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Review article

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LATEST ASPECTS OF ALDOSTERONE ACTIONS ON THE HEART MUSCLE

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The genomic action of aldosterone has already been known to the scientific community and is well-documented to a satisfactory degree. However, the existence of rapid, non-genomic aldosterone actions has repeatedly been proven. These actions are apparent to a lot of tissues, among which the cardiac tissue, with the cardiac cells being responsible for the secretion of endogenous aldosterone. In the genomic pathway, the connection between the hormone and its receptor results increased reabsorption of sodium and water and excretion of potassium. Thus, the genomic procedure reacts indirectly on cardiovascular system by altering the blood pressure. New studies have shed light on unknown aspects of the non-genomic mechanism, which is sometimes performed by means of mineralocorticoid receptor (MR), while others through an MR-independent pathway. It is believed that aldosterone exerts its non-genomic action with the help of a different receptor, probably a G protein coupled receptor. A possible target is protein kinase C (PKC), and PKCε is postulated increase the permeability of the membrane of the cardiac cells to sodium, resulting in delayed repolarization and prolongation of action potential. These findings totally agree with and account for the serendipitous finding of our laboratory, that there is a positive correlation between plasma aldosterone levels and left ventricle (LV) contraction duration. Also, aldosterone has been proven to exacerbate the oxidative stress and induce vasoconstriction by acting on the vascular resistance and the cardiac output. Finally, this article deals with the role of aldosterone in cardiac fibrosis and the latest aspects of aldosterone actions on the heart muscle as well as providing a historical overview of the landmarks pertaining aldosterone's research.

Key words: *aldosterone, mineralocorticoid receptor, heart fibrosis, cardiovascular system, renin-angiotensin system, RAS protein, epidermal growth factor receptor*

INTRODUCTION

The existence of aldosterone was first demonstrated in 1953 when Simpson and Tait (1) isolated it and described its characteristics. It is a steroid, lipid soluble hormone that belongs to the family of mineralocorticoids. It is produced mainly by the cortex of the adrenal glands and regulated by three primary factors.

The first and most important factor is the renin-angiotensin system (RAS). Renin is secreted by the juxtaglomerular apparatus of the kidney in response to low blood pressure (2). This hormone acts on angiotensinogen (2, 3), an α-2-glycoprotein, promoting the production of angiotensin I, which by means of the angiotensin converting enzyme (ACE) (2, 4) is converted into angiotensin II, a short lived (few minutes) but potent vasoconstrictor agent. During these few minutes angiotensin II, on one hand, causes directly and rapidly the contraction of the renal arterioles, while on the other, acts on the adrenal glands providing the signal for the secretion of aldosterone (2, 5). The other two regulating factors are the adrenocorticotropic hormone, and the concentration of potassium ions.

However, it has been shown both in experimental animals and humans that apart from the adrenal cortex, aldosterone is also produced by other parts of the body, such as the endothelium and smooth muscle cells of blood vessels (6) and the heart (7). Furthermore, in recent years, aldosterone has been thought to exert its action on target tissues *via* two different paths, one of which is carried out through a slow, genomic process, while the other through a faster, non-genomic one.

The interest in the cardiac actions of aldosterone begun to increase the last few years mainly due to accumulating evidence demonstrating the negative role this hormone plays in the development and maintenance of various pathological conditions of the cardiovascular system, such as hypertension, heart failure, fibrosis and hypertrophy of the heart muscle. This article attempts to gather the most recent data with respect to aldosterone actions on the heart and distinguish aspects that require further investigation. Furthermore we provide a review of the history of aldosterone, as time line of events, form its discovery to the present day (*Table 1*).

THE GENOMIC ACTION OF ALDOSTERONE

The genomic action of aldosterone in kidneys, described lately by many researchers (8-10), is inextricably linked to the function of the circulatory system, being one of the regulatory mechanisms of the arterial blood pressure.

