Review

Aldosterone and the heart: towards a physiological function?

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1. Introduction

Aldosterone (Aldo) plays an essential role in regulation of body sodium and potassium homeostasis by acting on epithelial tissues such as kidney and colon. Since about ten years the two dogmas which classically characterize Aldo, i.e., a synthesis in adrenal glands only and an action exclusively in epithelial tissues, have been challenged. Namely, Aldo receptors are present in the heart and specific actions of Aldo can be demonstrated in this organ. This suggests that cardiac tissue may be a genuine target for Aldo. Moreover, there are indications that an increase of plasma Aldo may be a risk factor in cardiovascular pathologies. For example, the CONSENSUS multicentric study has shown a relationship between plasma Aldo concentration and mortality in patients with congestive heart failure [1]. The very recent discovery of Aldo synthesis in rat heart [2] brings a new step to the evolution of our ideas concerning the physiology of this hormone. This demonstrates the existence of an Aldo tissue system – i.e., the presence in a cell or a group of cells of all the biochemical elements necessary for synthesis of a hormone and its specific receptors – making it possibly a local autocrine or paracrine action. This review makes the point of the literature dealing with effects and synthesis of Aldo in the heart.

2. Aldosterone receptors in heart

2.1. The intracellular mineralo-receptor

Aldo effects are mediated by binding of the hormone to its specific receptor: the mineralocorticoid receptor (MR). The MR is a nuclear zinc-finger transcription factor and forms a subfamily with glucocorticoid receptors (GR), progesterone and androgen receptors. The functional and structural organization of this receptor family displays three main domains: the variable N-terminal region, a well-conserved cysteine-rich DNA-binding domain (=90% amino acid identity) and a C-terminal steroid binding domain (=50% amino acid identity). In addition to epithelial tissues (kidney, parotid gland and colon) and non-epithelial tissues as brain and blood vessels, both the mRNA and protein MR have been found in rodent and human hearts [3,4]. In situ hybridization indicates that MR is expressed in cardiomyocytes, endothelial cells and fibroblasts [4]. In contrast, neither mRNA nor immunoreactive MR was detected over small intramyocardial blood vessels in rabbit and human heart [4].

2.2. MR specificity for Aldo

The high amino acid sequence identity in the ligand binding domains of MR and GR leads to substantial binding of glucocorticoids to MR. As circulating concentrations of glucocorticoids are commonly three orders of magnitude higher than those of Aldo, specific binding of Aldo to the MR involves several mechanisms. First, the NAD-dependent enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) converts cortisol and corticosterone into cortisone and 11-dehydrocorticosterone, respectively, which have negligible affinity for MR [5]. Conversely, Aldo is not a substrate for 11β-HSD2, because its 11-OH group is protected by cyclization with the unique aldehyde group at C-18. 11β-HSD2 is coexpressed with MR in human heart [4] confirming that cardiac tissue possesses the cellular machinery required for direct Aldo action. However, several aspects of 11β-HSD2 action in heart remain to be fully defined: 11β-HSD2 activity is about 100-fold lower in heart than in the typically Aldo-sensitive
renal collecting duct [4]. In agreement, the level of its mRNA is low since amplification by polymerase chain reaction was required to evidence its presence in human heart [6]. Second, MR may also discriminate Aldo from glucocorticoids independently of 11β-HSD2. Indeed, the off-rate of Aldo from MR is five times lower than that of glucocorticoids despite similar affinity constants [7]. Third, the transcortin and albumin bind 95% of circulating glucocorticoids whereas only 50% of aldosterone is albumin-bound. Thus, it is likely that selectivity of Aldo binding to MR in heart is achieved by several mechanisms that remain partly to be established [8].

2.3. MR mechanism of action

Unliganded MR is associated with a complex of chaperone proteins, including the heat shock protein HSP 90 and the immunophilin HSP 56, which maintain the receptor as inactive. This chaperone protein-associated MR displays high affinity for corticosteroids. Binding of steroid hormone induces dissociation of chaperone proteins and exposition of nuclear localization signals in MR. In the absence of hormone, MR is present in both cytoplasm and nucleus whereas in the presence of Aldo, activated MR rapidly accumulates in dynamic clusters in the cell nucleus [9]. In agreement with the high degree of homology of their DNA binding site, MR and GR bind to common nuclear glucocorticoid response elements (GRE) [10]. Regulation of transcription is also dependent on interaction with the transcription initiation complex and on diverse intracellular signaling pathways. In addition, increasing evidence indicates the existence of co-activators and corepressors acting as bridging factors between activated receptors and transcription initiation complex. MR is thus able to bind to specific DNA sequences and to regulate the expression of Aldo-responsive genes, like Na+/K+ ATPase in a human renal cell line [10]. In heart however, Aldo target genes are not fully identified.

