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Non-Genomic Effects of Aldosterone on Intracellular Ion Regulation and Cell Function in the Heart

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Abstract: Serum aldosterone levels are often elevated in patients with heart failure and are associated with poor clinical outcomes. Aldosterone can be produced in extra-adrenal tissues including the heart, and the local increase in aldosterone exerts deleterious effects on heart structure and function. Aldosterone has 2 types of effects on intracellular ion milieu and cellular function. One is the classical genomic effect in which aldosterone combines with the intracellular mineralocorticoid receptor, transfers to the nucleus, and stimulates synthesis of various proteins. Another is the non-genomic effect that expresses within minutes without synthesizing proteins. The non-genomic effects of aldosterone are less proved in the heart, but it has been shown that aldosterone rapidly activates Na^+ influxes *via* Na^+-K^+-$2Cl^-$ co-transport and Na^+/H^+ exchange, resulting in an increase in intracellular Na^+ concentration and intracellular alkalinization. These changes in intracellular ion milieu cause positive inotropy, cell swelling, and generation of reactive oxygen species. Thus, the non-genomic effects of aldosterone may contribute, in concert with the genomic effects, to cardiac hypertrophy, fibrosis, and remodeling. This review will discuss the experimental studies examining the mechanisms and physiological/patho-physiological relevance regarding the non-genomic effects of aldosterone in the heart.

Keywords: Aldosterone, non-genomic effect, ion concentration, cell volume, hypertrophy.

1. INTRODUCTION

The steroid hormone aldosterone is synthesized in the adrenal cortex in response to angiotensin II. The primary effect of aldosterone is to promote renal sodium absorption in exchange for potassium and to maintain electrolyte homeostasis and extra-cellular fluid volume.

Aldosterone levels are often elevated in patients with heart failure and are associated with poor clinical outcomes [1, 2]. The Randomized Aldactone Evaluation Study for congestive heart failure (RALES) has shown that a mineralocorticoid receptor (MR) antagonist, spironolactone, decreases mortality in patients with congestive heart failure [3]. The Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS) has also demonstrated a decreased mortality in patients with left ventricular dysfunction after myocardial infarction treated with an MR-specific antagonist, eplerenone [4]. In both studies, the MR antagonists improved clinical outcomes independently of the effects on blood pressure. The plasma concentration of aldosterone increases up to 0.1 μM in patients with heart failure, and the level of aldosterone in the myocardium is much higher than that in plasma [5, 6]. The rapid and direct effects of aldosterone on the heart are, therefore, considered to have significant clinical relevance and to be important as a therapeutic target for heart failure.

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Now it is well known that aldosterone has 2 types of effects on intracellular ion milieu and cellular function. One is the classical genomic effect and the other is the non-genomic effect that expresses within minutes without synthesizing proteins [7, 8]. The non-genomic effect of aldosterone was initially reported at 1964 in dog erythrocytes in which genomic mechanisms did not work because of the lack of nucleus [9]. There are several reports regarding the non-genomic effects of aldosterone in a variety of tissues including vascular endothelium [10], vascular smooth muscles [11], kidney cells [12], skin cells [13], bronchial epithelium [14], mononuclear leukocytes [15], and skeletal muscles [16]. In these tissues, aldosterone rapidly changed intracellular Na^+, Ca^{2+} and H^+ concentrations ($[Na^+]_i$, $[Ca^{2+}]_i$, and pH_i) by modifying ion fluxes through sarcolemma and *via* intracellular Ca^{2+} stores.

For cardiac myocytes, there are many reports on the genomic effects of aldosterone including the increases in Ca^{2+} and K^+ currents, the expression of T-type Ca^{2+} channels, and the activation of Na^+/H^+ exchange [17-19]. However, there are only a few reports on non-genomic effects of aldosterone, e.g. Na^+-K^+-$2Cl^-$ co-transporter (NKCCl) and Na^+/H^+ exchange. The aldosterone-induced modulation of these ion transporters can certainly affect intracellular ion regulation and cellular function. However, it is still undefined in cardiac tissues whether aldosterone actually alters intracellular ion concentrations, cell volume or myocardial contractility due to non-genomic effects.

