Identification and role of aldosterone receptors in the cardiovascular system

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The mineralocorticoid hormone, aldosterone, is synthesized predominantly in the zona glomerulosa of the adrenal gland. The final step of its biosynthesis involves the mitochondrial cytochrome P-450 enzyme aldosterone synthase encoded by the CYP11B2 gene, whose expression is under the control of several factors including angiotensin II, potassium, adrenocorticotropic hormone and sodium. Aldosterone is a key element involved in the control of salt and water homeostasis, most notably by regulating sodium reabsorption and potassium excretion across polarized tight epithelia. This hormone thereby participates to the regulation of blood pressure and is implicated in several human diseases such as hypertension and heart failure.

MECHANISM OF ALDOSTERONE ACTION

Most effects of aldosterone are mediated through its binding to an intracellular receptor, the mineralocorticoid receptor (MR) which belongs to the nuclear receptor superfamily [21]. MR is a highly specialized ligand-dependent transcription factor which is composed of three different functional domains ; the N terminal part (aa 1-602) which contains a transactivation function, the central DNA binding domain (aa
603-670) folds into two zinc fingers responsible for DNA interaction and the C terminal part (aa 671-984) constitutes the ligand-binding domain which also contains signals for nuclear localization, dimerization, transactivation and interaction with heat shock proteins, coactivors or corepressors. Figure 1 schematizes the mechanism of aldosterone action in a target cell. Such target cells include polarized epithelial cells of the distal nephron, the colon or of the salivary or sweat glands or non-epithelial cells of the central nervous and cardiovascular systems and mononuclear leukocytes. The inactivated receptor is found mostly in the cytoplasmic compartment complexed in a heterooligomeric structure with receptor-associated proteins most notably heat shock proteins 90, 70 but also immunophylins FKBP54 and cyclophilin CYP40. Upon aldosterone binding, MR undergoes conformational and phosphorylation changes, probably followed by a release of receptor-associated proteins, dimerization and translocation to the nuclear compartment. Inside the nucleus, MR interacts with hormone responsive elements located on promoter sequences and modulates expression of specific target genes. However, no such specific mineralocorticoid-responsive elements have yet been identified in the regulatory regions of mineralocorticoid-receptor genes. Moreover, although it is known that aldosterone stimulates the activities of several proteins involved in sodium transport (Na/K ATPase, amiloride sensitive sodium channel or ENaC) within cells of the distal tubule [34], it is not clear whether this occurs through direct transcriptional regulation. Recent evidence suggests that aldosterone might actually induce expression of sgk, a serine-threonine kinase which directly stimulates ENaC activity [5].

The specificity of aldosterone action in target tissues largely depends on the presence of an enzyme, the 11β-hydroxysteroid dehydrogenase type II (11HSD2). Indeed, MR displays a similar affinity both for mineralocorticoids and glucocorticoids, and given that the latter are present in a hundred to thousand fold excess in the plasma, 11HSD2 allows MR to remain free for aldosterone binding and effect by metabolizing the 11 hydroxysteroids into inactive 11 keto metabolites. In addition, at the receptor level other mineralocorticoid selectivity-conferring mechanisms seem to be necessary to fully account for receptor specificity, most notably the intrinsic properties of MR to discriminate among steroid ligands [12].

CARDIAC AND VASCULAR EFFECTS OF ALDOSTERONE

Besides the well known actions of aldosterone on sodium-transporting epithelia [1], it has become evident that aldosterone also exerts some direct effects on the cardiovascular system. Most notably, an involvement of aldosterone in the development of cardiac fibrosis has been recently documented. Weber et al. first reported that aldosterone excess leads to the development of fibrosis in hypertrophied ventricles independently of its hemodynamic consequences due to the renal actions [3, 35]. Long term treatment with aldosterone/sodium induced high blood pressure, left ventricle hypertrophy and cardiac interstitial and perivascular fibrosis [3, 27, 38]. Spironolactone administration prevents experimental interstitial fibrosis [2] and has also been reported to promote beneficial effects on human heart [20]. Although exact mechanisms involved in myocardial fibrosis remain unclear, it seems that aldosterone modifies type I and III collagen expression (stimulation of synthe-
Aldosterone actions in the vasculature have been also reported, which include an increased vasoreactivity to pressor agents [36], modulation of muscle tone and vessel elasticity and variations in electrolytes composition of the arterial wall [16, 24]. It is suggested that aldosterone effects could modify expression of adrenergic and angiotensin II receptors, thus potentiating vascular catecholamines and AII responses [15, 33].