2.4. Membrane receptors

Several rapid effects of Aldo have been described. For example, Aldo induces, in only 3 min an increase of sodium inward current and of intracellular calcium concentration in cultured vascular smooth muscle cells [11]. These effects do not involve protein synthesis, they are highly specific of mineralocorticoids and are not prevented by MR antagonists (for example spironolactone). These non-genomic mechanisms would be mediated by to date uncharacterized receptors. As described below, rapid Aldo effects are also observed in cardiac isolated cells, which suggests that this type of receptor may be present in the heart.

3. Aldosterone synthesis

3.1. Control of aldosterone synthesis in adrenals

Glucocorticoids and mineralocorticoids (mainly Aldo) are synthesized from cholesterol in the adrenal cortex. The first common step of their synthesis is the formation of pregnenolone from cholesterol by the mitochondrial enzyme P450scc (side chain cholesterol cleavage).

The final step of these synthetic pathways is catalyzed by two closely related cytochrome P-450 enzymes that display differences in their enzymatic activity, regulation and tissular distribution. The P-450 11β-hydroxylase (11β-OHase) is encoded by the CYP11B1 gene and synthesizes corticosterone from deoxyhydrocortisone (DOC) in the zona fasciculata-reticularis, whereas the P-450 aldosterone-synthase (Aldo-synthase) is encoded by the CYP11B2 gene and catalyzes synthesis of Aldo from DOC in the zona glomerulosa. Recent studies show that Aldo-synthase expression and activity is controlled by numerous factors, the main ones being Ang II, potassium and more weakly adrenocorticotropic hormone (ACTH) and sodium. Noticeably, Aldo-synthase and 11β-OHase are mitochondrial enzymes and thus are highly sensitive to tissue oxygen concentration. A large amount of experimental work has been devoted to elucidation of the control mechanisms of Aldo synthesis in the adrenal gland. These mechanisms have been studied mainly in human adrenocortical cultured cells [12] and are summarized in Fig. 1.

3.2. Aldosterone synthesis in heart

Besides the well-known and quantitatively predominant adrenal synthesis of Aldo, extra-adrenal sites of production have been detected in nonepithelial tissues, namely in blood vessels [13,14] and in brain. Previously, a 3β-hydroxysteroid dehydrogenase activity (this enzyme catalyzes pregnenolone conversion to progesterone in the Aldo biosynthesis pathway) had been evidenced in cardiac tissue supporting the hypothesis of a cardiac site of synthesis. The demonstration that the whole pathway of Aldo biosynthesis is present in cardiac muscle, and that it is functional, has been done recently [2]. Quantitative RT–PCR analysis gives evidence of Aldo-synthase and 11β-OHase gene expression in atria and ventricles of the young normal rat. Cardiac levels of 11β-OHase mRNA are sevenfold higher than those of Aldo-synthase mRNA, this ratio being similar in adrenals. Aldo and corticosterone production have been measured in the isolated rat heart (to avoid contamination by plasma steroids) using celite column chromatography coupled to radioimmunoassay. Aldo is detected in heart homogenate under baseline conditions and its level is markedly increased by Ang II and ACTH whereas that of the precursor DOC is decreased. Additional experiments aimed to study the chronic
Fig. 1. Main pathways of aldosterone synthesis and the control of Aldo-synthase gene expression in human adrenal cortex. As discussed in the Section 3.1, it is likely that this scheme is also valid in heart. Ang II binding on Ang II type I subtype receptor (AT1-R) increases intracellular calcium concentration (Ca$^{2+}$). Similarly, extracellular potassium, by decreasing the membrane potential, activates the voltage-sensitive Ca$^{2+}$ channel and increases (Ca$^{2+}$). Ca$^{2+}$-calmodulin-dependent phosphorylation of cAMP response element binding protein (CREB) allows its binding to the cAMP response element (CRE) localized in the CYP11B2 5’ flanking region and thereby activates Aldo-synthase gene transcription. ACTH induces CREB phosphorylation and Aldo-synthase gene transcription via the adenyl cyclase. The proximal CRE is not sufficient to support CYP11B2 expression and other sequences are required such as the orphan nuclear receptors steroidogenic factor 1 (SF-1) and chicken ovalbumin upstream promoter transcription factor (COUP-TF). Abbreviations: Em: membrane potential; PLC: phospholipase C; PKC: protein kinase C; PKA: protein kinase A; PIP: phosphatidyl inositol diphosphate; IP3: inositol triphosphate; CaMK: calmodulin kinase.