This review discusses the non-genomic effects of aldosterone on cellular ion regulation, function, and physiological/patho-physiological implications in cardiac myocytes.

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2. GENOMIC AND NON-GENOMIC EFFECTS OF ALDOSTERONE

Genomic effects of aldosterone imply the binding to its intracellular receptor and the translation of the ligand-receptor-complex to the nucleus, followed by the modulation of transcriptional and translational processes. The receptors that transmit these effects have been cloned and represent the superfamily of steroid receptors, including the classic intracellular MR [13].

Rapid non-genomic effects of aldosterone have been ascribed to undefined membrane receptors and intracellular signaling pathways. These kinds of aldosterone effects were not blocked by spironolactone, actinomycin D or cycloheximide, suggestive of the distinct mechanisms from the MR, and gene transcription and translation. In vascular smooth muscles and mononuclear leukocytes, aldosterone stimulated phosphoinositide hydrolysis and thus inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG) formation within minutes [20]. The increase in IP_3-induced Ca^{2+} release from intracellular Ca^{2+} stores could explain the aldosterone-induced increase in $[Ca^{2+}]_i$ in these cell types. Aldosterone increased $[Ca^{2+}]_i$ and cAMP even in the mice skin cells where MR is knocked out [13]. These effects were not induced by micromolar concentrations of hydrocortisone or blocked by spironolactone. In human bronchial epithelial cells, aldosterone reduced $[Ca^{2+}]_i$ *via* a non-genomic mechanism, involving the activation of endoplasmic reticulum (ER) Ca^{2+}-ATPase and adenyl cyclase- and PKA-coupled signaling pathways [14]. In human distal colon and in mouse cortical collecting duct, aldosterone increased $[Ca^{2+}]_i$ *via* protein kinase C (PKC) [21, 22].

In cardiac myocytes, molecular steps that mediate non-genomic effects of aldosterone remain unclear. Mihailidou *et al.* reported that aldosterone-induced activation of NKCC1 was mediated by εPKC in rabbit ventricular myocytes [23]. Another report by Mano *et al.* suggested a specific plasma membrane receptor coupled with phospholipase C [24].

On the other hand, recent reports have indicated that the classical MR seems to participate at least in some rapid non-genomic effects of aldosterone, because MR blockers abolished them [25]. However, we and another study have shown that spironolactone could by itself modify intracellular ion concentrations and cell function independently of MR [26, 27]. Furthermore, under physiological conditions, MRs in cardiac myocytes are normally occupied by endogenous glucocorticoid because 11 β-hydroxysteroid dehydrogenase is not usually expressed in cardiac myocytes and there are high levels of circulating cortisol [28]. Thus, at the present state, the specific membrane receptors for aldosterone are not yet cloned, and the involvement of MR remains undefined. Future studies are necessary to elucidate the receptor-intracellular signaling pathways of the non-genomic effects of aldosterone.

3. NON-GENOMIC EFFECTS OF ALDOSTERONE ON INTRACELLULAR ION REGULATION

(a) Na^+

Regarding non-genomic effects of aldosterone on $[Na^+]_i$, there have been several reports that showed a rapid rise in intracellular Na^+ activity in rabbit papillary muscles [29], and increases in $[Na^+]_i$ in rat ventricular myocytes under normal and high $[Na^+]_o$ conditions [26, 30]. These aldosterone-induced Na^+ increases were independent of the MR.

We examined the non-genomic effects of aldosterone on $[Na^+]_i$ in isolated rat ventricular myocytes using a laser scanning confocal microscopy and a Na^+-sensitive fluorescent dye Sodium-green [27]. Aldosterone rapidly elevated $[Na^+]_i$ at the concentrations of 0.1 μM to 10 μM (Figs. **1A** & **B**). The aldosterone-induced increase in $[Na^+]_i$ was not abolished by the co-administration of actinomycin D, an inhibitor of transcription. This data confirmed that aldosterone actually modified $[Na^+]_i$ by rapid non-genomic effects. The extent of the increase in $[Na^+]_i$ and the concentration of aldosterone varied among the studies (presumably from differences in animal species, specimens and temperature), but the increase was estimated to be 1~1.5 mM [24, 29-31].

