Review Article

# Aldosterone in Heart and Kidney Diseases

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In recent years our knowledge and understanding of the contribution of steroid hormones to cardiovascular and renal disease has increased significantly. The action of aldosterone is well known. It is recommended for the control of sodium ($Na^+$) secretion via mechanisms that depend on the function of mineralocorticoid receptors. Aldosterone is a steroid hormone that is produced by the cholesterol in the glomerular zone of the adrenal cortex and it is the most important mineralocorticoid of the human organism.[1,2] It was described for the first time fifty years ago, when it was isolated from the urine of patients with heart failure and was found to cause $Na^+$ retention and to contribute to the development of oedemas, a condition that later came to be known as renin-dependent, or secondary aldosteronism.[3] In contrast to the classical view concerning the synthesis and function of aldosterone, recent data support the importance of its synthesis in locations outside the adrenal glands. Indeed, it seems that aldosterone is produced by the endothelial and smooth muscle cells in the heart, in blood vessels, and in the brain.[4-6] It acts via the epithelial mineralocorticoid receptors, mainly in the kidneys but also in the sweat and salivary glands and in the colon, while to a lesser degree it also affects epithelial receptors in the brain and cardiovascular system.[7-9] It plays a major role in regulating the volume of extracellular fluid, through its effect on $Na^+$ secretion and on potassium ($K^+$) homeostasis.[2,7-9] These effects ($Na^+$ retention and $K^+$ elimination) are mainly exerted on the distal convoluted and collecting ducts, where it promotes reabsorption of $Na^+$ and, via a change in the electrical gradient, excretion of $K^+$, hydrogen and magnesium.[2] Apart from the changes that depend on mineralocorticoid receptors, in gene expression, aldosterone is able to exert rapid non-genomic effects that are independent of mineralocorticoid receptors. Thus, apart from regulating $Na^+$ homeostasis, aldosterone causes inflammation and fibrosis in the cardiovascular system and the kidneys. In this context it coordinates the development of fibroblasts and myofibroblasts, controlling collagen deposition in the heart, blood vessels and kidneys.[9-12]

## Mechanisms regulating aldosterone secretion

### *Factors that stimulate aldosterone secretion*

The main regulators of aldosterone secretion in the organism are the renin-angiotensin system, $K^+$ levels, and adrenocorticotropic hormone (ACTH).[13-16] The renin-angiotensin system is the most important system for the regulation of extracellular fluid and mediates the response of mineralocorticoid receptors to uptake and volume loading.[2,17] A reduction in $Na^+$ uptake and a shrinkage in the effective plasma volume stimulate the production of renin by the cells of the juxtaglomerular apparatus. The renin causes release of angiotensin I in the systemic circulation, via angiotensinogen hydrolysis. The angiotensin I is converted by the action of angiotensin converting enzyme to

angiotensin II, which in its turn stimulates the production of aldosterone via the AT1 receptors, which are located in the glomerular zone of the adrenal glands.[17,18] Apart from the systemic circulation, it appears that in the tissues (including the kidneys) there are many enzymes (proteinases), such as calicreine, catepsine G and chymase, which convert angiotensin I to angiotensin II.[19-21] In particular, the chymase pathway appears to represent the main route of angiotensisn II production in the diseased (atheromatous) aorta, while there are indications from *in vitro* studies that angiotensin II is produced in the heart via the chymase pathway.[19]

Serum $K^+$ concentration is the second most important regulatory system of aldosterone release. Increases in serum $K^+$ concentration (even changes of the order of 0.1 mEq/L) cause a significant increase in aldosterone secretion, and *vice versa*. ACTH, apart from stimulating the production of glycocorticoids in the adrenal cortex, also regulates the production of mineralocorticoids, including aldosterone.[18] This stimulation pathway acquires particular significance in cases of heart failure, where the observed increase in ACTH (together with the increase in other, less important, substances, such as endothelin, vasopressin and catecholamines) contributes to an increase in the secretion rate of aldosterone by five to twenty times.[15,18,22-24] In addition, in heart failure, because of the reduced glomerular filtration rate in the context of a low cardiac output, renin release increases and in consequence so does aldosterone secretion.[25] Apart from increased production, however, in heart failure hepatic venous congestion and reduced hepatic blood flow interfere with the metabolism of aldosterone, resulting in reduced aldosterone clearance and consequently retention of $Na^+$ and water.[25]

