ISSN 1669-5402 (Print) ISSN 1669-5410 (Online)

Physiological Mini-Reviews

Edited by the Argentine Physiological Society.

Vol. 2, N° 6, January 2007.

http://www.mini.reviews.safisiol.org.ar

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[ISSN 1669-5402 (Print); ISSN 1669-5410 (Online)]

Edited by the **Argentine Physiological Society**

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Ciudad de Buenos Aires Argentina.
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http://www.mini.reviews.safisiol.org.ar

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(Resol. IGJ 763-04)

New aspects of aldosterone action; cardiovascular effects.

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The steroid hormone aldosterone plays a major role in the control of blood pressure and extracellular volume homeostasia, by regulating renal sodium reabsorption and potassium excretion[1-6]. The main steps of the mechanism of action of aldosterone are illustrated schematically on **(Fig.1)**. Aldosterone binds to the mineralocorticoid receptor (MR), which is a ligand-dependent transcription factor that belongs to the superfamily of nuclear receptors, (including mineralo- and glucocorticoid receptors, androgen, estrogen and retinoic acid receptors, among others), all having a common organization. Binding of aldosterone-MR complexes on specific regions of the promotors of regulated genes leads to their transcriptional activation or repression. This will ultimately trigger (primarily or secondarily) an increase in the activity or number of epithelial sodium transporters. In a typical target cell for aldosterone, such as the renal collecting duct principal cell, the sodium of the tubular fluid enters the cell through amiloride-sensitive apical sodium channels (ENaC, for Epithelium Sodium Channel), and is then extruded from the cell to the peritubular space by the Na+-K+- ATPase located in the basolateral membrane[7]. Both transporters are coordinately activated in the presence of aldosterone, leading to an increase in transepithelial sodium reabsorption. Aldosterone action is restricted to the distal parts oh the nephron (distal tubule and collecting duct), after the bulk of sodium reabsorption occuring in the proximal tubule and loop of Henle; this effect is however critical, and variations in aldosteronemia lead to precise and physiologically relevant adjustments of sodium reabsorption. Alterations in these adjusments, as observed in Addison's disease, in syndromes of resistance to aldosterone (such as pseudohypoaldosteronism) or hyperaldosteronism, illustrate the critical role of aldosterone in regulation of sodium handling.

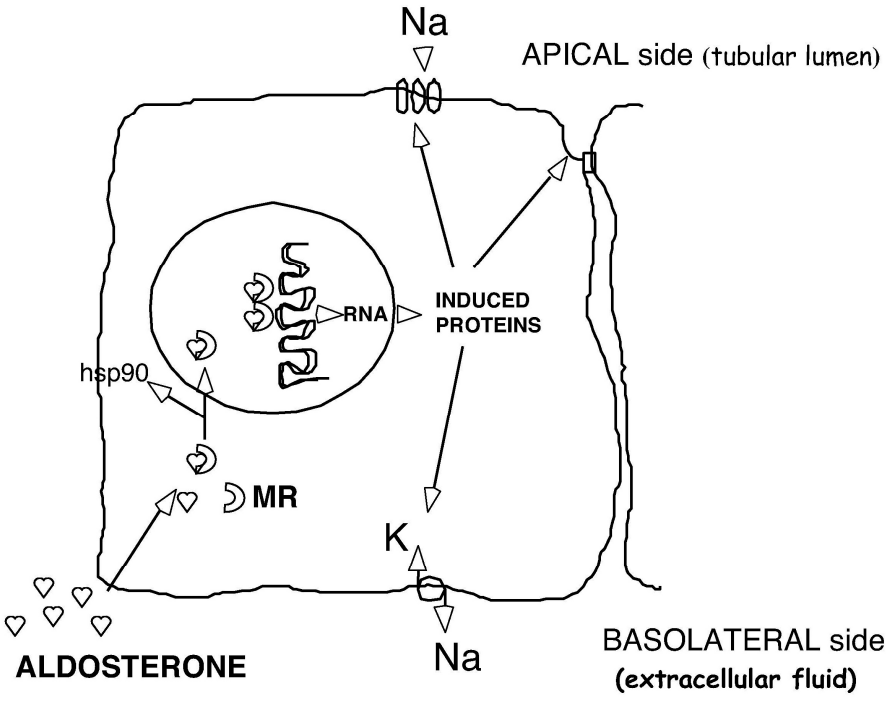
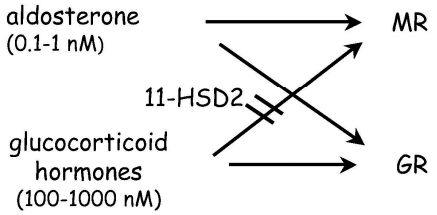