The non-genomic effects of aldosterone on membrane Na^+ regulatory proteins have been observed in various cell types. In human mononuclear leukocytes and vascular smooth muscle cells, aldosterone activated Na^+/H^+ exchange, resulting in intracellular alkalinization [15, 32]. In vascular smooth muscle cells, the involvement of NKCC1 in aldosterone-induced changes in Na^+ flux was also described [11].

In cardiac myocytes, $[Na^+]_i$ is principally determined by the balance between Na^+ influxes *via* Na^+/H^+ exchange and the fast Na^+ channel, and Na^+ extrusion by Na^+/K^+ -ATPase. The Na^+ influx *via* NKCC1 seems to be less important [33, 34].

The aldosterone-induced increase in $[Na^+]_i$ was abolished by the inhibition of Na^+/H^+ exchange or NKCC1 (Fig. **1D**). These findings were also found in other studies [29, 30]. Mihailidou *et al.* demonstrated that the increased Na^+ influx by acute activation of NKCC1 stimulated Na^+/K^+ pump current [29], but aldosterone directly inhibited Na^+/K^+-ATPase [31]. We confirmed that a rapid increase in $[Na^+]_i$ by aldosterone occurred even after the inhibition of Na^+/K^+-ATPase, and bumetanide prevented the aldosterone-induced increase in $[Na^+]_i$ [27]. Therefore, both Na^+/H^+ exchange and NKCC1 are likely to contribute to the increase in $[Na^+]_i$ induced by a non-genomic effect of aldosterone, whereas the inhibition of Na^+/K^+-ATPase might also participate.

(b) Ca^{2+}, Mg^{2+}

Previous reports have shown that aldosterone increased $[Ca^{2+}]_i$ within 1 h in human mononuclear leukocytes, cultured skeletal muscle cells and porcine aortic endothelial cells [16, 35, 36]. In these tissues, the aldosterone-induced increase in $[Ca^{2+}]_i$ was thought to be mainly mediated by phosphoinositide hydrolysis. The production of IP_3 caused a Ca^{2+} release from intracellular IP_3-sensitive stores.

In adult cardiac myocytes, this mechanism seems to be rather small since Ca^{2+} -induced Ca^{2+} release from the sarcoplasmic reticulum is more important for excitation-contraction coupling. Actually, there are no reports that showed the changes in Ca^{2+} transient by the non-genomic effects of aldosterone. It was demonstrated that aldosterone increased L-type Ca^{2+} currents as the genomic effect but did not change it within 24 hours [17]. We and another report