### *Factors that inhibit aldosterone secretion*

Apart from the factors that stimulate aldosterone secretion, there are others that do the opposite. Thus, hormones that cause elimination of $Na^+$, such as natriuretic peptides, inhibit aldosterone secretion, especially in response to aldosterone II, by up to 50%.[26,27] Other factors that inhibit aldosterone secretion are dopamine, reduced intake of $Na^+$ and $K^+$ in food (changing the response of aldosterone to $Na^+$ retention and $K^+$ loading), and finally heparin.[2,18,26] Heparin inhibits aldosterone secretion through an as yet undetermined mechanism (probably via binding the AT1 receptors in the glomerular zone of the adrenal glands, which synthesise aldosterone), four to eight days after the start of its administration.[18,28] The inhibitory action of heparin on aldosterone secretion may become clinically significant (with the appearance of hyperkalaemia) in patients with insulin-dependent diabetes mellitus and renal failure, as well as in those who are taking $K^+$ supplements or pharmaceutical agents that cause $K^+$ retention (e.g. amiloride).[29] Digitalis, in contrast to earlier views, does not appear to affect aldosterone secretion.[30-32]

## Biological action pathways of aldosterone

Aldosterone exerts its biological effect via two pathways. The first to be discovered was the slow, or genomic pathway, in which aldosterone modifies genetic expression. The biological effect starts to appear about one hour after the drug is administered and its full expression requires hours or days.[33,34] The action of aldosterone via the genomic pathway is mediated by the standard mineralocorticoid receptors, although there are strong indications that other, non-classical receptors are involved.[35]

The second pathway is the so-called rapid, non-genomic pathway. It is mediated by receptors in the cytoplasm and the cellular membrane.[36] The action of aldosterone via this route is exhausted within about ten minutes.[36] Despite the striking differences between the two aldosterone action pathways, recent experimental data suggest that there may well be interactions between them.[37]

## Effects of aldosterone on target organs

According to the latest data, the biological effect of aldosterone on target organs may be summarised as follows:[38-54]

At the level of the blood vessels it causes endothelial dysfunction, vascular inflammation, vascular remodelling, and perivascular fibrosis, resulting in the development of arterial hypertension, atherosclerosis, and myocardial ischaemia or infarction. In the brain it causes damage to the cerebral vessels and baroreceptor dysfunction, leading to arterial hypertension and a predisposition to stroke. In the kidneys it causes inflammation of the blood vessels and renal fibrosis, with consequent arterial hypertension and renal failure. In the myocardium it causes left ventricular hypertrophy, myocardial fibrosis, left ventricular remodelling, stimulation of the sympathetic nervous system, together with parasympathetic withdrawal and inhibition of myocardial norepinephrine uptake. These manifestations result in an increase in circulating catecholamine concentrations, thus predisposing to heart failure, myocardial infarction, reentry arrhythmias and sudden cardiac death.