Figure 1*. Scheme of the mechanism of action of aldosterone in the renal collecting duct, a prototype of aldosterone-sensitive cell. Aldosterone enters the cell by diffusion and binds to the mineralocorticoid receptor (MR), liberating associated proteins as hsp90. The hormone-receptor complex translocates into the nucleus. It binds to the promotor of regulated genes, to induce (or repress) transcription of early induced genes. Later on, these early events lead to enhanced transepithelial sodium transport, through coordinated up-regulation of the apical sodium channel ENaC and of the basolateral Na/K/ATPase.*

This classical simple scheme of aldosterone action has to be revisited however, to take into account the complexity of the biological response[1, 2].

First, the mineralocorticoid receptor MR displays similar affinity for aldosterone and glucocorticoid hormones, that are much more abundant in the plasma (100-1000 fold) than aldosterone **(Fig. 2)**. This should lead to permanent occupancy of the MR by glucocorticoid hormones, inducing permanent and maximal sodium retention, independent of plasma aldosterone levels. This is in contradiction with observations made in animals and humans, where sodium excretion clearly depends on aldosteronemia. Mineralocorticoid selectivity depends on the enzyme 11b hydroxysteroid dehydrogenase type 2 (11-HSD2) that metabolizes circulating glucocorticoid hormones (cortisol in man, corticosterone in rodents) into inactive 11-dehydro-derivatives (cortisone, 11-dehydrocorticosterone) with very low affinity for MR **(Fig. 2)**. 11-HSD2 is coexpressed with MR in the distal nephron, in the terminal parts of the colon and in the salivary and sweat gland ducts[1, 2]. Mutations which inactivate 11-HSD2 have been identified in patients suffering from the Apparent Mineralocorticoid Excess syndrome[8]. These patients exhibit severe hypertension, kypokaliemia and alcalosis, contrasting with their very low renin and aldosterone plasma levels. Chronic ingestion of liquorice also promote high blood pressure by altering 11-HSD2: glycyrrhetinic acid, the active metabolite of liquorice, is a potent inhibitor of the enzyme. This inhibition may be reversed after withdrawal of liquorice, leading to normalization of blood pressure. Thus, it is clear that the enzyme 11-HSD2 plays a key role in mineralocorticoid selectivity and consequently in regulation of arterial blood pressure. However, many questions remain unanswered. Regulation of the enzyme in physiology or pathophysiology is unknow; however it could play a significant role in regulating blood pressure levels. Partial alterations in 11-HSD2 activity (less severe than in the syndrome of Apparent Mineralocorticoid Excess) may occur in patients with essential hypertension[9]. From a general point of view, 11-HSD2 may be an important determinant in the setting of blood pressure levels, which may be regulated by several yet unidentified factors; alternatively, the enzyme may be the (unknown) target for some therapeutic agents, explaining some of their adverse effects.

-The MR has similar affinity (0.1-0.5 nM) for aldosterone and for glucocorticoid hormones
- glucocorticoid hormones are 100-1000 fold more abundant than aldosterone in the plasma



A major mechanism of mineralocorticoid selectivity: the enzyme 11 beta hydroxysteroid dehydrogenase (11-HSD2),metabolizes glucocorticoids into inactive derivatives, thus preventing permanent illicit occupancy of the MR by glucocorticoids

Figure 2*. Determinants of mineralocorticoid selectivity. Corticosteroid receptors (MR and GR) can be occupied by aldosterone and glucocorticoid hormones. The enzyme 11 beta hydroxysteroid dehydrogenase type 2 (11-HSD2) metabolizes glucocorticoids in aldosterone-regulated cells.*

