Aldosterone a Neurosteroid?

Neurosteroids are produced in the CNS from cholesterol or circulating precursors where they probably serve autocrine and paracrine functions [48]. We speculated that synthesis of aldosterone in cells expressing the MR would give aldosterone a stoichiometric advantage over glucocorticoids in the absence of 11 β -HSD2. All the requisite components for aldosterone synthesis from cholesterol have been documented in the rat and human brain [39, 49–52]. Minced brain parts from intact and adrenalectomized rats synthesize aldosterone and precursors from endogenous as well as tritiated substrates, with similar efficiency [39]. The aldosterone content of the brain reflects that of the plasma in intact rats on diets of different salt content [53]. Aldosterone concentrations in brains of adrenalectomized rats are low but consistently measurable and significantly higher than that of their plasma,

which is usually below the limits of detection. These data suggest that extra-adrenal aldosterone synthesis does occur in the brain, but that most of the aldosterone in the brain derives from the adrenal gland [53]. The relevance of local aldosterone synthesis has yet to be proven; however, the Dahl SS rat may offer some insight.

Salt-induced hypertension in the Dahl SS rat is prevented by the central infusion of an MR antagonist and by ablation of the anteroventral area of the third ventricle, a maneuver that prevents mineralocorticoid-salt hypertension, even though circulating aldosterone in Dahl SS rats is normal or low [54]. The hypertension is also mitigated by the central infusion of an inhibitor of the aldosterone synthase enzyme 19-ethynyl deoxycorticosterone [38] and subseizure doses of trilostane, a 3β -hydroxysteroid dehydrogenase inhibitor [55]. These findings suggest that excessive or unregulated aldosterone production within the brain is part of the complex pathogenesis of hypertension in the Dahl SS rat.

If extra-adrenally produced aldosterone is relevant for homeostasis, it must also be regulated. Adrenal aldosterone production is tightly regulated, primarily by angiotensin II [56] which increases the expression of aldosterone synthase [57]. The renin-angiotensin system (RAS) of the hypothalamus, but not the brainstem, appears to be regulated by sodium status in a manner similar to that of the systemic RAS [58]; however, expression of aldosterone synthase mRNA in the brain was not found to be altered by sodium depletion in a different study [59]. As the aldosterone production by the adrenal gland far exceeds that by the brain even when maximally suppressed by a chronic high salt diet, it has been impossible to accurately measure the effect of sodium consumption on the extra-adrenal production of aldosterone [53]. The central infusion of angiotensin-converting enzyme inhibitors and angiotensin receptor and MR inhibitors, but not blockade of the systemic RAS or MR, was reported to decrease sympathetic drive and increase tissue and circulating inflammatory cytokines produced by coronary ligation [23, 60]. While not conclusive, these data suggest that synthesis of aldosterone within the brain may be regulated by a local RAS.

Functional Specificity of the MR

Unlike MR of transport epithelia, the consequences of activation of MR in different parts of the brain and heart differ depending on the MR ligand [36, 61]. Aldosterone, but not corticosterone, activates MR in circumventricu-

lar areas associated with blood pressure control to increase blood pressure [62]. Aldosterone replacement in the adrenalectomized animal does not restore normal corticosterone-mediated functions of the hippocampal MR, including modulation of arousal and stress responses and some forms of memory and cognitive functions [35–37].

The area of study with perhaps the greatest potential of finding mechanisms for both ligand- and cell-specific functions of the MR is the differential distribution of proteins that interact with steroid receptors. Chaperone proteins, coactivators and corepressors, underlie diversity and cell specificity of other steroid-receptor-mediated signals; however, little is known about the transcriptional modulation of the MR. The ligand-binding conformation of the MR monomer is stabilized by binding to several cell-type-specific chaperone proteins, including heat shock protein 90. These may alter the binding affinity for aldosterone compared to the glucocorticoids in some cells. Upon binding to a ligand, the MR sheds the chaperone proteins and dimerizes either as a homo- or heterodimer with the GR in the nucleus, before or at the time it binds to hormone response elements in the promoter regions of specific genes, increasing the expression of these genes by recruiting requisite transcriptional machinery [63–67]. MR and GR are coexpressed in many cells of the brain, including cerebellar Purkinje and hippocampal pyramidal cells [49, 68], and heterodimerization of MR and GR appears to be important in the transcriptional regulation of glucocorticoid-responsive genes in the brain [69, 70]. Whether GR and MR heterodimerization figures in the control of blood pressure and neurohumoral control of inflammation is another important area of study.

Transcription efficiency is modulated by coactivators or corepressors, proteins that bind activated receptors. To date, there are no cofactors or hormone response elements known to be specific for the MR; however, mRNA of two steroid receptor coactivator (SRC) protein family members, SRC-1a and SRC-1e, has been reported in specific areas of the brain. Expression of SRC-1a mRNA was greater in the arcuate, paraventricular and ventromedial nuclei of the hypothalamus, the locus coeruleus and the trigeminal motor nucleus, as well as the anterior pituitary [71], areas associated with blood pressure homeostasis as well as mineralocorticoid hypertension. It is plausible that cell-specific protein interactions with the MR alter its ligand specificity or transcriptional efficiency.

Conclusion

Experimental evidence that the inappropriate activation of MR by mineralocorticoids in specific areas of the brain and heart participates in the pathogenesis of several forms of hypertension and exacerbates end organ failure led to a renewed interest in the role of aldosterone and MR in heart failure and to several clinical studies that demonstrated that MR antagonists clearly and significantly ameliorate symptoms and outcomes of patients with severe congestive heart failure or who are at risk of cardiac or renal failure [72–74]. Use of MR antagonists in such patients has now become the standard of care. The salutary effects may be due to blocking MR in the heart,

vessels, macrophages and/or cardiovascular centers in the brain; however, what effects MR antagonists at these relatively low therapeutic doses may have on hippocampal mediated functions, most of which are unrelated to cardiovascular homeostasis, is unknown. It is critical that more be learned about the basic biology and function of the MR in the various areas of the brain to guide the development of more specific therapeutic agents.

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Erratum

In the article by Zitzmann et al., entitled ‘The Novel mTOR Inhibitor RAD001 (Everolimus) Induces Antiproliferative Effects in Human Pancreatic Neuroendocrine Tumor Cells’ which was published in *Neuroendocrinology* 2007;85:54–60, the actual concentrations of RAD001 are 500-fold higher than indicated throughout the whole paper. However, the authors assert that concentrations of 20 nM are sufficient to cause dephosphorylation of p70S6K, upregulation of pAkt and downregulation of cyclin D1, resulting in potent antitumor effects (fig. 1). The authors regret this substantial error in the original publication.

Fig. 1. RAD001 induces potent antitumor effects. BON cells were treated with the indicated concentrations of RAD001. **a** 72 h later, cell viability was measured with Cell Titer 96 kit (Promega). Values are demonstrated as the average and SD of 3 independently performed experiments. $p < 0.001$ vs. untreated control for all concentrations. **b** The expression of pp70S6K, pAkt and cyclin D1 was evaluated after 2 h (pp70S6K, pAkt) and 24 h (cyclin D1) of incubation by Western blot analysis. One representative experiment of 3 performed is shown.