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Review

Non-genomic actions of aldosterone: From receptors and signals to membrane targets

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ABSTRACT

In tissues which express the mineralocorticoid receptor (MR), aldosterone modulates the expression of membrane targets such as the subunits of the epithelial Na^+ channel, in combination with important signalling intermediates such as serum and glucocorticoid-regulated kinase-1. In addition, the rapid 'non-genomic' activation of protein kinases and secondary messenger signalling cascades has also been detected in aldosterone-sensitive tissues of the nephron, distal colon and cardiovascular system. These rapid actions are variously described as being coupled to MR or to an as yet unidentified, membrane-associated aldosterone receptor. The rapidly activated signalling cascades add a level of fine-tuning to the activity of aldosterone-responsive membrane transporters and also modulate the aldosterone-induced changes in gene expression through receptor and transcription factor phosphorylation.

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Abbreviations: ASDN, aldosterone sensitive distal nephron; BMP, bone morphogenetic protein; cAMP, cyclic adenosine monophosphate; CCD, cortical collecting duct; CHO, Chinese hamster ovary; CREB, cAMP response element binding protein; EGF(R), epidermal growth factor (receptor); ENaC, epithelial Na^+ channel; ERK, extracellular stimulus regulated kinase; GILZ, glucocorticoid-induced leucine zipper protein; HEK, human embryonic kidney; Hsp, heat shock protein; JNK, c-Jun N-terminal kinase; MAP, mitogen activated kinase; MDCK, Madin–Darby canine kidney; MR, mineralocorticoid receptor; NHE, Na^+/H^+ exchanger; NO, nitric oxide; PHA, pseudohypoaldosteronism; PI3K, phosphoinositol 3-kinase; PKC/D, protein kinase C/D; PLC, phospholipase C; ROMK, renal outer medullary K^+ channel; SGK, serum and glucocorticoid induced kinase; SRC, steroid receptor co-activator; TH, tyrosine hydroxylase; VSMC, vascular smooth muscle cell.

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1. Introduction

The binding of steroid hormones to their cognate receptors induces the dissociation of heat shock proteins (Hsp), dimerization of the receptor and translocation into the nucleus. Here the hormone-receptor complex acts as a ligand-dependent transcription factor, binding to hormone response elements (HREs) in the promoters of various target genes, thereby regulating their transcription. Steroid hormone receptors also induce rapid extranuclear signalling effects including the activation of kinase signalling cascades and increases in second messenger production, and these effects are not dependent on transcription/translation. Many routes of cross-talk exist between the rapid effects which occur within seconds/minutes and the later genomic effects which take hours/days, both pathways integrating to mediate the final physiological outcome.

Aldosterone acts as a key mediator of sodium homeostasis by tightly controlling ion transport in the kidney through both genomic and non-genomic mechanisms. Aldosterone binds to the mineralocorticoid receptor (MR) and induces the expression of a number of genes including the renal outer medullary K^+ (ROMK) channel, Na^+/K^+-ATPase and the epithelial Na^+ channel (ENaC) (Asher et al., 1996; Beesley et al., 1998; Kolla and Litwack, 2000). Aldosterone also mediates rapid non-genomic effects such as the activation of the PKC–PKD and ERK1/2 MAPK protein kinase cascades through the transactivation of the epidermal growth factor receptor (EGFR), via the non-receptor tyrosine kinase, c-Src. Signalling cascades coupled to EGFR transactivation either directly modulate membrane targets through their phosphorylation or alternatively modulate the expression of membrane targets through the phosphorylation of transcription factors such as CREB or MR. Fig. 1 shows a summary of aldosterone-induced rapid non-genomic effects initiated in the cytoplasm such as transactivation of EGFR and kinase signalling and the latent genomic effects in the nuclear compartment such as the induction of expression of ENaC or the serum and glucocorticoid-induced kinase, SGK-1.