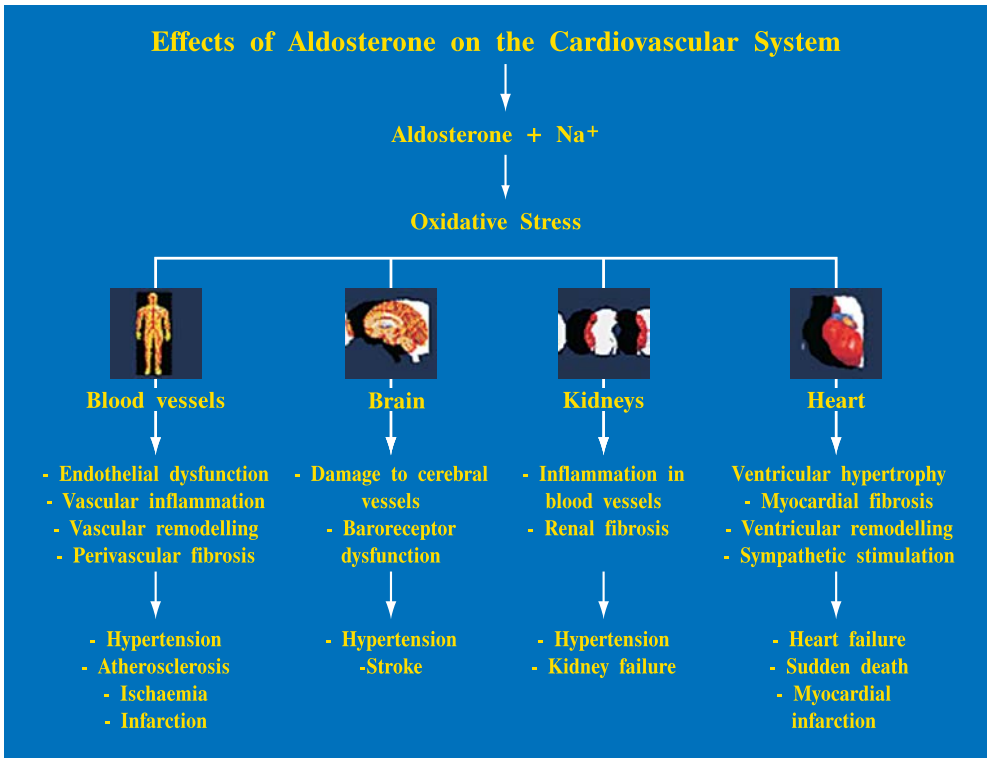


**Figure 1.** Summary schematic representation of the effect of aldosterone on target organs and the underlying pathophysiological mechanisms.

The effect of aldosterone on target organs and the underlying pathophysiological mechanisms are shown schematically in figure 1.

### *Aldosterone and cardiovascular diseases*

As far as cardiovascular diseases are concerned, as mentioned above the heart and blood vessels have the capability for local, extra-adrenal production of aldosterone. Even though the locally produced quantities of aldosterone are insufficient for its systemic effects, they can exert a significant effect on the local regulation of extracellular fluid, with pathological consequences.[55] The same stimuli that trigger aldosterone production in the adrenal glands (angiotensin II, $K^+$, ACTH, etc.) also cause its local production in the myocardium. As a result of local production, intracardiac aldosterone levels exceed those in the circulation.[56] Aldosterone acts on the myocardium via both the biological pathways discussed above: the slow pathway (via the mineralocorticoid receptor, which is found on various heart muscle cells) and the rapid pathway.[55,57] However, apart from mineralocorticoid receptors, expression of the genomic pathway also requires the enzyme 11 beta-hydroxysteroid dehydrogenase, which promotes binding of aldosterone to its receptor.[55] The importance of 11 beta-hydroxysteroid dehydrogenase, whose activity has been confirmed in the heart, is special, given that glycocorticoids bind to the same receptor and circulate in much greater quantities than do mineralocorticoids.[55]

From the above we can see that in the heart there are all the prerequisites for aldosterone to have a local, paracrine action.[57] Indeed, nowadays there are strong indications, from clinical and experimental studies, that aldosterone, like other neurohormones, is responsible for the appearance of cardiovascular complications, independently of the "classical" effect of mineralocorticoids on the kidneys and the changes in arterial blood pressure.[38] Aldosterone has been shown to play a crucial role in the development of myocardial fibrosis, since the administration of an aldosterone antagonist inhibits the deposition of collagen in hypertensive rats that are given aldosterone and $Na^+$.[38,58,59] The mechanisms through which aldosterone causes vascular damage at the level of the cardiovascular system are not known.[38] The view has recently been expressed that the fibrosis induced by aldosterone is not due to direct action of the hormone on the extracellular matrix, but is rather the result of is-

chaemic and necrotic changes in the myocardium. This hypothesis is reinforced by the fact that the administration of eplerenone, an aldosterone inhibitor, together with excision of the adrenal glands, contributed to a reduction in vascular lesions and the extent of myocardial necrosis in an experimental model where arterial hypertension was caused by angiotensin II administration.[45,60] Also, in another experimental study, in which arterial hypertension was induced in rats by administration of aldosterone and $Na^+$, inflammatory changes were seen in the coronary arteries, with perivascular aggregation, mainly of macrophages, and lesions of the fibrinoid necrosis type.[38,41] The inflammatory reaction was preceded by expression in the vascular media of the proinflammatory cytokines macrophage chemoattractant protein-1 (MCP-1) and osteopontin, which becomes apparent within four to eight days, while collagen deposition and fibrosis do not occur for about four weeks.[38] In the latter study, too, eplerenone administration reduced the blood pressure and the size of vascular and myocardial lesions. Finally, it appears that the collagen deposition and fibrosis caused by aldosterone are also facilitated by disturbances of the fibrinolytic system (increase in plasminogen activator inhibitor-1), by increased concentrations of transforming growth factor beta (TGF-β), and by free oxygen radicals.[61]

The detrimental effects of aldosterone on cardiovascular structures are not exerted solely by causing arterial hypertension, but are also accomplished through additional, non-haemodynamic mechanisms: that is, independently of angiotensin II. This is shown by two pieces of data: first, aldosterone causes vascular damage even under the simultaneous administration of angiotensin-converting enzyme inhibitor (ACEI), and second, the administration of an aldosterone antagonist exerts a vasoprotective effect when angiotensin II is administered.[8,46] In an experimental study on rats in which mineralocorticoids and $Na^+$ were administered, it was found that the development of myocardial fibrosis was independent of angiotensin II levels, left ventricular hypertrophy or $K^+$ levels.[62] Moreover, the administration of spironolactone in experimental models inhibited the development of myocardial fibrosis.[63]