Year	Event	Reference
1898	First identification of renin by Tigerstedt and Bergmann	(88)
Early 1950's	Identification of Ang I, Ang II and ACE by Skeggs *et al*.	(89)
1953	Isolation of aldosterone and description of its characteristics by Simpson and Tait.	(1)
1954-1956	Description of primary aldosteronism (PA), a syndrome of aldosterone overproduction, by Conn et Louis.	(90)
1956	First crystallization of aldosterone from patients with congestive heart failure by Luetscher *et al*.	(91)
1956	Giroud and Ayres, independently, confirmed the production of aldosterone by zona glomerulosa of adrenal glands	(92, 93)
1960	Presentation of the model for a genomic action of steroids, such as aldosterone, by Clever and Karlson.	(94)
Early 1960's	Identification of a new mineralocorticoid receptor antagonist, spironolactone.	(95)
1963	Clinical observation of a decrease of cardiac output and an increase in peripheral resistance 5 min after aldosterone injection in normotonic subjects by Klein and Henk.	(61)
1964	Observation of a rapid modulation of sodium flux caused by aldosterone by Spach and Streeten. A growing hypothesis for a non genomic effect of the hormone.	(96)
1971	Hypothesis of a non genomic action of aldosterone, which does not regulate gene transcription and is not affected by transcriptional inhibitors by Mashui and Markert (Later extended by Blackmore in 1991).	(97, 98)
1984	Confirmed postulation of non-genomic effects of aldosterone by Moura and Worcel.	(60)
1986	Observation of a diurnal variation of aldosterone secretion in the morning (high levels) and in the evening (lower levels), a feature regulated by ACTH (Richards *et al*.).	(99)
1987	Analytical description of the nuclear receptor MR, finding in the renal cytosol and its composition with certain functional domains by Arriza.	(100)
1987	First identification of aldosterone effects in the CNS (de Kloet *et al*).	(101)
1987-1988	Definition of the principal regulatory factors of aldosterone synthesis by Muller and by Quinn and Williams, angiotensin II, the concentration of extracellular potassium and ACTH.	(5, 102)
1988	Characterization of 11-HSD type 2 enzyme complex in colocalization with MR in epithelial tissues, in placenta and in vascular tissue by Funder and by Stewart, independently.	(103, 104)
1992	Karl Weber showed that aldosterone-high salt status in rats induces cardiac hypertrophy and global cardiac fibrosis.	(105)

As mentioned above, one of the early signals triggering the production of aldosterone is the drop in arterial blood pressure and the subsequent activation of renin-angiotensin system (RAS). Aldosterone, once produced in the adrenal glands, migrates to the renal collecting ducts and binds to the mineralocorticoid receptor (MR) in the cytoplasm of the target cells. MR in its inactive form is bound to chaperones of the Hsp70 and 90 families and various immunophilins (11), from which it is released in the presence of aldosterone.

Then the complex aldosterone-MR migrates as a dimer to the cell nucleus where it binds to specific sites of the genome initiating the transcription of certain genes, including those encoding the SGK kinases, the family of Ras proteins and the CHIF protein, necessary genes for the activation of the sodium channel ENaC and the sodium-potassium pump (12). The complex's action could be regarded as those of a transcriptional factor, while it is also believed that it promotes the insertion of the transporters to the cell membranes (13). Outcome of all these actions is the elevated reabsorption of sodium in juxtaglomerular capillaries. Together with the sodium ions a significant quantity of water is reabsorbed, sufficient to increase the blood volume in the circulation, which as expected, increases the mean arterial pressure too.

The enhancement of the action of sodium-potassium pump by aldosterone, the final operator of renin-angiotensin-aldosterone system, explains the reason why potassium ions are involved in the regulation of the hormone's production. It is known that the operation of the pump in the renal collecting ducts includes not only the reabsorption of sodium ions into the bloodstream, but also the excretion of potassium ions in the urine. In this case, just like in other negative feedback systems, the regulated variable provides a negative signal to its regulator. In simple terms, when the levels of potassium ions in the plasma are significantly increased, the production of aldosterone is stimulated in order to cause the excretion of the excess.

All the above processes require the presence of the aldosterone receptor, the MR, which is located in the cytoplasm of aldosterone target cells and seems to be an essential factor in the action of aldosterone, as indicated by the severity of abnormalities in the various types of pseudohypoaldosteronism.

