Non-genomic Effects of Aldosterone

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For many years, aldosterone was considered to be a long-term regulator of salt retention, acting as an endogenous ligand on the mineralocorticoid receptor (MR) of target organs such as the kidney, colon and salivary glands (Grossmann and Gekle, 2009).

Interest in non-genomic aldosterone effects was only apparent when a link was made between the pathophysiological processes in the renocardiovascular system, and the non-classical MR targets of aldosterone. Studies indicated that aldosterone was an active contributor to inflammatory and remodelling processes within the renocardiovascular system, and thus causing fibrosis, endothelial dysfunction and hypertrophy (Qin *et al*, 2003; Young and Funder, 2003).

Early findings

Early cellular studies of canine erythrocytes indicated retardation of sodium exchange across the cellular membrane, in the presence of aldosterone (Spach and Streeten, 1964). As mature erythrocytes lack the nucleus, the classical MR pathway could not explain the effects observed. Despite the effectiveness of this model to study the nongenomic effects of aldosterone, it is worth noting that the canine erythrocytes differ to humans in terms of ion transport pathways across the membrane; for example, the canine erythrocytes have the Na^+/Ca^{2+} exchanger, which is substituted for the Na^+/K^+ pump in human and other mammalian erythrocytes (Bernhardt *et al*, 1988).

Classical mineralocorticoid target organs

In classical mineralocorticoid target organs such as the kidney and the colon, pH can be used to detect the rapid effects of aldosterone. Many studies have identified a transient acidification, followed by a significant alkalisation within their pH study samples (Figure 1; control). The acidifications could be due to the rise in Ca^{2+} concentrations within the cells (within 1 min of aldosterone treatment), and the rapid alkalisation could be due to the activation of the Na^+/H^+ exchanger (NHE) across the membrane in M1 cells (Markos *et al*, 2005).

Evidence of interactions between rapid aldosterone and classical MR pathways were demonstrated by Grossmann *et al*, 2008. The activation of the MAP kinases ERK1/2 was believed to be dependent solely on genomic mechanisms. However comparable progesterone and oestrogen receptors studies demonstrated that deletion constructs containing only the MR domains E/F were sufficient to express non-genomic properties of aldosterone (Grossmann *et al*, 2008).

By inhibiting the ERK1/2 phosphorylation, it was possible to regulate the cytosolic Ca^{2+} levels which in turn, have a direct effect on the activation of NHE (Gekle *et al*, 2001).

In recent studies NHE3, a sodium-hydrogen antiporter involved in maintaining the balance of sodium was found to block the HCO_3^- reabsorption through an MR-independent pathway in the thick ascending limb of the loop of Henle (Good, 2007). By contrast, NHE3 activity in the proximal tubule was said to be stimulatory by the effects of aldosterone (Drumm *et al*, 2006). A possible explanation to this could be that pH sensitivity influences the signalling processes. As NHE1 induced a pH shift across the membrane of the cell, it would in turn affect the NHE3 activity within the renal ultrafiltrate by activating membrane transporters responsible for K^+ recycling in the distal tubule such as K^+_{ATP} channels. Observations indicated that aldosterone-induced K^+_{ATP} channel activity was detected within 2 min in A6 amphibian renal principal cells. Inhibition of such activity was achieved using amiloride, an inhibitor of NHE activity or by simply altering cytosolic pH from 7.15 to 7.4 (Urbach *et al*, 1996).

Other studies have also found relationships with the activation of protein kinase C (PCK; in M1-CCD cells), intracellular calcium, and phosphorylation of

epidermal growth factor receptor (EGFR) and NHE (Grossmann and Gekle, 2009).

Non-classical target tissues

Non-genomic effects have been observed in both epithelial and non-epithelial tissues, showing insensitivity to inhibitors of transcription (actinomycin D) and translation (cycloheximide; Falkenstein *et al*, 2000; Losel *et al*, 2003). Observations on human mononuclear leukocytes, vascular smooth muscle cells (VSMC), and rat cardiomyocytes revealed an increase of inositol trisphosphate, intracellular Ca^{2+}, the repression of PKC activity, and the activation of the Na^+/K^+ pump when exposed to aldosterone (Ivanova *et al*, 2008).

Another notable observation was that these effects were neither blocked by spironolactone and/or other MR antagonists, supporting the theory of an alternative signalling pathway for aldosterone (Good, 2007).

A cardiovascular study on 17 coronary heart disease patients revealed an elevation in systemic vascular resistance, cardiac output, and cardiac index (Wilcoxon test, $P < 0.02$–0.05) compared with a placebo group after intravenous administration of 1mg of aldosterone. The effects were apparent within 3 minutes, and later dissipated at around 10 minutes after the dose, supporting the idea of rapid aldosterone effects (Wehling *et al*, 1998). Nevertheless, there has been no evidence of a non-classical aldosterone receptor within literature.