Apart from myocardial fibrosis, aldosterone, when combined with $Na^+$ loading, is also responsible for the development of fibrosis in the arterial wall.[64] In this case, too, the vascular remodelling is caused by the direct action of aldosterone on the vessel wall and has no correlation with levels of angiotensin II. The vascular remodelling is due to inflammation of the media, as mentioned above, and the final outcome of the inflammatory process is an increase in vessel stiffness, with the well-known detrimental consequences.[43] The deleterious effect of aldosterone on the elastic properties of arteries has also been shown by the powerful inverse correlation between aldosterone serum concentrations and large artery compliance, as determined from a study of reflected arterial waves using a special tonometer.[47]

As regards myocardial hypertrophy, here, too, aldosterone has been shown to facilitate the development of left ventricular hypertrophy by direct action, independently of the levels of angiotensin II.[65] Left ventricular hypertrophy is known to be responsible for the appearance of diastolic dysfunction, diastolic heart failure, myocardial ischaemia, and an increase in the incidence of rhythm disturbances and sudden cardiac death.[17] It has also been observed that serum aldosterone levels show a linear correlation with left ventricular mass.[65]

### *Aldosterone and heart failure*

The role of aldosterone in the pathophysiology of heart failure is particularly important, and aldosteronism is a constant finding in patients with heart failure.[66] In patients with congestive heart failure, as mentioned above, circulating aldosterone concentrations may be as much as twenty times higher than the upper normal levels.[67] The increased aldosterone levels are mainly due to increased production, but a secondary cause is reduced clearance because of a reduction in hepatic blood flow.[24] The increased aldosterone production in heart failure is due firstly to its increased production in the adrenal glands via the renin-angiotensin system, which is induced by the reduced glomerular filtration rate, and secondly to its increased local (cardiac) production.[55]

Although the well-established treatment of heart failure with ACEI theoretically inhibits aldosterone production, in practice the reduction is a transient phenomenon that is described by the term "aldosterone escape".[67] The transient inhibition of aldosterone is due to various reasons, for example, non-administration of ACEI because of low blood pressure. However, even at maximum dosage ACEI do not completely inhibit the adrenal production of aldosterone induced by angiotensin II.[68] Strong stimuli for renin production, such as an upright posture, bodily activity, and a significant reduction in $Na^+$ uptake (<3 g daily) may overcome the inhibitory action of ACEI.[68] In addition, aldosterone is also produced independently of the action of angiotensin II and $K^+$ concentrations make a significant contribution.[68,69] Thus, in heart failure, where $K^+$ supplements are often administered along with

diuretics, aldosterone is also produced via alternative pathways.[68,69] Finally, the reduced metabolic clearance of aldosterone and the biological activity of its metabolites may also contribute to the "escape" phenomenon.[67]

As stated above, aldosterone is also produced locally in the heart, though this does not contribute to systemic concentrations of the hormone. However, it is not clear to what extent local production plays a role in the development of heart failure or how much ACEI can alter its autocrine and paracrine actions.[68] The significant contribution of aldosterone to the manifestations of the heart failure syndrome, regardless of aetiology, is also confirmed by the beneficial effect that aldosterone receptor blockers have in those patients, which will be analysed in a later section.[70,71]

There are various hypotheses regarding the mechanisms through which aldosterone receptor blockers contribute to an improvement in the prognosis of patients with heart failure. These mechanisms include the effect, mentioned above, of aldosterone inhibitors on the regulation of extracellular fluid volume ($Na^+$ retention, loss of $K^+$ and $Mg^{2+}$), as well as their effects on the autonomic nervous system, baroreceptor reflexes and vascular tone.[72] However, of particular importance appears to be the reduction in cardiac fibrosis that is caused by the action of aldosterone on the turnover of the extracellular matrix, which has an important role in the cardiac remodelling that is seen in left ventricular hypertrophy of hypertensive aetiology, in dilated cardiomyopathy, and after myocardial infarction.[38,58,59,72] Cardiac fibrosis is of vital importance to diastolic dysfunction and to the heart's pumping capability, while it also provides a favourable substrate for the development of both ventricular and supraventricular arrhythmias, thus contributing to a worsening of the heart failure and increasing the incidence of sudden cardiac death.[73,74] In one study of patients with heart failure, six months' administration of spironolactone reduced serum levels of biochemical indexes of cardiac fibrosis (carboxyterminal peptide of procollagen type I, aminoterminal peptide of procollagen types I and II), providing indirect indications of inhibition of the fibrotic process.[72] Finally, at the vascular level, it is important to note its contribution to the appearance of endothelial dysfunction, mainly via a reduction in the bioavailability of nitric oxide and via an increase in the oxidative load and an improvement in the flow-induced vasodilation seen in patients with heart failure after spironolactone administration.[18,75,76]