regulation of this cardiac steroidogenic system have shown that it behaves in a very similar way to that of the adrenal gland. The Aldo synthesis pathway is sensitive to low sodium/high potassium diet and to Ang II, whereas the corticosterone synthesis pathway responds positively to Ang II and ACTH. Thus, it is likely that Aldo synthesis in heart is controlled by pathways similar to those described in adreno-cortical cells, as depicted in Fig. 1.

4. Effects of aldosterone in heart

4.1. Cardiac fibrosis

One of the best documented effects of Aldo on the heart is the induction of an important fibrosis, with detrimental consequence for cardiac pump. Karl Weber’s group has found that fibrosis appears in rat heart only if Aldo and sodium intake are chronically increased [15]. The role of sodium in the pathogenesis of fibrosis is crucial but still unresolved. To confirm the specific effect of the hormone, it has been shown that inhibition of Aldo binding to MR by a low dose of spironolactone prevents only fibrosis but not the associated hypertension and left ventricle hypertrophy, whereas a high dose prevents all secondary effects of the treatment [16]. Whereas hypertrophy and atrial natriuretic peptide mRNA increase are restricted to the left ventricle, fibrosis appears in both ventricles [15,17,18] supporting the contention that this Aldo-induced cardiac response is independent of hemodynamic factors.

One characteristic feature of this experimental model of hypertension is the long interval of time observed between the increase of plasma Aldo (obtained by osmotic minipumps) and the modifications of cardiac structure [19]. Indeed, type I and III procollagen increase in both ventricles at 2 weeks only, whereas progressive cardiac fibrosis
is found at 1 month. At 2 months total MatrixMetalloProtease-2 activity is increased and localized by in-situ zymography within the coronary arteries, suggesting a role in vascular remodeling [20]. Thus, cardiac alterations are late, precluding a direct modulation of cardiac collagen synthesis by Aldo in vivo. The early mechanisms by which Aldo induces fibrosis are far from being understood, and previous results have shown that Aldo-salt or DOCA-salt cardiac fibrosis may be prevented or reduced by several classes of inhibitors, making it difficult to postulate a simple mechanism of fibrosis development. Cardiac Ang II receptor may be a target for Aldo, possibly leading to increased responsiveness of cardiac cells to Ang II. Indeed, ventricular density of AT1 receptors is increased in rats treated for one month with Aldo-salt [21,22] this increase being prevented by both spironolactone and losartan [22]. The growth promoting effect of Ang II has been well described, and it may thus participate in the stimulation of myofibroblast proliferation observed at sites of fibrosis in Aldo-salt or Ang II treated animals [23]. Ang II-induced intracellular calcium increase may also be involved in this process, since the calcium channel blocker mibefradil prevents Aldo- or Ang II-induced cardiac fibrosis [23]. Increased cardiac bradykinin receptor binding is seen in Aldo-salt excess [24] and blockade of bradykinin B2-receptors prevents the Ang II-induced cardiac fibrosis [25]. The postulated pathway of bradykinin action is also via stimulation of myofibroblast proliferation, possibly mediated by prostaglandin release [23]. The intervention of other fibrogenic hormones like endothelin-1 in Aldo-salt cardiac fibrosis is also likely, given that it is overexpressed in the endothelium of coronary arteries and endocardium of DOCA-salt treated rats [26] and the endothelin antagonist bosentan decreases cardiac fibrosis in the same model [27]. Fig. 2 summarizes these observations.

4.2. Ionic transports

A series of observations indicate that Aldo can modify the cardiac expression or activity of proteins involved in control of ionic homeostasis and of intracellular pH. Aldo increases the expression of the major sub-units of Na⁺/K⁺ ATPase in isolated cardiac myocytes [28] but this effect is not found in heart in vivo [19]. It also increases the Na⁺/K⁺ co-transporter activity, and thereby enhances inward Na⁺ flux and Na⁺/K⁺ ATPase activity [29]. In cultured neo-natal rat myocytes Aldo raises the Cl⁻/HCO₃⁻ exchanger activity at 6 days of culture and the Na⁺/H⁺ antiport activity at 9 days [30].