Rapid effects of aldosterone via nongenomic molecular mechanisms have also been postulated in cultured vascular smooth muscle and endothelial cells [7]. They involve modifications of inositol-1,4,5-triphosphate (IP3) and diacylglycerol production, stimulation of protein kinase C pathway and increases in intracellular calcium and cAMP concentrations [6, 7]. In vivo experiments performed in patients demonstrate changes in systemic vascular resistance, cardiac output and cardiac index after an iv injection of aldosterone [37]. It remains to determine the mechanisms of such effects and to establish a possible involvement of aldosterone membrane receptors which have still to be identified. Specific membrane binding sites could eventually be related to the classical mineralocorticoid receptor as recently described for the cell membrane and nuclear estrogen receptor [26].

MINERALOCORTICOID RECEPTOR EXPRESSION IN THE CARDIOVASCULAR SYSTEM

Direct effects of aldosterone on cardiac and vascular functions indeed require the presence of its specific receptor in target cells. MR expression, at both mRNA and protein level, has been demonstrated in the heart [18, 19, 25] and large blood vessels [13, 19, 23]. In the rabbit model, we have provided evidence for the presence of MR in the cardiovascular system [19]. Using aldosterone binding assays, we demonstrated the presence of specific aldosterone binding sites whose biochemical and structural characteristics were identically to those of the renal receptor. Indeed, the affinity constant for aldosterone, Kd determined by Scatchard analysis, was 0.25 nM. Mineralocorticoid specificity of these sites was confirmed by steroid competition studies in which two anti-mineralocorticoid compounds, spironolactone and ZK 91587 displaced tritiated aldosterone binding whereas RU486, a glucocorticoid antagonist was totally ineffective. Density gradient analysis together with the use of an anti-hsp90 antibody which increased sedimentation coefficient of cardiac MR from 9S to 11S indicated that the receptor was complexed with the heat shock protein hsp90 under an heterooligomeric structure. Quantification of MR content in various rabbit organs indicated that the cardiac and aortic MR concentration (18 and 11 fmol/mg cytosolic protein) was approximately one half and one third of that of the kidney (35 fmol/mg prot) respectively. Immunohistochemical studies using the monoclonal anti-idiotypic antibody H10E which recognizes the ligand binding domain of the receptor revealed the presence of immunoreactive material in the heart, especially in the cardiomyocytes and endothelial cells of the atria and ventricles. MR were also identified in the blood vessels, localized in the vascular smooth muscle and endothelial cells of the large arteries.

We further investigated aldosterone receptors in the cardiovascular system in man [18]. For this purpose, we used molecular and immunological probes to identify the hMR at both mRNA and protein levels in human heart samples. In situ hybridization techniques allowed to detect specific signals over atrial and ventricular cardiomyocytes while intramyocardial vascular structures did not display such signal. Quantification of hMR mRNA content by image analysis showed that specific hMR mRNA hybridization signal (antisense minus sense labeling) expressed in arbitrary units per surface area was approximately five times lower over cardiomyocytes than distal parts of the nephron. We also examined the presence of 11HSD2 in the human heart. A low but significant NAD-dependent catalytic activity was observed in small biopsy samples. These results have been confirmed by others and extended by RT-PCR analysis which further demonstrated the presence of 11HSD2 transcripts in the human heart [30]. Altogether, it appears that the cardiovascular system possesses all the molecular components required for direct and selective aldosterone actions. The precise nature of aldosterone action in the cardiovascular system is obviously a major field of future research. As mentioned above, by analogy with renal mineralocorticoid effects, MR might modulate transcription of target genes involved in sodium transport. Such aldosterone-induced or -repressed proteins could be constituent or regulatory elements of ion transporters or channels but some of them might likely be cardiac or vascular-specific. Finally, one has to keep in mind a possible interaction between mineralocorticoid effects and other signal transduction pathways such as that recently described between the mineralocorticoid receptor and the protein kinase A cascade [22]. Cross-talk between signaling pathways might be particularly relevant in the cardiovascular system.