## Studies of survival after administration of aldosterone receptor blockers to patients with heart failure

### *Spironolactone administration in patients with severe chronic heart failure: the RALES study*

At the clinical level, there have been two important multicentre studies of the administration of aldosterone receptor blockers to patients with left ventricular systolic dysfunction.[70,71] The first of these was the RALES study, which included 1663 patients with severe heart failure, NYHA functional class III and IV and ejection fraction <35%.[70] The patients were already under treatment with loop diuretics (100%), ACEI (~95%), digitalis (73%) or β-blockers (~11%). They were randomised to 25 mg spironolactone (822 patients), with the possibility of halving or doubling the initial dose, or placebo (841 patients). The primary endpoint of the study was death from any cause. It was discontinued after 24 months, earlier than originally planned, because an interim analysis proved the efficacy of spironolactone. Indeed, in the spironolactone group there was a very significant reduction in mortality, by 30%, while the risk of hospitalisation for acute destabilisation of heart failure reduced by 35%.

The reduction in mortality was attributed to a lower risk of death because of a deterioration of the heart failure, but also to a reduction in the incidence of sudden death from a cardiac cause. The reduction in the risk of death or hospitalisation for worsening heart failure became clear by the end of the second month of the study and persisted throughout. In addition, spironolactone administration was also associated with an improvement in functional capacity during the study. The mean dose of spironolactone administered was 26 mg, and apart from being effective the drug also proved to be safe. Severe hyperkalaemia (≥6.0 mEq/L) was observed in 2% of the spironolactone group and 1% of the placebo group. However, it must be remembered that patients with serum creatinine >2.5 mg/dL and serum $K^+$ levels >5.0 mEq/L were excluded from the study. Gynaecomastia or breast pain occurred in 10% of patients receiving spironolactone, compared to 1% in the placebo group, and was a frequent reason for discontinuing treatment. The RALES study was the first randomised, large scale, multicentre study to show the cardioprotective action resulting from the blocking of aldosterone receptors by spironolactone administration.

These impressive results led spironolactone to become an established treatment for patients with chronic heart failure who met the criteria for inclusion in the RALES study with a IIa indication.[77] It now remains for

further studies to evaluate the possible benefit from administration of the drug in milder forms of heart failure, as well as in other conditions, such as diastolic heart failure, left ventricular hypertrophy of independent aetiology, and so on.

### *Administration of eplerenone to patients with post-infarction left ventricular dysfunction: the EPHESUS study*

The second large scale, randomised clinical study in which aldosterone receptor blockers were administered was the EPHESUS study.[71] The study included 6632 patients 3-14 days (mean 7.3 days) after acute myocardial infarction, with concomitant left ventricular dysfunction (ejection fraction <40%) and heart failure (presence of third heart sound, pulmonary congestion on chest X-ray, and rales on chest auscultation). Apart from the appropriate and maximised drug treatment (including reperfusion therapy), 3319 patients also received placebo and 3313 were given 25 mg eplerenone, titrated up to 50 mg. Eplerenone is a selective blocker of the mineralocorticoid receptor, which does not bind glycocorticoid, progesterone or androgen receptors.[78] The study had two primary endpoints: the first was death from any cause and the second was death from a cardiovascular cause or hospitalisation for cardiovascular events (worsening heart failure, acute myocardial infarction, stroke, or ventricular arrhythmia). The follow-up period was 16 months. Both primary endpoints showed a significant benefit to the eplerenone group. The percentage of deaths from any cause was 14.4% in the eplerenone group compared to 16.7% in the placebo group (15% reduction, relative risk, RR=0.85 for the eplerenone patients), while for the second primary endpoint the corresponding results were 26.7% and 30% (RR=0.87). The 17% reduction in cardiovascular mortality in the eplerenone group was mainly due to a reduction in sudden deaths from cardiac cause to 21% (note that 75% of the patients were taking β-blockers, compared with only 11% in the RALES study), while the reduction in hospital admissions for cardiovascular causes was due to a 15% reduction in admissions for heart failure.[71,79] The risk of severe hyperkalaemia was statistically significantly greater in the eplerenone group (5.5% versus 3.9% in the placebo group, 1.6% greater): however, it should be noted that in this study, too, patients with serum creatinine >2.5 mg/dL and serum $K^+$ >5.0 mEq/L were excluded. In addition, because of eplerenone's selectivity for the mineralocorticoid receptor the incidence of gynaecomastia and breast pain was similar in the two groups.