1994	Aldosterone increases collagen I synthesis in cardiac fibroblasts by Robert *et al*.	(106)
1994	Isolation of a candidate membrane protein from human lymphocytes, a hypothesis for a putative membrane receptor of aldosterone responsible for the MR-independent rapid signaling pathway.	(107)
1994	Evidence for the existence of a local RAA system by Samani *et al*.	(108)
1995	Christ *et al*., identified that aldosterone increases IP3, DAG and [Ca^{2+}], stimulating the translocation of PKC from membrane to cytosol, a protein with several action in the non-genomic axis of hormone.	(34, 109)
1996	Zange *et al*., observed a modulation on oxidative metabolism in calf muscle 8 min after aldosterone administration.	(110)
1997	Identification of rapid activation of Na^{+}-H^{+} exchanger , an effect on membrane proton conductance by Gekle *et al*., as well as activation of MAPK signaling cascade.	(43, 111)
2002	Epidermal growth factor receptor in COS cells activates the non-genomic aldosterone signaling, such as MAPK cascade by Krug *et al*.	(45)
2002	Tillmann *et al*. observed a proarrhythmogenic effect of aldosterone within minutes after its administration, a prolongation of monophasic action potentials.	(48)
2002	Eplerenone and ACE inhibitors, enalapril, reduce blood pressures in non insulin dependent diabetes mellitus patients (Epstein, 2002.) An evidence of correlation of insulin and blood pressure.	(112)
1999 and 2003	Presentation of RALES and EPHESUS trial by Pitt *et al*. MR antagonists, spironolactone and eplerenone, respectively, at low doses, significantly reduce the risk of mortality and morbidity in patients with severe heart failure. An absolute confirmation of the severe effects of cardiovascular MR activation by aldosterone.	(28, 113)
2003	Qin *et al*. highlighted the protective role of glycocorticoids against the severe effects of aldosterone.	(114)
2004	Young and Funder suggested that eplerenone even decreases cardiac fibrosis in animal models.	(115)
2004	Mihailidou *et al*. demonstrated that PKCε mediates the rapid non genomic effects of aldosterone in rabbit cardiomyocytes, by phosphorylating transmembrane sodium channels (Na^{+}-K^{+}-2Cl^{-} cotransporter, K^{+}/Na^{+} pump)	(39)
2008	McEneaney *et al*., demonstrated that aldosterone activates also PKD in M1-CCD cells, through the transactivation of the EGFR *via* the c-Src kinase.	(38)
2011	GPCR30 expression is responsible for the MR-independent vascular effects of aldosterone. A hypothesis expressed by Gros *et al*., for the unidentified membrane aldosterone receptor.	(35)
2013	Identification of an existing cross-talk between MR/Ang II type 1 receptor and MAPK pathway, responsible for the induction of atrial fibrotic responses by aldosterone, in HL-1 cells by Tsai *et al*.	(83)

The MR is encoded by the gene NR3C2, placed on chromosome 4q31.1 and consists of ten exons, the first two of which are not translated. Thanks to Pearce and Funder is now known, that MR is expressed also by cardiomyocytes (14), as well as by the vascular endothelium (15). To MR, not only aldosterone, but also glucocorticoid hormones, such as cortisol bind with high affinity. However, some mechanisms have been described, which enhance the binding of aldosterone to MR despite the large excess of glucocorticoids.

More specifically, the enzyme 11β-HSD2 (11β-hydroxysteroid dehydrogenase), which among other tissues is expressed in the heart (7), the vascular endothelium and the muscular layer, (16, 17) has the ability to metabolize glucocorticoids, thereby reducing their affinity with the aldosterone receptor. A typical example of the action of this enzyme is the conversion of cortisol to cortisone, which is accompanied by the production of a molecule of NADH (or NADPH). Furthermore, it has been speculated that this molecule, inhibits glucocorticoid-MR complexes that despite the existence of the enzyme were able to form (18).

Regarding the structure of MR recent studies have come to the following conclusions: MR consists of three distinct structural domains, the N-terminal, the C-terminal and the central (19). The central domain contains two zinc fingers, which are necessary for binding to the DNA, as well as a signal for the translocation of the receptor in the nucleus. The central domain exhibits a remarkable 94% homology between the glucocorticoid receptor and MR, demonstrating that these receptors bind to the same binding sites on the genome (20).

The C-terminal region is involved in the hormone binding to the receptor. This is also the area to which the Hsp proteins are bound, when the receptor is inactive in the absence of aldosterone. Finally, at the amino terminus, sub regions AF1a and AF1b (21) are identified and found to interact with the receptor co-activators and co-repressors (22). Such a co-activator is the elongation factor ELL, whose action was described by Pascual-Le Tallec and his team (19).

The genomic actions of aldosterone pertaining to the renal system and indirectly the cardiovascular have been greatly clarified. Few issues require further study, including the

relationship between aldosterone and the ACE. It has been shown that aldosterone is involved in various cardiopathies *via* a positive feedback mechanism. This mechanism involves the interaction of aldosterone with ACE, which in turn stimulates the RAS system once more (23). It is not known yet whether this action of aldosterone involves both pathways, the slow genomic and the fast non-genomic one, but the successful outcome of spironolactone (MR antagonist) administration, in cardiac patients, confirms the participation of the MR.