Although 11-HSD2 plays a key role in mineralocorticoid selectivity, other factors also intervene[1-3] such as the intrinsic properties of the MR, its capacity to form homo-or heterodimer (with the glucocorticoid receptor, GR) or its interaction with other transcription factors. Because of the structural homology between MR and GR, it is easy to understand cross occupancies of MR and GR by aldosterone and glucocorticoid hormones. Several studies [3] have evidenced a ligand-dependent distinct behaviour of the MR, which depends on the nature of the ligand, aldosterone, antialdosterone molecules (such as spironolactone) or glucocorticoids, despite the fact that MR displays the same affinity for all these ligands. It has been shown that aldosterone-MR complexes are much more stable than cortisol-MR (or spironolactone-MR) complexes. The efficiency of nuclear translocation of MR-ligand complexes is much higher in the presence of aldosterone than of glucocorticoids. These observations suggest that the « active » conformation of MR (i.e. efficient to interact with the transcriptional machinery) is ligand-dependent, with aldosterone being more efficient than glucocorticoid hormones. Recent structural data[10, 11] have documented the interactions of aldosterone within the hydrophobic pocket of the ligand-binding domain of the MR, and showed that this position differs from that of cortisol. On the other hand, several groups have demonstrated that MR can transactivate a reporter gene (for example the promotor of the mouse mammary tumor virus MMTV coupled to luciferase) more efficiently when bound to aldosterone: indeed concentrations of aldosterone ten-fold lower than of glucocorticoid hormones are required to transactivate these reporter genes[12]. Thus, it appears that the receptor itself plays a role in mineralocorticoid selectivity.

By analogy with the functioning of other receptors of the same family, it is assumed that MR binds to DNA regulatory elements (promotors) as dimers. These dimers may be homodimers (MR-MR) or heterodimers, formed together with other receptors, particulary the glucocorticoid receptor. By transfection experiments where the relative amounts of MR and GR were varied, it has been demonstrated that transcriptional activity varies according to the nature of the dimer: MR-MR or GR-GR homodimers, or MR-GR heterodimers[3].

This phenomenon likely occurs in vivo, and may be at the origin (at least partly) of the complex and tissue-specific effects of corticosteroid hormones. For example, several authors have shown that the full mineralocorticoid response, as monitored on sodium transport, is due to occupancy of both MR and GR (simultaneously or successively). Since each of these receptors may bind aldosterone or glucocorticoid hormones, it offers a great regulatory flexibility due to the different posssible combinations of receptor-ligand complexes:

- MR-MR, occupied by aldosterone alone, or partly by glucocorticoid hormones,

- GR-GR, occupied by glucocorticoids alone, or partly by aldosterone,

- MR-GR, with variable proportions of each ligand.

These variable associations depend on the level of activity of 11-HSD2 and on the respective intracellular concentration of each ligand (indeed, some glucocorticoid hormones are likely to be present in the cell, despite the very high metabolizing efficiency of 11-HSD2). In addition, the relative amounts of MR and GR vary among cell types.

Another aspect of mineralocorticoid specificity is related to tissue-specific differential effects of the hormone. At variance with the almost ubiquitous expression of GR, MR expression is restricted to some tissues[3]. They can be classified schematically as classical target tissues (where aldosterone controls sodium reabsorption) and as new targets (where MR has been evidenced, but its function remains elusive). Classical target tissues include first the terminal parts of the nephron (distal tubule and collecting duct), but also the colonic epithelium and the excretory ducts of sweat and salivary glands. However, aldosterone effects may differ in these classical target tissues. For example, as a result of variations in plasma aldosterone (adrenalectomized rats compared to aldosterone-injected rats) aldosterone increases the level of mRNA expression of the sole a subunit of the amiloride-sensitive sodium channel ENaC in the kidney, while in the colon the regulated subunits are the b and the g subunit. These effects are probably due to interactions of MR with yet unidentified tissue-specific transcription factors.