4.3. Rapid (non-genomic) effects

Rapid effects of Aldo have been evidenced in several cell types [11] including cardiac myocytes. Aldo decreases protein kinase C activity in isolated cardiomyocytes, but not in fibroblasts, from neonate rat [31]. These rapid effects (2–10 min), which are not fully inhibited by spironolactone, are presumably mediated by a yet unknown membrane receptor and do not involve protein synthesis. They may have pathophysiological consequences since a recent study from Wehling’s laboratory
performed in patients during cardiac catheterization describes rapid changes of systemic vascular resistance and cardiac output 10 min after Aldo infusion [11].

4.4. Polymorphisms of aldo-synthase

Since Aldo has effects on the heart, genetic variations in Aldo synthesis could influence cardiac structure and function. A diallelic polymorphism (-344C/T) has been identified in the promoter region of the human CYP11B2 gene. In multiple regression analyses, the CYP11B2 phenotype was strongly associated with increased left ventricular size [32] and modestly with essential hypertension [33]. In another study, this polymorphism was associated with elevated plasma Aldo and arterial stiffness in patients with essential hypertension [34].

5. Function(s) of cardiac aldosterone

5.1. Characterization of the cardiac steroidogenic system

Future investigations aimed to characterize this system have to address two crucial points: First, the presence and functional integrity of the whole catalytic cascade leading to the formation of Aldo must be demonstrated under physiologic conditions. In this view, unpublished results from our laboratory evidenced the expression of the P450scc gene (P450scc catalyzes production of pregnenolone from cholesterol) within cardiac tissue suggesting that the whole pathway of Aldo synthesis is present in the heart. Second, the site where the steroidogenic system is located in the heart remains to be defined; both the coronary vascular cells and the cardiac myocytes must be considered. Vascular endothelial and smooth muscle cells have been shown to produce Aldo [13,14]. Since cardiac Aldo-synthase mRNA level is low, detection of its expression in the whole heart is technically delicate. However, in preliminary experiments using RT–PCR, we detected both Aldo-synthase and 11β-hydroxylase gene expression in isolated neonatal cultured rat myocytes. The determination of the subcellular localization of various components of the cardiac steroidogenic system may allow insights into the functional integration of the system in cardiac physiology. Indeed, if the site of synthesis of a cardiac hormone is close to its site of action (and a comparative study of the localization of biosynthesis enzymes and of receptors would allow this question to be answered), the response of such a local system is necessarily very short. One may thus think that an hormonal system with rapid adaptation is able to modulate in the short term the properties of a group of cells, thus ensuring a local fine-tuning relative to the broad influence of circulating hormones.

6.1. Physiological significance

To date, direct evidence for the biological significance of the cardiac steroidogenic system is still lacking. However, the discovery of a local steroidogenic system which responds both short and long term to physiological stimuli suggests a paracrine or autocrine action for these cardiac-generated steroids. Measures of Aldo concentration in myocardium furnish an interesting result. Indeed, a value of about 16 nM is found, i.e. 17-fold higher than the mean plasma value [2]. Possible explanations for this are slower Aldo degradation in heart than in plasma, intracellular segregation or local delivery into extracellular space instead of release into the bloodstream. Anyway, this relatively high Aldo concentration in heart again supports a putative physiological role. Such a role is yet not known. As suggested by previously mentioned studies, cardiac Aldo may be involved in the control of ionic movements [28,29]. Ang II and Aldo may have interactions in myocardium, as already mentioned since Ang II enhances cardiac Aldo synthesis. On the other hand, Aldo increases AT-1 receptor mRNA and density [21,22] and potentiates Ang II-stimulated hypertrophy [13]. These actions may thus sensitize cardiac responses to circulating or locally produced Ang II. Finally, cardiac Aldo may also be involved in cardiac remodeling [15–18] or in generation of ventricular arrhythmias through the control of norepinephrine uptake [35]. In this respect, future studies of cardiac Aldo synthesis in pathological situations should provide fruitful information.

7. Conclusion

One of the major recent advances in cardiac endocrinology is the discovery of tissular hormonal systems, i.e. the presence in a cell or a group of cells of all the biochemical elements necessary for synthesis of a hormone and its specific receptors. These local systems open the possibility for local autocrine or paracrine actions. A series of arguments discussed above underline the potential for a physiological role, independently from plasma hormones, of this newly discovered Aldo-generating pathway in cardiac function. In conclusion, the potential biological significance of this system represents an exciting new area of research.

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