At this point it is necessary to mention the aldosterone antagonists, *i.e.* molecules which bind to MR, prevent aldosterone binding and thus block its action. One of them, used for therapeutic purposes for half a century now, is spironolactone. Until now it is the most common drug for the treatment of primary and secondary hyperaldosteronism, hypertension and heart failure. Nevertheless, it exhibits high affinity not only for the MR but for the progesterone receptor as well (24) and displays in men unwanted antiandrogenic activity (25). For these reasons there was need to develop a more suitable drug for blocking the action of aldosterone. The solution to this problem came with the discovery of eplerenone. This antagonist displays *in vivo* efficacy and potency comparable to spironolactone. However, unlike it, it appears to act very selectively limiting the side effects. Moreover, it is metabolized in a very short time and its metabolites are inactive (26).

Both aldosterone antagonists are characterized as potassium sparing diuretics. The origin of this name is easily understood: because of their administration aldosterone is unable to bind to the MR and effect the reabsorption of sodium. Thus, sodium is excreted in the urine. Furthermore, due to reduced action of K^+/Na^+ pump, potassium is not excreted; instead it remains in the body (spared), resulting in its increased concentration in interstitial fluid.

THE NON GENOMIC ACTION OF ALDOSTERONE

Further studies on the action of aldosterone began when it was discovered that this hormone acts on the cardiovascular system through mechanisms other than those related to renal functions. The involvement of aldosterone in cardiovascular diseases was, firstly, confirmed by the existence of antagonists spironolactone and eplerenone (27) and the statistical significant results of RALES (the effect of spironolactone on morbidity and mortality in patients with severe heart failure) and EPHESUS (eplerenone- a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction) studies, by Pitt *et al.* (28, 29). When these agents were administered to patients, a drop in blood pressure and an increased chance of survival in patients were documented and especially eplerenone disposes an increased specificity and function.

The non-genomic action of aldosterone is documented in a very short time (a few minutes < 10 min) and for this reason sometimes it is referred to as the rapid action of aldosterone. It is brought about via two different pathways, one of which involves the MR in common with the genomic action, while the other is MR independent and probably related to the existence of another receptor. The above theory has been documented by experiments conducted by Grossmann *et al.* (30) in both human and animal cells. After the cells were exposed to aldosterone, it was found that the ERK $^1/_2$ (extracellular signal-regulated kinase $^1/_2$) and JNK $^1/_2$ (c-Jun N-terninal kinase $^1/_2$) were activated only in the presence of MR, while in contrast, the intracellular calcium increased regardless of the existence of the MR (31).

In the fast non-genomic mode of action, aldosterone appears to induce the activation of second messengers such as triphosphate inositol (IP_3), Ca^{2+} ions, protein kinase C (PKC) and protein kinase D (PKD). The interest for IP_3 peaked when it became evident that addition of aldosterone in human mononuclear leukocytes stimulated the Na^+/H^+ antiporter within minutes (32). At the same time, it was already known that other hormones that activate the same antiporter function by hydrolyzing the phosphatidylinositol bisphosphate to IP_3 and diacylglycerol (33). These data prompted Crist *et al.* (34) to carry out experiments which conclusively demonstrated the importance of IP_3 in the non-genomic pathway of aldosterone action.

Little is known for the new putative receptor possibly involved in the fast, non-genomic pathway. The focus recently lies with the receptor coupled to G proteins. It is actually an estrogen receptor, which is involved in the regulation of intracellular calcium and the synthesis of phosphatidylinositol triphosphate. Its presence seems to be necessary in the MR-independent pathway of aldosterone action, as demonstrated by experiments on smooth muscle and endothelial cells (35). Therefore, many questions about the non-genomic aldosterone actions may be answered through the extensive study of this receptor (*Fig. 1*).