The presence of MR has been clearly assessed in other tissues, in particular in cells devoided of vectorialized sodium transport processes, as neurons (in particular in the hippocampus), cardiomyocytes and keratinocytes. The consequences of MR activation in these tissues are currently investigated, in normal as well as in pathological situations. Importantly these tissues have low or undetectable 11-HSD2 activity; thus it is likely that cortisol (not aldosterone) is the ligand occupying the MR in such organs (in addition to binding to the GR). Neuronal effects of aldosterone may be important for cognitive processes and memory[13]. Direct cardiovascular effects of aldosterone (besides the cardiovascular consequences of an altered functioning of the renin-angiotensin system) have been suggested; in addition vascular and cardiac synthesis of aldosterone have been reported, raising the question of a local production and action independent of adrenal production of corticosteroid hormones. Furthermore there are data in favor of an involvement of aldosterone in the constitution of cardiac fibrosis appearing together with left ventricular hypertrophy[14-16]. Two large clinical trials[17, 18] pointed on the beneficial effects of MR blockade in humans. The RALES study demonstrated that spironolactone (a classical MR antagonist) treatment (in addition to standard therapy) decreased morbidity and mortality in patients with severe heart failure, while the EPHESUS trial showed that eplerenone (a second generation MR antagonist) greatly improved patients with acute myocardial infarction and left ventricular systolic dysfunction. Half of the benefits in both RALES and EPHESUS was attributed to a decrease in sudden death, likely due to cardiac arrhythmia. As aldosterone displays pleiotropic effects, it is difficult to define the origin of the beneficial effects of MR antagonism in heart disease. The mechanisms responsible for these favorable effects may rely on consequences of impaired renal Na and K handling, on myocardial fibrosis inhibition and attenuation of cardiac sympathetic activity, thereby reducing arrhythmogenic substrates, but also on cardiac ion channel remodeling. It is also interesting to distinguish effects linked to ligand modifications from those due to receptor expression levels. While extensive literature adress the former aspect, little information is available on the role of the MR or the GR in cardiac functions. To this purpose, we have designed a general experimental strategy allowing to modulate these steroid hormone receptors specifically in the heart of mice, independently of systemic effects and without alteration of circulating ligands[19, 20].

We have generated transgenic mouse models with conditional, inducible cardiac-specific manipulation of MR and GR expression in cardiomyocytes only **(Fig. 3)**. The targeted approach rules out the possibility of secondary effects due to changes in renal ion homeostasis or to general aldosterone/glucocorticoid-induced disturbances and focuses on the specific role of MR/GR in the cardiomyocytes.

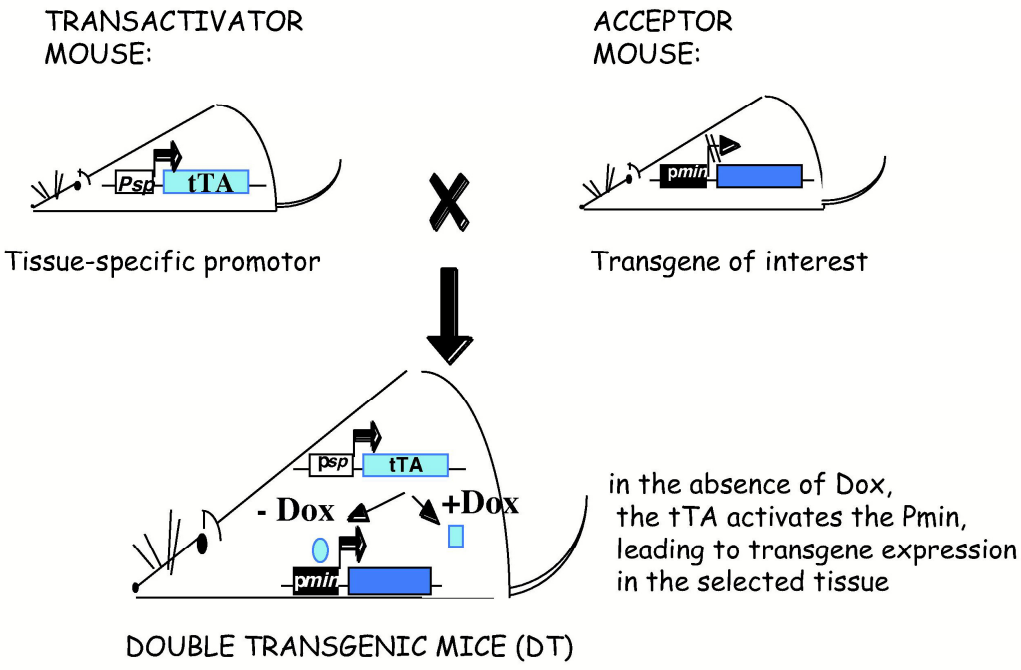


***Figure 3**. Scheme of generation of transgenic mouse models with conditional, inducible tissue-specific expression of a transgene. The tet system is a binary system including a transactivator mouse (expressing the tTA transcription factor in a tissue-specific manner, allowing spatial control) and an acceptor mouse (expressing the transgene of interest under the control of the tetO inducible promoter, allowing time-dependent control). The tet system is therefore only functional in double-transgenic (DT) mice after mating of the mono-transgenic trains. Transgene expression in DT then depends of the ligand Doxycycline (Dox), acting as a molecular switch. Adding Dox at different time, from embryonic development to adulthood, allows repression/induction of transgene expression and time-dependent control of the beginning or end of the pathological process.*