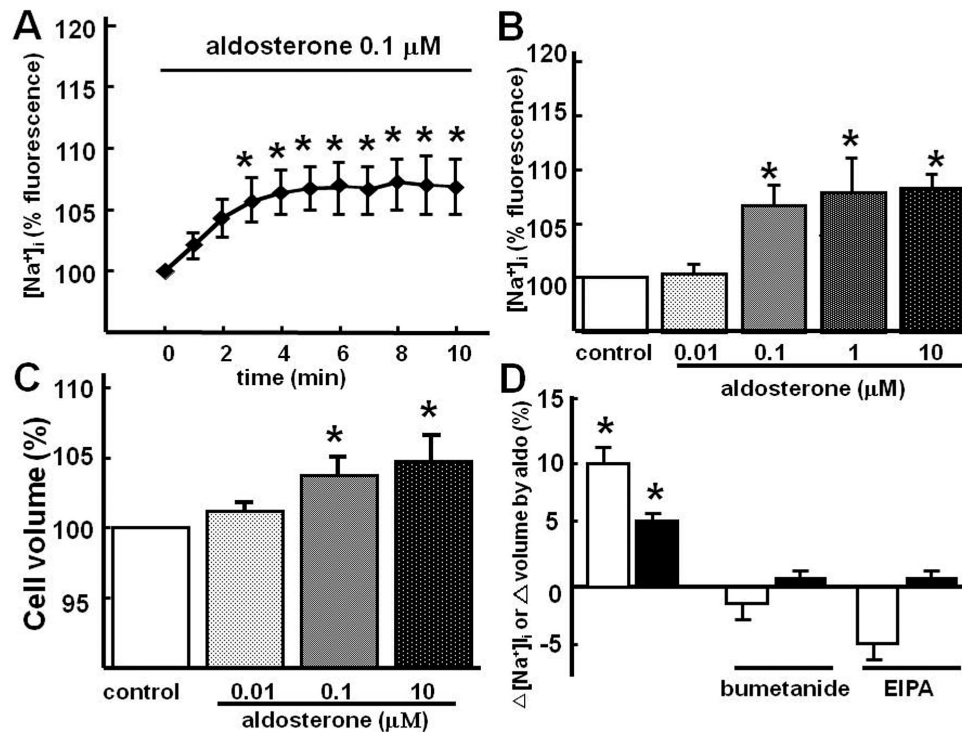

Fig. (1). Effects of aldosterone on $[Na^+]_i$ and cell volume.
(**A**) The time-dependent change in $[Na^+]_i$ during the perfusion of 0.1 μM aldosterone.
(**B**) The concentration-dependent changes in $[Na^+]_i$. The changes in $[Na^+]_i$ were expressed as % changes of fluorescence intensity of sodium-green. (**C**) The concentration-dependent change in cell volume. Cell volume was measured by 3-dimensional reconstruction from optical sections of a calcein-loaded myocyte.
(**D**) Changes in $[Na^+]_i$ (empty columns) and cell volume (filled columns) by aldosterone (0.1 μM). The aldosterone-induced increases in $[Na^+]_i$ and cell volume were abolished by the preincubation of bumetanide (an inhibitor of NKCC1) or 5-(N-ethyl-N-isopropyl) amiloride (EIPA, an inhibitor of Na^+/H^+ exchange). Data is shown as means±SE from 5-8 experiments. * $p<0.05$ *vs.* control by ANOVA. (Data is taken from Matsui *et al.* ref. 27).

also showed no immediate change in the profile of twitch Ca^{2+} transients in adult cardiac myocytes [26, 27]. Only one report exhibited a nifedipine-sensitive rapid increase in $[Ca^{2+}]_i$ in aldosterone-treated neonatal rat cardiac myocytes [24].

These findings, however, provoke the question why a significant increase in $[Na^+]_i$ due to the activation of NKCC1 and Na^+/H^+ exchange does not augment Ca^{2+} transients. Under physiological conditions, the increase in $[Na^+]_i$ is expected to elevate $[Ca^{2+}]_i$ by decreasing Ca^{2+} efflux (or increasing Ca^{2+} influx) *via* Na^+/Ca^{2+} exchange [34]. The possible explanations are that (1) the increase in $[Na^+]_i$ by aldosterone was too small to affect Ca^{2+} regulation, (2) aldosterone increased cellular Ca^{2+} gain but the concomitant increase in cell volume minimized the increase in $[Ca^{2+}]_i$, and (3) other unknown non-genomic pathways cancelled the effects of the increase in $[Na^+]_i$. Regarding the last possibility, we observed that aldosterone directly accelerated the Na^+/Ca^{2+} exchange current in guinea pig ventricular myocytes (personal data).

Mg^{2+} is an abundant divalent intracellular cation and acts to regulate energy state and $[Ca^{2+}]_i$. Trans-membrane Mg^{2+} fluxes are linked to Na^+-dependent Mg^{2+} exchange activity, but the detailed molecular mechanisms that regulate these fluxes are still unknown [37]. It has been reported that hyperaldosteronism is associated with Mg^{2+} loss in urine. Although there is no study examining the effect of aldosterone on Mg^{2+} regulation in the heart, the changes in Mg^{2+} status may influence the intracellular Ca^{2+} regulation and cellular function.