Contradictory to the findings by Good, a more flexible and water soluble MR antagonist termed RU28318 was able to inhibit the non-genomic actions of aldosterone

(Figure 1; Mihailidou and Funder, 2005). This potassium salt was found to selectively reduce MR binding and prevent ACTH-induced and angiotensin II-induced aldosterone biosynthesis (Perroteau *et al*, 1984).

Another study by Grossmann *et al* in 2005 deduced three possible aldosterone signalling pathways: genomic (MR dependent), non-genotropic (MR dependent) and non-genotropic (MR independent). The research focused on the MR in Chinese hamster ovary, human embryonic kidney cells and primary human aortic endothelial cells. After trasfecting the cells with human MR, there was a correlation between concentration of aldosterone with the phosphorylation of ERK1/2 and c-Jun NH_2-terminal kinase (JNK) 1/2 kinases. It was noted that the phosphorylation effects were down regulated by spironolactone, and involved cSrc kinase as well as EGFR (Grossmann *et al*, 2005).

Studies conducted on the relationship between prolonged genomic and non-genomic actions of aldosterone have revealed that EGFR signalling components play a vital role in the cross-talk between genomic and nongenomic aldosterone regulation. EGFR is widely known for its induced cell proliferating and remodelling roles in the renocardiovascular system. cSrc has been previously associated with aldosterone-bound MR and MAP kinases, having EGFR as intermediate in addition to the activation of PKD1 in M1-CCD cells through the Na^+/H^+ exchanger pathway. Consequently, upregulation of EGFR would result in ENaC subunit intracellular trafficking, a process influencing the Na^+ content within cells and ultimately, the proliferation of cardiomyocytes (McEneaney *et al*,
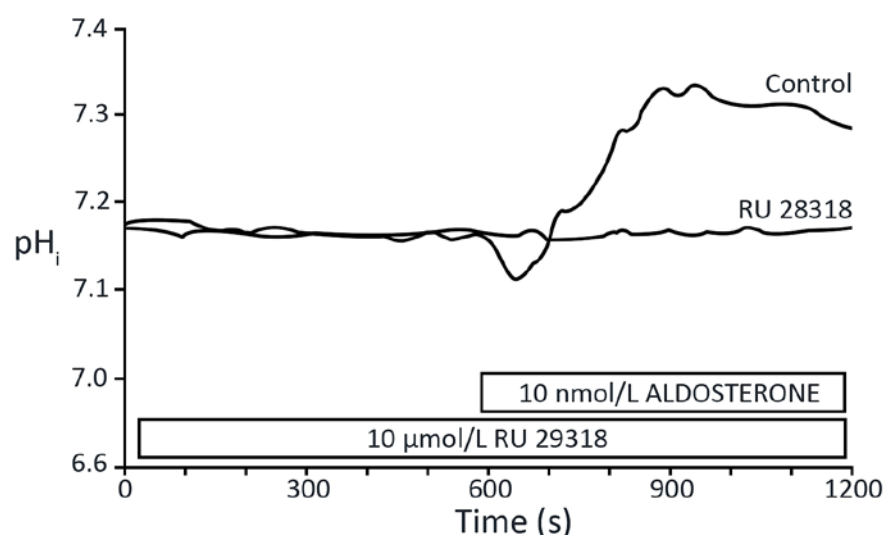

Figure 1 Rapid nongenomic effect of aldosterone on intracellular pH in vascular smooth muscle cells. RU28318 inhibitory effect is compared to a control. The unchanged pH levels in the RU28318 sample indicate the success of the antagonistic effect (Mihailidou and Funder, 2005).

2007). Notably, cell proliferation has also been observed in VSMC through the phosphorylation of EGFR (Min *et al*, 2005).

Collagen III secretion was investigated in deletion constructs of the MR (without DNA-binding domain) in an attempt to distinguish between genomic and non-genomic effects of aldosterone involving the EGFR pathway. Observations indicated that inducing the cells with aldosterone resulted in H_2O_2-stimulated increase in collagen III secretion (Grossmann *et al*, 2008). Evidence from these findings indicated a longer-lasting effect of aldosterone on cells, demonstrating a more varied reaction rate and thus complicating the distinguished effects between genomic and non-genomic aldosterone signalling pathways.

An alternative pathway had also been suggested during the analyses as elevated cytosolic Ca^{2+} levels were detected in correspondence to aldosterone increment in both control and human MR transfected cells, thus indicating a far more complex signalling system (Grossmann *et al*, 2005; illustrated in Figure 2).
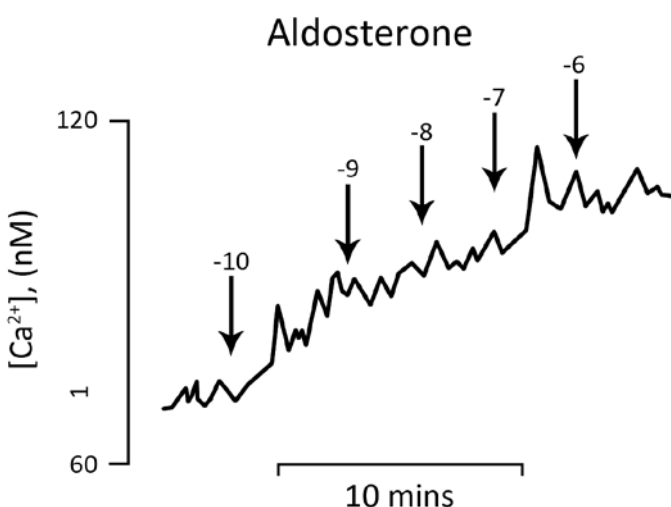

Figure 2 The relationship between Ca^{2+} and aldosterone concentrations in vascular smooth muscle cells over a 10 minute period (Mihailidou and Funder, 2005).