Conditional down-expression of the endogenous murine MR in the heart (using an MR antisense as transgene) unexpectedly led to profound structural cardiac remodeling associated with severe dilated cardiomyopathy and fibrosis[21]. Most interestingly, cardiac failure and fibrosis were strongly reduced upon retrieval of transgene expression. More recently we have overexpressed the MR in the heart, i.e. a procedure opposite to that mentioned above[22]. Cardiac MR over-expression during gestation led to a high rate of perinatal death that could be prevented by spironolactone. In surviving MR overexpressing mice, major electrocardiographic abnormalities were evidenced, as spontaneous and triggered ventricular arrhythmias and torsades de pointe, that are likely at the origin of the observed early sudden death. This was associated to ion channel remodeling, as measured by patch-clamp on isolated cardiomyocytes. Interestingly, these mice do not present cardiac structural alterations or fibrosis.

Cardiomyocytes also express the GR; glucocorticoids have been identified as a risk factor for heart failure. The specificity of MR versus GR signalling pathways is complex to analyze as both receptors can bind either aldosterone or glucocorticoids, depending upon their plasma concentrations. Moreover MR/GR expression levels may be altered in cardiovascular diseases. Comparison of the phenotype of cardiac GR- and MR-overexpressing mice should allow to understand the contribution of each receptor in cardiac pathophysiology. We are now producing and studying mice with conditional inducible cardiac-specific expression of human GR (hGR). No major structural cardiac remodeling nor early lethality are observed. Unexpectedly, cardiac GR overexpression

reduces heart rate and depresses cardiac conduction leading to atrio-ventricular blocks (AVB) and decreased heart rate variability. These preliminary results reveal that GR activity may be important for the regulation of heart conduction and sinus node function. Thus, although closely related, the MR and GR appear to exert some distinct specific cardiac effects, when overexpressed in transgenic mice. Both models result in local MR/GR imbalance, pointing on a crucial role of MR and/or GR signaling in cardiac physiology. Future work should help to dissect the molecular and cellular pathways that are differentialy activated in these models.

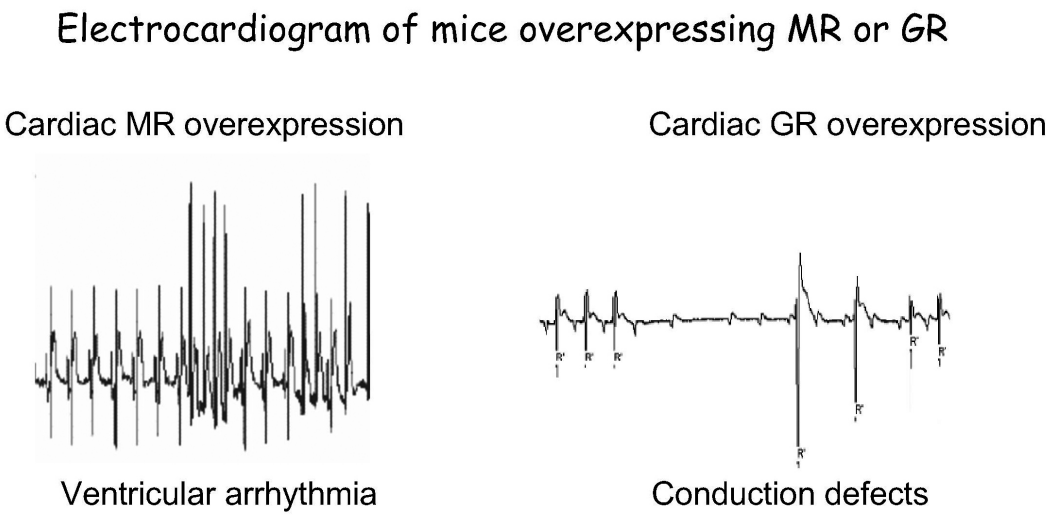


Figure 4*. Electrocardiographic features of recordings in mice overexpressing the MR or the GR.*

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