Moreover, numerous protein kinases and especially PKC appears to play a critical role in the non-genomic action of aldosterone. The PKC family is involved in a variety number of cellular processes, such as proliferation, apoptosis or trafficking. There are different identified isoforms with distinct structure and functions, such as the classical isoforms (PKCα, βI, βII, γ), the novel isoforms (PKCδ, ε, η, θ) and the atypical isoforms (PKCζ, λ, τ). Among these isoforms PKCα, β, δ, ε have attracted the interest because of their role in the cardiovascular system. Related experiments established that aldosterone contributes to the activation of PKC in rat neonatal cardiomyocytes but not in fibroblasts (36). This demonstrates that the interaction between aldosterone and PKC is a process specific to particular cell types. Notably, in VSMCs the intracellular signaling pathway of aldosterone leads to the activation of PKC, probably, in a MR-independent manner. Aldosterone, also, induces the activation of protein kinase D isoform (PKD kinase), which in turn constitutes a substrate for the novel PKC isoforms, PKCβ, PKCε. The activation of PKC mobilizes the increase of intracellular [Ca^{2+}] and the sequential activation of sodium-proton exchanger type 1 (NHE1), bringing an intracellular alkalinisation. Based on research of different scientific groups some information pertaining to the production of different isoforms of PKC in response to aldosterone has arisen. PKCα, identified in the collecting ducts, is produced through direct interaction with aldosterone, (37) while PKCδ and ε are activated *via* the epidermal growth factor receptor (EGFR) (38). Furthermore, it seems that PKCα, β, δ, ε, contribute to the pathophysiological deflection of cardiac tissue and the inhibitory targeting of these isoforms is quite attractive. Summarizing, the activation of different PKC isoforms by aldosterone may act as an enhanced transducer of the non-genomic cellular responses.

Mihailidou *et al.* (39) went a step further into studying the PKC documenting that PKCε has the ability to phosphorylate transmembrane sodium channels (Na^+-K^+-$2Cl^-$ cotransporter, $K^+/N\alpha^+$ pump) in rabbit cardiomyocytes. Thanks to this phosphorylation the Na^+-K^+-$2Cl^-$ cotransporter is activated and the increased intracellular sodium concentration increases the permeability of the membrane for this ion. These experiments give a very convincing explanation to the question raised by De Mello and Motta (40) few decades ago, who noticed a decrease in the rate of repolarization in respective cells after the addition of aldosterone.

Concerning the activation of the EGFR by aldosterone, it seems that aldosterone mediates its genomic effects *via* the transactivated phosphorylation of the EGFR through ERK $^1/_2$ kinase. Grossman *et al.* (31) found that aldosterone treatment induced the expression of EGFR in aorta smooth muscle cells, rendered them more sensitive to the receptor. EGFR acts, also, as