(c) H^+

The activation of Na^+/H^+ exchange causes intracellular alkalinization because of H^+ extrusion. In fact, several reports have shown an aldosterone-induced rapid increase in pH_i in a variety of tissues including the heart [26, 30, 38, 39]. The involvement of Na^+/H^+ exchange was judged by the ability of amiloride derivatives to abolish the response. The increase in cytosolic pH_i has important meaning because it causes not only positive inotropy (due to the leftward shift of pCa-ATPase curve of myofilaments), but also cardiac hypertrophy and remodeling as discussed below.

4. PHYSIOLOGICAL AND PATHO-PHYSIOLOGICAL IMPLICATION OF NON-GENOMIC EFFECTS OF ALDOSTERONE

(a) Cell Volume

Aldosterone has been recognized as a key regulator of fluid and electrolyte balance in many organs including the kidney. The aldosterone-induced rapid increase in cell vol-

ume was reported in human mononuclear leukocytes and also in neonatal rat cardiac myocytes [30, 40].

A recent report demonstrated that an acute exposure to elevated $[Na^+]_o$ caused rapid shrinkage of cardiac myocytes as a result of fluid loss, and aldosterone strongly suppressed it by increasing Na^+ entry into cardiac myocytes [30]. Thus, the principal effect of aldosterone is to maintain Na^+ homeostasis and to protect myocytes from osmotic stress. As shown in Fig. (**1C**), we also found that aldosterone increased cell volume in adult rat ventricular myocytes [27, 41]. Since the aldosterone-induced increase in cell volume was blocked by an amiloride derivative or bumetanide (Fig. **1D**), the activation of Na^+ influxes was associated with the increase in cell volume [27, 42].

(b) Contractility

Aldosterone was initially found to decrease cardiac output within 5 min, while peripheral vascular resistance and blood pressure increased [43]. More recently, aldosterone induced a negative inotropic response in human myocardial trabeculae [44]. On the other hand, in Langendorff-perfused and working rat hearts, aldosterone increased left ventricular developed pressure immediately [45, 46]. Spironolactone and eplerenone did not block these positive and negative inotropic effects. Therefore, *in vivo* evidence of the non-genomic effect of aldosterone on cardiac contractility has not yet been confirmed, since the changes in coronary flow and peripheral vascular resistance also occurred.

As mentioned above, aldosterone did not change Ca^{2+} transients but increased pH_i. The non-genomic effect of aldosterone appears to cause positive inotropy due to the leftward shift of the pCa-ATPase curve in myofilaments. In our previous study, however, the perfusion of aldosterone did not alter cell shortening in isolated rat myocytes, developed tension in rat papillary muscles, or left ventricular developed pressure in Langendorff-perfused rat hearts [27]. There seems to be species difference in contractile response to aldosterone, presumably due to the difference in response of pH_i. Actually, we found that aldosterone increased pH_i in guinea pig ventricular myocytes but did not in rats (personal data).

(c) Cardiac Hypertrophy, Fibrosis, and Remodeling

Basic and clinical studies have shown that aldosterone-induced cardiac hypertrophy, fibrosis and remodeling are suppressed by MR antagonists, suggesting the genomic effects of aldosterone [3, 4, 47, 48]. However, as mentioned above, the non-genomic effects of aldosterone involve the modulation of various membrane proteins that are associated with downstream hypertrophic signals (Fig. **2**). In patients with hypertension or heart failure, local aldosterone levels are often elevated, and the aldosterone induces local expression of angiotensin-converting enzyme, creating a vicious cycle involving the renin-angiotensin-aldosterone system. Thus, the non-genomic effects of aldosterone, in concert with genomic effects, can have some patho-physiological relevance.