Knowing this information, it's possible to speculate that the RU28318 antagonist (Figure 1) could be an inhibitor to the non-genotropic (MR independent) signalling pathway (suggested by Grossmann *et al*), relating the Ca^{2+} elevation under aldosterone conditions to the pH levels observed by Mihailidou and Funder (2005).

Research in the endothelial dysfunction sector had linked aldosterone to nitric oxide (NO) release in endothelial cells which are said to further affect the contractions of smooth muscle cells below it, and consequently affect medial arterial hypertension (Schmidt *et al*, 2006).

In addition, the cSrc pathway could also be utilised to generate NADPH oxidase, a key component in increasing the reactive oxygen species (ROS) generation in VSMC, rat neonatal myocytes and mesangial cells. In cadiomyocytes, high levels of ROS were associated with increased apoptosis rates as well as the activation of MMP-2 and MMP-9, crucial enzymes known for their roles in tissue healing, remodelling and cancer cell metastasis (Hayashi *et al*, 2008). Furthermore, cSrc could also activate p38, a kinase molecule involved in stimulating collagen synthesis and profibrotic actions. These distinct pathways were observed in vascular myocytes from spontaneously hypertensive rats (Callera *et al*, 2005). From these studies, there are apparent evidence of pathophysiological effects resulting from the interactions between both classical and non-classical MR aldosterone signalling pathways leading to induced remodelling and inflammation effects. These profound effects ultimately make the cells more vulnerable to additional stress (Figure 3).

In summary, non-genomic effects of aldosterone are vast and occur in both classical and non-classical target tissues. Within classical MC target organs such as the kidneys, research has indicated that aldosterone has a profound influence over not only salt retention, but also electrolyte homeostasis, pH and cell volume.

Renocardiovascular studies have demonstrated a strong association of aldosterone with cell proliferation, inflammation, remodelling and endothelial dysfunction rendering cells vulnerable to any additional stress.

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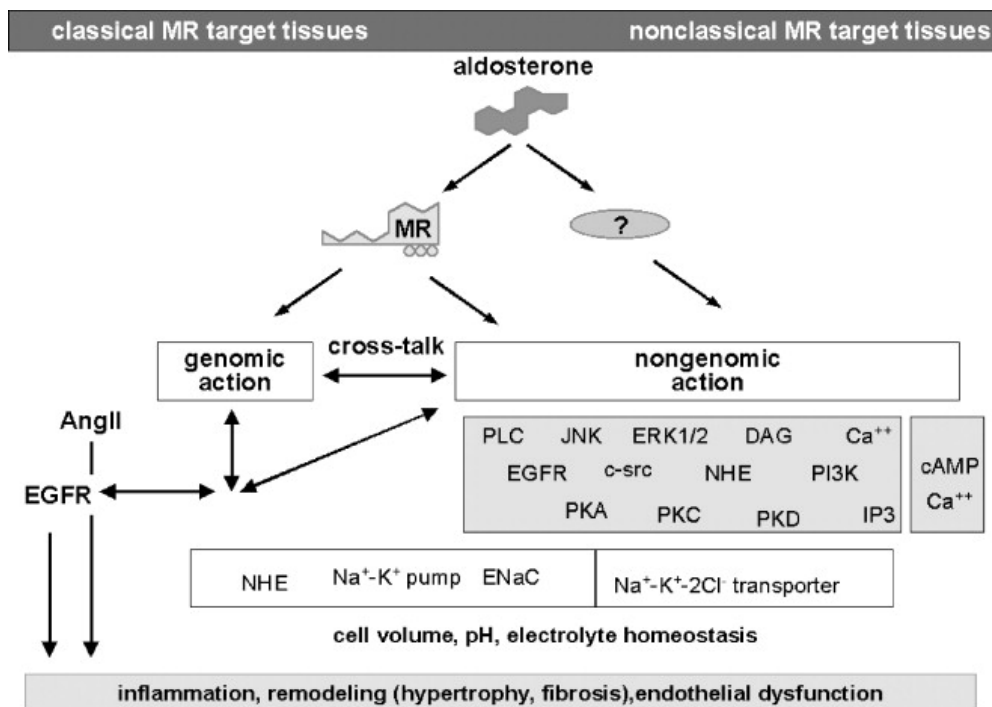
Figure 3 An overview of the currently distinguished pathways involving the aldosterone signaling (Grossmann and Gekle, 2009).

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