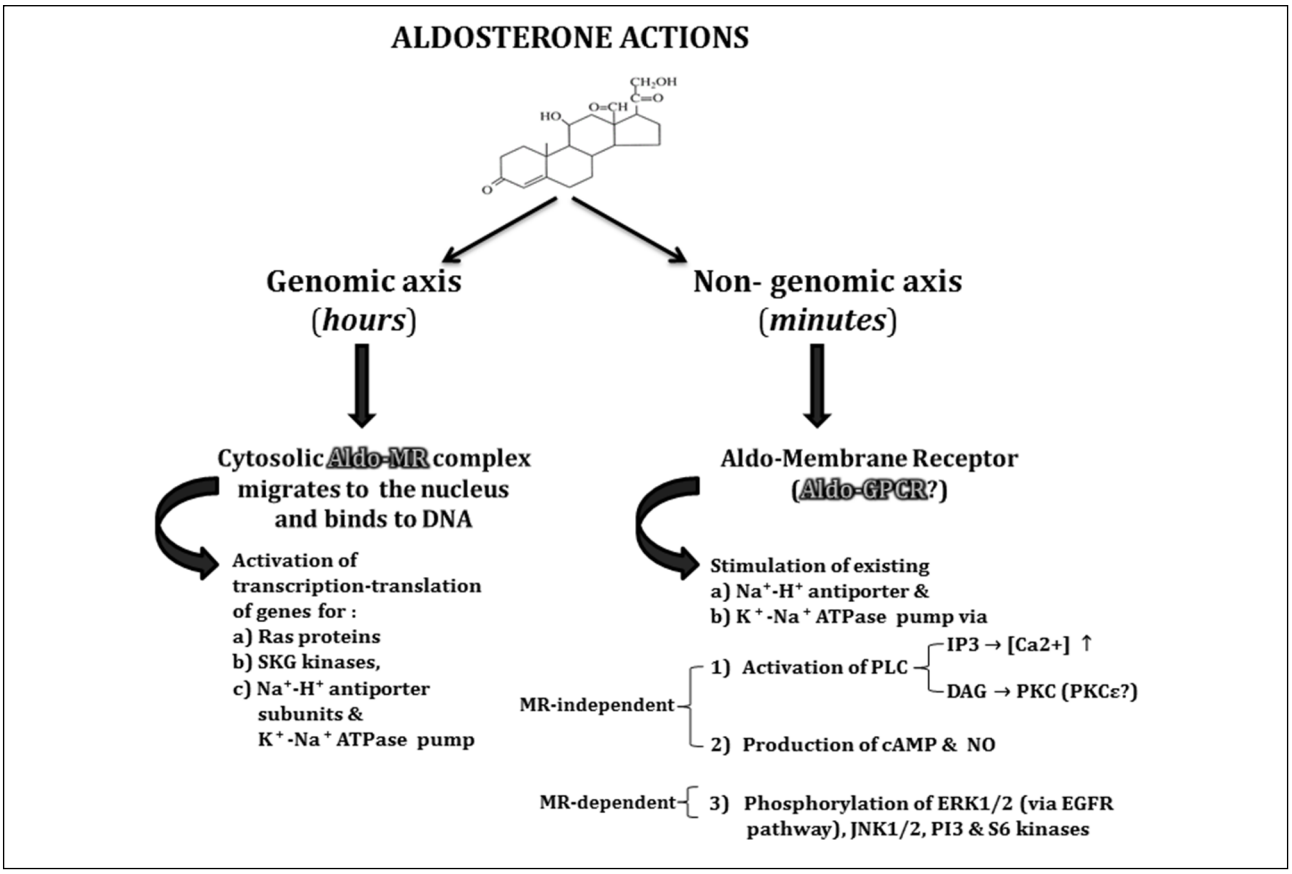

Fig. 1. Schematic representation of the acute non genomic actions of aldosterone (right, in minutes) as opposed to the genomic actions (left, in hours). In the first case, activation of transcription of key genes from the complex Aldo-MR, is not induced but rather the activation of existing molecules of Na^+ -H^+ antiporter and pump K^+-Na^+ ATPase is stimulated, *via* MR-dependent or MR-independent distinct intracellular pathways.

intermediate signaling station capable of amplify the rapid responses of hormone (41). As a consequence, there is an accurate regulation and a crosstalk between genomic and non genomic signaling responses, through the interaction of aldosterone and EGFR. Combing the aforementioned findings with the activation of AngII-AT1 receptor signaling cascades through the phosphorylation of EGFR, it becomes clear that EGFR plays a critical role in the total RAA axis (42). The activation of receptor by AngII is cell specific, including various cells types, such as VSMCs, cardiac myocytes, fibroblasts, COS-7 cells, C9 hepatic cells, glomerular mesangial cells. According to the tissue involved, the final outcome of the EGFR transactivation varies from vascular remodeling and hypertrophy of VSMCs to renal lesions, as well as induction of fibrotic genes in cardiac fibroblasts. Especially, EGFRis involved in the fibrosis induced by angiotensin II and endothelin-1. In fibroblasts, aldosterone upregulates both its expression and its activation. It initially activates the fast path through the ERK $^1/_2$, which in turn phosphorylates the EGFR (43-45). On the other hand, it was found that the complex of aldosterone-MR enhances transcription of the mRNA of the EGFR (46) leading to a consequent contraction of the arteries (47).

Although these actions are referred to cardiovascular system, EGFR transactivation has, indirectly, an important and an expanded role to the whole function of organism, which needs to be further illuminated.

The ability of aldosterone to make less negative the action potential of cardiac cells can also account for some of the cases of cardiac arrhythmias. Tillmann *et al.* (48) studied the monophasic action potential in patients with supraventricular arrhythmias compared with aldosterone levels and found a significant increase in its duration, a few minutes after intravenous administration of the hormone. The prolongation of the action potential is rapid and decreases in a very short time after the hormone administration. This fact, in combination with the Schneider *et al.* (49) postulation that non-genomic actions of aldosterone decline despite continuous presence of the hormone led Macdonald and Struthers (50) to the conclusion that the most important role in heart is played by periodic pulses of endogenous aldosterone production.