### *Aldosterone receptor blockers in the acute phase of infarction*

Apart from the subacute phase, examined in the EPHESUS study, aldosterone receptor blockers have also been given in the acute phase of infarction. In one study, 150 patients with a first anterior acute myocardial infarction were randomised to receive, apart from the usual medication, either 25 mg spironolactone or placebo, within 24 hours of the onset of symptoms, after the patients had undergone successful primary percutaneous angioplasty.[80] After one month the patients had a new coronary angiography and ventriculography, which showed that those in the spironolactone group had a significantly improved ejection fraction, as well as a significant reduction in left ventricular end-diastolic volume. Consequently, the administration of spironolactone to patients who were already taking ACEI was associated with more favourable left ventricular remodelling. It is also significant that the ejection fraction in these patients was 46%, demonstrating that a benefit may also be expected in patients without significant left ventricular systolic dysfunction. In the spironolactone group there was also a significant reduction in the aminoterminal peptide of procollagen type III. This explains the favourable left ventricular remodelling, which was attributed to the spironolactone-induced inhibition of fibroblast proliferation and therefore of collagen synthesis in the heart's extracellular matrix. It should also be noted that aldosterone inhibitors appear to have no effect on infarct healing.[81] The above mentioned study, in combination with EPHESUS, showed the benefit from the administration of aldosterone receptor blockers in patients with myocardial infarction. However, future studies will need to determine the ideal time to start the administration and the ideal duration of treatment.

### *Aldosterone receptor antagonists and restenosis after percutaneous coronary angioplasty*

A new area in which aldosterone receptor antagonists have been used is percutaneous coronary angioplasty. As is well known, restenosis is the main disadvantage and limitation of angioplasty. The accumulation of collagen on the arterial wall is the pathophysiological basis of the phenomenon. Since aldosterone inhibitors are known also to inhibit fibroblast proliferation, in a study of pigs undergoing percutaneous angioplasty the incidence of restenosis was recorded one month after administration of eplerenone per os, spironolactone per os, subcutaneous aldosterone, and in a control group

where no treatment was given.[82] A statistically greater luminal area compared to the control group was seen only in the eplerenone group, while aldosterone had the opposite effect. However, larger scale studies are needed to confirm these preliminary, though clearly interesting findings.

### Aldosterone and the nervous system

It has already been mentioned that aldosterone is responsible for changes in the tone of the autonomic nervous system, specifically stimulation of the sympathetic and suppression of the parasympathetic system.[83] The administration of spironolactone to patients with heart failure who are already under treatment with ACEI has been shown to have a parasympatheticomimetic effect, causing a reduction in heart rate, mainly during the morning hours (6-10 a.m.) when spectral analysis in the time domain showed prolongation of the R-R interval.[83,84] The favourable effect of spironolactone, especially in the morning, is of particular importance, given that the incidence of fatal arrhythmias, and consequently sudden cardiac deaths, is greater during this period.[83] Also, in another study, spironolactone administration for four days significantly lowered all the indexes of QT dispersion (QTcmax, QTd and QTcd), a reduction that in this case, too, was apparent at 6 a.m.[84]

Another effect of aldosterone on the nervous system is a reduction in baroreceptor sensitivity.[85] An acute increase in blood pressure is known to trigger a baroreceptor-mediated increase in parasympathetic tone, resulting in a slowing of the heart rate.[86] The reduction in baroreceptor reflex sensitivity is of particular importance, given that it facilitates the occurrence of potentially life-threatening ventricular arrhythmias.[87]

### Aldosterone and kidney diseases

The connection between aldosterone and kidney diseases, known since the nineteen fifties, has followed a tortuous path. The effect of aldosterone on progressive renal disease was described during the period 1954-1964. This initial period was followed by another, during which this relationship was forgotten. In 1996, Greene et al signalled the start of the second period of aldosterone research.[88]

In 1955, Conn described the first case of a patient with an adrenal tumour that secreted aldosterone and he named the disease "Conn's syndrome". In 1964 the same investigator presented 145 cases of patients with primary aldosteronism, of whom 85% had proteinuria. In that study there was no correlation between aldosterone and renal damage.[89,90]

In 1992 Weber and Brilla[91] claimed that the myocardial fibrosis shown by some experimental models was due, in some cases, to activation of the renin-angiotensin system and consequently to an increase in aldosterone levels. This view was soon supported by another study.[92]

Based on these studies, in 1996 Greene et al provided the first indications that aldosterone was probably involved in the pathogenesis of progressive renal disease.[88] These observations arose from studies of experimental models of subtotal nephrectomy, in which antagonists of the AT1 angiotensin II receptor were administered (angiotensin receptor blockers – ARB, losartan, and ACEI**,** enalapril). The reduction in aldosterone levels after the administration of ACEI and ARB was associated with a reduction in renal damage.