Actually, the aldosterone–induced activation of Na^+/H^+ exchange, Ca^{2+} overload, myocyte swelling, apoptosis, and the increase in matrix metalloproteinase (MMP) activity have been reported to contribute to cardiac hypertrophy and

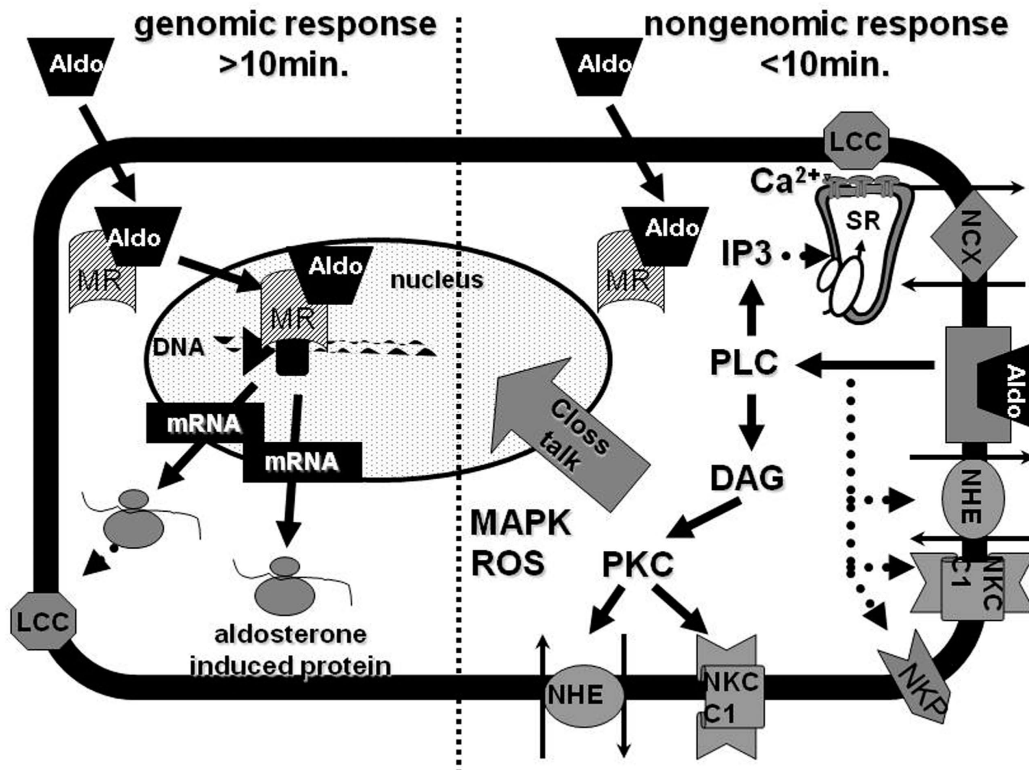

Fig. (2). A scheme for genomic and non-genomic effects of aldosterone in a cardiac myocyte. Aldo: aldosterone, MR: mineralocorticoid receptor, LCC: L-type Ca^{2+} channel, NHE: Na^+/H^+ exchange, NCX: Na^+/Ca^{2+} exchange, NKCC1: Na^+-K^+-$2Cl^-$ co-transporter, NKP: Na^+-K^+ pump, SR: sarcoplasmic reticulum, ROS: reactive oxygen species.

remodeling [24, 30, 49, 50]. Second messenger pathways that link these patho-physiological effects of aldosterone include PKC (especially its Ca^{2+}-independent isoform, PKCε), mitogen/ extracellular signal-regulated kinase (MEK), extracellular signal-regulated kinase 1/2 (ERK 1/2), and the generation of reactive oxygen species (ROS) [23, 24, 49]. It is reported that aldosterone rapidly increased generation of ROS by activating NADPH oxidase [49]. The increase in ROS generation can relate to myocyte damage, inflammation, and activation of MMP.

5. CONCLUSIONS

Aldosterone exerts several non-genomic effects on cellular ionic regulation and function in the heart (as well as in other tissues). The various non-genomic effects of aldosterone have been described during the past decades, but the identification, characterization, and cloning of the membrane receptor for them remain the major goals. The second messengers and the interaction with MR-dependent genomic actions also remain to be elucidated. The physiological and pathophysiological relevance of non-genomic effects of aldosterone should be further characterized, and the development of novel specific antagonists may yield therapeutic benefits for a variety of cardiovascular diseases.

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