While investigating in our laboratory (Laboratory of Experimental Physiology) the effects of endogenous vasoactive substances on cardiovascular and hormonal parameters in 25 healthy anaesthetized New Zealand White male rabbits, a serendipitous positive correlation between left ventricle (LV) contraction duration and plasmtaldosterone levels came to our attention. By means of LV pressure (LVP) tracings (taken invasively), maximal and minimal change over time in LVP (LVmaxdp/dt and LVmindp/dt) and Δd (time interval between LVmaxdp/dt and LVmindp/dt = LV contraction duration) were estimated. A positive linear regression was found between aldosterone (measured by RIA) and Δd ($\Delta d = 0.1126 + 0.00019 \times$ aldosterone, $r = 0.47$, $P = 0.018$). Stepwise regression analysis demonstrated that peripheral blood pressure and aldosterone were the most important determinants of Δd and that the effect of aldosterone was independent of confounding factors (51). We have found a similar regression in cholesterol-fed atherosclerotic NZW rabbits (52). Since electrical and mechanical events in cardiac muscle overlap considerably in time (53) it might be

speculated that the aforementioned non-genomically mediated increase of monophasic action potential duration by aldosterone would result, by analogy, in (non-genomically mediated) increase of myocardial contraction duration (Δd). Our observation that plasma rennin activity does not correlate to Δd, also suggests that the effect may be attributed directly to aldosterone. Therefore, experimental confirmation of our unexpected finding may provide insight into another possibly non-genomic mechanism of aldosterone action on the heart muscle.

A major effort on studying the rapid response of aldosterone in the heart was made in 2004, when Okoshi *et al.* (54) conducted experiments in cell populations from neonatal mice ventricular myocardium. Thanks to these experiments it was demonstrated that aldosterone is directly related to the hypertrophy of these cells and indeed it became officially accepted the hypothesis of earlier scholars (55, 56) that the mechanisms involved in the phenomenon are independent of the MR. More specifically, it was found that cardiac hypertrophy happens *via* the activation of ERK, JNK and PKCα by aldosterone. The kinases then increase the atrial natriuretic peptide and the mRNA for heavy chain of myosin, A and B (57).

A further action of aldosterone in the cardiovascular system observed in recent years is associated with the reactive oxygen species (ROS). It is known that small concentrations of ROS in normal cells play a beneficial role in cell signaling. However, in cases of oxidative stress abnormally increased concentration of these compounds inevitably leads to cell damage and vasodilation. The existence of aldosterone in these cells has been shown to adversely affect the above situation, as concluded by Landmesser *et al.* (58) after experiments on mice.

In particular, aldosterone *via* the c-src protein stimulates the production of NADPH oxidase in the vessels (59) and alters the activity of endothelial nitric oxide synthase (eNOS). Therefore, the existence of aldosterone and its effect on these enzymes results in the overproduction of O_2^- in the cell affected by oxidative stress. Furthermore, in presence of aldosterone, eNOS produces O_2^- instead of NO. The macroscopic consequence of this phenomenon is vasoconstriction instead of the vasodilation that would have taken place under physiological condition (20, 60). Thus, aldosterone could be considered as part of the group of vasoconstrictor substances.

The first ascertainment that aldosterone is associated with peripheral vascular resistance and cardiac output was made in 1963 (61), and since then became the subject of research of many scientists. Schmidt *et al.* and Wehling *et al.* held a series of experiments in humans using non-invasive and invasive methods in order to confirm these findings. Thus, it is now known that aldosterone increases systemic vascular resistance and decreases cardiac output and cardiac index through non-genomic processes (62, 63).

These experiments represent a first attempt to shift the scientific interest from *in vitro* observations to studies on living human bodies. The same tactic was followed by Chai *et al.* (64, 65) making possible a direct comparison of the action of aldosterone on the myocardium of mice and humans. He reported that aldosterone has a positive inotropic effect on the rat heart (64), whereas in the human heart trabeculae it exerts a negative one (65). A third experimental process from Mihailidou *et al.* (39) also demonstrated negative inotropic effects of aldosterone in rabbit cardiomyocytes.

The correlation of aldosterone with cardiac fibrosis is an extended issue, which is still under investigation. However, there are many research studies, thanks to which the involvement of aldosterone in fibrosis has become undeniable. Rickard *et al.* (66, 67) contributed significantly to this by showing that the blockade of MR in macrophages (66) and murine cardiomyocytes (67) reduces cardiac fibrosis and increased blood pressure. Also, studies conducted in animals and patients with adrenal adenoma, (68) showed that aldosterone causes heart tissue remodeling and fibrosis.

The views on the way in which aldosterone and other factors cause fibrosis were gathered in a recent article by Kong *et al.* (69) in which the following possible mechanisms are indicated:

1. Aldosterone causes inflammation in vascular walls and overexpression of cytokines and chemokines (70) which are believed to participate in the process of fibrosis.
2. Regulates macrophage function, as mentioned above.
3. Provides the starting signal of fibrosis in cardiomyocytes.
4. Regulates the synthesis and proliferation of collagen fibres.