The administration of spironolactone in these models did not reduce glomerulosclerosis, even though it temporarily reduced proteinuria, blood pressure, and cardiac hypertrophy. The pathophysiology of these findings is interpreted in terms of the renin-angiotensin system.

Experimental studies have shown that mineralocorticoid receptor blockers protect the kidneys by preventing the occurrence of glomerular and tubulointerstitial damage in spontaneously hypertensive rats to which N-nitro-L-arginine methyl ester has been administered, without affecting the blood pressure or glomerular haemodynamics.[93] As in the heart, the mechanism via which aldosterone causes glomerular damage is related to the production of free oxygen radicals and to the increased expression of inflammation molecules such as osteopontin, MCP-1, interleukin-6 and interleukin-1β.[94,95]

Nevertheless, the roads that lead to progressive renal disease, especially from inflammation in the glomerular and tubulointerstitial fibrosis, are not known. It has, however, been determined that mineralocorticoid receptor blockers reduce the renal expression of plasminogen activator inhibitor-1 at the same time as reducing kidney damage.[96]

Particular importance should be attached to the observation that, while aldosterone does not induce the renal expression of TGF-β, it nevertheless causes a rapid increase in its secretion in the urine via a transcription mechanism that depends on the receptors, while being independent of the $K^+$ concentration.[97] It has been shown that TGF-β administration in an ex-

perimental model of renal disease increases the production of extracellular matrix proteins and plasminogen activator inhibitor-1, while reducing the production of metalloproteins.[98]

However, in experimental models of contralateral ureteral obstruction that show a lack of integrin-$\beta_6$ it has been shown that aldosterone induces the expression of plasminogen activator inhibitor-1 and interstitial fibrosis via a mechanism that is independent of TGF-β.[98]

The renin-angiotensin system is known to play a fundamental role in the establishment of progressive renal disease.[61,99] In addition, the contribution of both ACEI and ARB to the slowing or the inhibition of progressive renal disease has been determined in both experimental and clinical studies.[100] Although aldosterone levels have not been measured on a systematic basis, when measured they were found to be reduced. In a study where enalapril was given for a month, aldosterone levels reduced from the baseline value of 234 pg/mL to 135 pg/mL.[101]

The contribution of aldosterone to kidney damage is further reinforced by observations from clinical data. In patients with kidney failure who were administered captopril for six months, aldosterone levels reduced from 266 pg/mL to 105 pg/mL.

A low-albumin diet slows progressive renal disease, while at the same time it reduces aldosterone levels, both in healthy controls and in patients with kidney diseases.[102-105]

There is a mass of data concerning the participation of angiotensin II in renal pathophysiology. Nevertheless, there is accumulated experience suggesting that aldosterone is an important and independent factor causing renal damage, via haemodynamic mechanisms as well as through direct cellular effects.[106]

Elevated plasma aldosterone levels are a consistent finding in patients with kidney failure, and are independent of $K^+$ concentration and renin activity.[106] The aldosterone level rises as creatinine clearance falls. Thus, at creatinine clearance values <70 mL/min there is an increase in aldosterone levels to three or four times the upper normal limits.[107] This observation has been confirmed by many clinical studies.[101,108-110] In a study of eight patients with mean creatinine clearance 14 mL/ min, five of them had aldosterone levels above normal limits.[108] In a study of nine patients with insulin clearance 27 mL/min aldosterone levels were four times normal levels.[101]

The significance of this aldosteronism in progressive renal disease has not been probed as far as one might expect, although Walker noted a significant association between aldosterone level and the rate of progressive renal disease in a long-term study of patients with diabetic nephropathy.[109]

In a study of 252 members of 58 families with hypertension, a significant relationship was found between levels of aldosterone and microalbuminuria.[110] Aldosterone levels in the urine were found to be the most powerful prognostic factor for albumin elimination.