MR ISOFORMS EXPRESSION AND REGULATION

The genomic organization of the human MR gene has recently been established [41]. The hMR gene spanning over 75 kb, is composed of 10 exons. Exons 2-9 encode for the functional domains of the receptor molecule.
Interestingly, the first two exons (1α and 1β) are untranslated sequences that splice alternatively into the common exon 2, leading to two distinct mRNA isoforms (hMRα and hMRβ). This was highly suggestive of alternative promoters utilization. Indeed, we have identified two functional promoters P1 and P2, corresponding to the 5′-flanking regions of the two untranslated exons 1α and 1β respectively. These alternative promoters not only differ strikingly by their basal transcriptional activity but are submitted to differential corticosteroid regulation [40]. This could account for the complex regulatory control in directing hMR gene expression in a tissue-specific and developmental manner.

Tissue-specific expression of hMR isoforms has been subsequently studied by in situ hybridization using exon specific riboprobes on various human biopsies [39]. We showed that both hMRα and β transcripts were coexpressed in aldosterone target cells, i.e. kidney cortical collecting ducts, cardiomyocytes, sweat gland ducts, keratinocytes and colonic enterocytes, at approximately the same level. However, relative abundance of hMRα and β transcripts compared to that of exon 2 containing mRNA strikingly differs among tissues. In kidney, the signals corresponding to 1α and 1β mRNAs were clearly lower than that given by the exon 2 specific probe and therefore fully accounted for the expression of the common exon 2 containing mRNA. In contrast, this is not the case in the heart where 1α- and 1β-containing transcripts are expressed in the cardiomyocytes at approximately 4 fold higher levels than exon 2 containing messengers. Our results strongly suggest the existence of other cardiac hMR mRNA variants which could arise from tissue-specific mRNA splicing leading to translational products distinct from the wild type MR.

The important question that remains to be elucidated is the regulatory mechanisms controlling MR expression in a tissue-specific manner. Experimental models reveal that the steroid status does not seem to modify the MR levels in the heart [11] but it is likely that various physiological stimuli and/or physiopathological situations (heart failure, myocardial infarction, alterations of hemodynamic parameters) might influence the expression of cardiac or vascular MR.

LOCAL ALDOSTERONE BIOSYNTHESIS IN THE Cardiovascular SYSTEM

The description of extra-adrenal production of steroids has considerably challenged the conventional notions of classical endocrinology. This is the case for the cardiovascular aldosterone system. Indeed, local aldosterone production and secretion have been recently identified in the blood vessels and the heart. Aldosterone synthase gene expression (CYP11B2) was demonstrated in both atria and ventricles of rat heart by RT-PCR [29] as well as in the rat mesenteric and human pulmonary arteries [14, 31]. CYP11B2 gene expression increased after adrenalectomy, angiotensin II and potassium treatment and was stimulated by low sodium/high potassium diet. Vascular aldosterone synthesis [14, 32] and aldosterone production by isolated heart was unambiguously demonstrated by specific radioimmunoassays [29]. Aldosterone secretion was enhanced by angiotensin II and ACTH. It is worth noting that expression of renin and angiotensinogen has also been described in the rat and human heart [8, 10], leading to production of angiotensin II [9] which in turn might be implicated in the regulation of the local aldosterone synthesis. Altogether, the local autocrine renin-angiotensin-aldosterone system in the cardiovascular system emphasizes the potential pathophysiological implications of aldosterone actions (table 1).

TRANSGENIC MICE MODELS TO STUDY MR FUNCTION IN CARDIOVASCULAR PATHOPHYSIOLOGY

In order to get further insights into the molecular and cellular mechanism of aldosterone action, we have undertaken to establish transgenic mouse models in

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which hMR is overexpressed in a tissue-specific manner. To direct recombinant hMR expression in normally expressing cells, we used the two promoters of the hMR gene which were shown to clearly differ by their relative potency and their tissue-specific utilization in vivo, as determined by means of a targeted oncogenesis strategy [17]. Several lines of genetically engineered mice have been generated and their phenotypic characterization is currently under investigation. Functional evaluation of cardiovascular, renal and endocrine functions should allow to identify specific abnormalities pointing to direct or indirect effects of aldosterone on the cardiovascular system. Experimental models of human diseases such as hypertension or cardiac fibrosis could represent interesting systems to further investigate the role of MR in vivo and should also facilitate our understanding of pathophysiological mechanisms of aldosterone action in the cardiovascular system.

In summary, the cardiovascular system is able to produce locally aldosterone and represents an important mineralocorticoid target tissue. Molecular mechanisms of the mineralocorticoid signaling pathway through an endocrine and/or autocrine system and its physiological significance remain to be determined. This research opens interesting pharmacological perspectives.

REFERENCES