As for the last possible action of aldosterone, namely collagen production, it is found that a significant role is played by the calcium ions. In rats treated with aldosterone and an inhibitor of calcium channels, it was observed a decreased production of collagen (71). Still, it is believed that aldosterone alone causes increased levels of calcium in the smooth muscle cells of the vessel wall, (72) as well as in other tissues of the body, such as the cells in the renal cortical collecting ducts (73). It was also shown that calcium levels in these cells were not affected by the presence of aldosterone antagonists (74). This was confirmed after a few years by Arima *et al.* (75).

Another factor associated with cardiac fibrosis is the consumption of salt. Yu *et al.* (76) not only proved experimentally the effect of salt levels in the action of aldosterone, but also associated factor transforming growth factor β1 (TGF-β1) with the phenomenon. TGF-β1 is produced by fibroblasts of the heart and is activated by macrophages contributing this way to the differentiation of fibroblasts and collagen production. Conversely, potassium is not thought to play a single role in this process (77).

Finally, recent research on the action of aldosterone introduced a new molecule in the list of possible factors leading to fibrosis (78). This is galectin-3, a protein produced in fibroblasts and endothelial cells and whose activity is associated with cardiac fibrosis, inflammation and heart failure (79). Elevated levels of aldosterone cause a proportional increase in this protein and administration of spironolactone and eplerenone appears to act positively by limiting fibrosis.

It becomes, widely, perceived that abnormal aldosterone levels have deleterious effects on cardiovascular system, without missing out that this hormone, by definition, is involved in the raise of peripheral arterial resistance and blood pressure, as well as in the enhancement of vasoconstriction. A contradictory factor of the aforementioned actions, atrial natriuretic peptide exhibits various and opposite effects on the cardiovascular system. It is secreted by the atria of the heart and binds to specific receptors (NPR_A, NPR_C). Moreover, it is, already, known that ANP is capable of reducing the peripheral resistance and the arterial pressure, after intravenous administration (80). According to a study of Kallaras *et al.* (81). ANP has the ability to decrease aorta stiffness in cholesterol-fed anesthetized rabbits and this effect expands its clinical significance. Additionally, ANP possesses diuretic and natriuretic actions on the renal system, while it, also, has effects on the central nervous system. To conclude, we could note that ANP possesses useful effects, regarding the physiology of cardiac tissue and this feature could benefit therapeutic value, reversing aldosterone actions.

The immediate goal of scientists is the search of new and effective methods for dealing with various heart and vascular diseases (*e.g.* hypertension) associated with abnormal levels of aldosterone. These efforts so far are based on the findings on the structure and function of aldosterone. An important finding for the gene of aldosterone synthase (CYP11B2) was made in 2008 when it was reported that the T-344C polymorphism in this gene is associated with atrial fibrillation (82). Recent experiments of Tsai *et al.* (83) confirmed this view and added additional data on the potential mechanism demonstrating the involvement of MAP kinase.

Another innovative idea was proposed very recently by a group of researchers from the United Kingdom. Robertson *et al.* (84) conducted experiments in both normal human adrenal cells and in samples of adenomas overexpressing aldosterone. Silencing a gene essential for the maturation of microRNA they found that the expression of CYP11B2 gene was altered. Further investigations led to the conclusion that different miRNA molecules bind to the 3' untranslated region of the CYP11B2 gene thus regulating its expression. On the basis of these data it is suggested a new way of addressing hyperaldosteronism, which aims not to the gene itself, but to the necessity for its expression miRNAs.

Finally, new information is constantly brought to light suggesting the interaction of aldosterone with other molecular pathways. In specific, Mokra *et al.* (85) make remarkable comments on the potential role of phosphodiesterase 3 (PD3) and its inhibitors in the regulation of aldosterone, while Korolczuk *et al.* (86) focus on the nephrotoxicity caused by cyclosporine A possibly through the stimulation of the RAA system. Recent experiments (87) also support the involvement of angiotensin II in the atherogenic process, a fact that augments the scientific options with regard to the treatment of more cardiovascular diseases.

Although the classical actions of aldosterone are mediated through genomic pathways, there is growing evidence for the existence of important non-genomic responses, which are becoming widely recognized. This is in part a result of the growing awareness of the effects of aldosterone on non-epithelial sites (*e.g.* heart and vessels), in which non-genomic actions could partner with genomic ones. The non-genomic story has apparently been stifled by the as yet unknown identity of the mediating receptor.

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