Experimental and clinical studies have shown that aldosterone is a powerful and independent (from angiotensin II) "link" between arterial hypertension and the development of vascular remodelling and the appearance of hypertensive renal disease.[61] Indeed, experimental models that were subjected to subtotal nephrectomy and adrenalectomy exhibited a lesser degree of arterial hypertension, albuminuria and renal parenchymal fibrosis than did rats who underwent subtotal nephrectomy but not adrenalectomy.[111] In patients who had taken enalapril for one year spironolactone was given in a dosage of 25 mg/day for four weeks. At the end of that period an additional reduction of 54% was seen in the daily urine albuminuria, without any changes in creatinine clearance or serum $K^+$ levels. In contrast, a reduction in mean blood pressure by 10 mmHg was seen at the end of the four-week period.

At the clinical level, preliminary studies suggest that spironolactone limits albuminuria in patients with chronic renal disease and in those with diabetic nephropathy.[112,113] In addition, in patients with idiopathic hypertension there is a strong correlation between aldosterone levels in the urine and the incidence of microalbuminuria.[100]

In the 4E study the ratio of albumin to creatinine in the urine, which is a risk factor for cardiovascular disease, decreased significantly in the group that was taking eplerenone and enalapril compared to those who were taking just one of those drugs.[114] In patients with mild to moderate hypertension eplerenone reduced the above ratio when the two drugs were administered in dosages equivalent to those that cause a reduction in blood pressure.[115] In elderly individuals with systolic hypertension eplerenone reduced the ratio to a greater extent than did amiloride when given in haemodynamically comparable doses.[116]

### *Effect of aldosterone on experimental models of hypertension*

At the level of the small renal arteries and arterioles aldosterone causes fibrinoid necrosis in the media, with

consequent tissue necrosis and fibrosis, while eplerenone administration limits the extent of the renal damage.[45] The role of aldosterone was studied in spontaneously hypertensive rats, in relation to the changes observed in the mineralocorticoid receptors of the cytosome in these vessels compared to rats with normal blood pressure (Wistar-Kyoto).[117] After six weeks the hypertensive animals showed an increase in the concentration of aldosterone receptors in the cytosome and a reduction in $Na^+$ and urine volume, even though the plasma aldosterone concentrations were the same in both types of animal. Furthermore, it has been shown that the aldosterone-induced thrombotic microvascular disease is independent of blood pressure values and is thus due to direct action.[40]

Although the clinical and experimental data support the place of mineralocorticoid receptor blockers in the prevention of progressive renal disease, many questions remain unanswered. The effect of mineralocorticoid receptor blockers has not been investigated at an advanced stage, such as in progressive renal disease. Their effect in patients with kidney failure is likely to be limited by the risk of hyperkalaemia. In this context, since the publication of the RALES study the incidence of hyperkalaemia in patients taking mineralocorticoid receptor blockers and ACEI has increased substantially.[118]

## Conclusions

Initially there was a perception that aldosterone was produced exclusively by the adrenal glands and that its effect was limited to the epithelium of the urinary tubules, where it caused $Na^+$ retention and $K^+$ elimination. Recent data suggest that there are also extra-adrenal production sites, at which aldosterone, via the mineralocorticoid receptor, exerts a paracrine effect. Experimental models have shown that some of its biological effects are achieved independently of the renin-angiotensin system. Nowadays, it is recognised that aldosterone has a mass of toxic effects on target organs such as the heart, blood vessels, nervous system and kidneys, where in brief it causes oxidative stress, endothelial dysfunction, inflammation, fibrosis, and autonomic nervous system dysfunction, conditions that lead to cardiovascular remodelling, fibrosis and renal damage. While aldosterone is able to affect the vessels via mineralocorticoid receptors, through mechanisms that may depend on them or not, data from genetic experimental models suggest that their activation plays a fundamental role in the pathogenesis of fibrosis of the cardiac muscle. The roads leading from inflammation to fibrosis and remodelling depend on tissue factors such as an increase in the expression of the glycoprotein osteopontin (causing adhesion, migration, cell hyperplasia and deposition of extracellular matrix), or an increase in the expression of plasminogen activator inhibitor-1. Randomised, multicentre studies have already made the benefit clear, in terms of survival after administration of aldosterone receptor blockers, in patients with severe heart failure and post-infarction left ventricular dysfunction. In the research field, data are eagerly awaited concerning the administration of aldosterone receptor blockers in other clinical conditions where its damaging effect is known. The first clinical studies have shown a reduction in circulatory indexes of collagen distribution, an improvement in endothelial dysfunction in some cases and a reduction in albumin excretion.

The administration of mineralocorticoid receptor blockers should be accompanied by regular measurements of serum $K^+$ levels for the prompt detection and management of hyperkalaemia, especially in cases with concomitant renal disorders or initial $K^+$ levels $>5$ mEq/L.

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