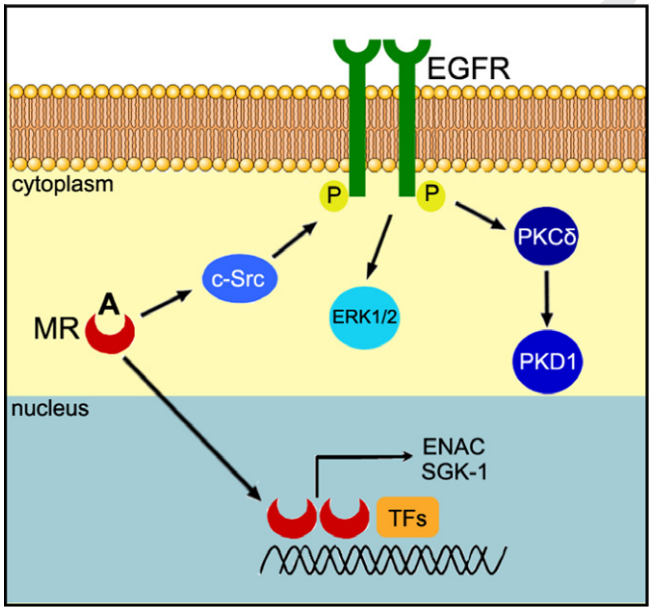

Fig. 1. Rapid versus genomic effects of aldosterone. Aldosterone diffuses across the basolateral membrane and binds to the mineralocorticoid receptor (MR), inducing dimerization and translocation to the nucleus. Here the hormone-receptor complex binds to GRE response elements, recruits other transcription factors (TFs), and acts as a ligand-dependent transcription factor inducing the expression of genes such as ENaC and SGK-1. Aldosterone binding to the MR also induces rapid kinase signalling cascades in the cytoplasm, including the activation of extracellular stimulus regulated kinase 1/2 (ERK1/2), protein kinase C delta (PKCδ) and protein kinase D (PKD), through the transactivation of the epidermal growth factor receptor (EGFR) via the non-receptor tyrosine kinase, c-Src.

The classical nuclear MR is responsible for transducing numerous aldosterone-induced rapid signalling effects, as demonstrated through the sensitivity of these responses to MR antagonists such as spironolactone or eplerenone. However, other studies found that rapid aldosterone-mediated effects are not affected by MR antagonism. The identity of this alternative aldosterone receptor to date remains elusive. In order to examine MR-dependency in rapid non-genomic responses, Grossmann et al. performed a study using heterologous expression of human MR in Chinese hamster ovary (CHO) and human embryonic kidney (HEK)-293 cells (Grossmann et al., 2005). Aldosterone induced rapid extracellular stimulus regulated kinase (ERK)1/2 and c-Jun N-terminal kinase (JNK)1/2 signalling responses, which were spironolactone-sensitive. Conversely, aldosterone also induced a spironolactone-insensitive rapid increase in intracellular Ca^{2+} concentration ($[Ca_i^{2+}]$) in both MR-transfected and mock-transfected cells (Grossmann et al., 2005). This study clearly outlines two different mechanisms for aldosterone-mediated rapid signalling events; MR-dependent and MR-independent pathways. The "unknown" aldosterone receptor may be an as yet undiscovered novel receptor, or a well characterized signalling molecule. For example, aldosterone binds directly to the C2 domain of protein kinase C alpha (PKCα), with a binding affinity of between 0.5 and 1 nM resulting in PKCα autophosphorylation (Alzamora et al., 2007). Furthermore, numerous reports have proposed that the G protein coupled receptor, GPR30, is a novel estrogen receptor. Estrogen binds to GPR30, resulting in intracellular Ca^{2+} mobilization and nuclear phosphatidylinositol 3,4,5-triphosphate (PIP_3) synthesis (Revankar et al., 2005). Recently, the rapid responses to aldosterone in smooth muscle have been linked to the GPR30-coupled signalling pathway, where the expression of GPR30 is required for the MR-independent rapid effects of aldosterone (Gros et al., 2011). The capacity for GPR30 to bind multiple steroid ligands is controversial and its promiscuity needs to be better understood.

In the case of other steroid hormone receptors, rapid responses are mainly mediated by a small proportion of classical nuclear steroid receptors localized to the plasma membrane. The estrogen receptor (ER) is associated with a subset of lipid rafts termed caveolae (Kim et al., 1999; Razandi et al., 2002). Caveolin-1, the major protein component of caveolae has been implicated as a structural scaffold, for the organization of cytoplasmic signalling complexes (Okamoto et al., 1998). Palmitoylation of ERα enhances the interaction of this receptor with caveolin-1 (Acconcia et al., 2005). Moreover, a conserved palmitoylation motif in the E domain of estrogen receptors ERα and β, progesterone receptors PR-A and B as well as the androgen receptor (AR) was shown to be required for membrane localization and rapid signalling events (Pedram et al., 2007). More recently it was shown that heat shock protein 27 (Hsp27) binds ERα and promotes its palmitoylation and its interaction with caveolin-1 and this same mechanism was extended to both AR and PR (Razandi et al., 2010). The glucocorticoid receptor (GR) colocalized with c-Src in caveolae and caveolin was required to mediate rapid PKB activation and induce cell proliferation (Matthews et al., 2008). The androgen receptor also localizes to caveolin-rich membrane fractions, and over-expression of caveolin-1 potentiates ligand-dependent AR activation (Lu et al., 2001). To date there is no indication of lipid-modification of MR and this steroid receptor lacks the conserved palmitoylation motif mentioned above. Recent evidence points to a fraction of MR localized at the membrane through interaction with the epidermal growth factor receptor (EGFR); disruption of cholesterol-rich membrane domains by cyclodextrin perturbed this MR–EGFR interaction (Grossmann et al., 2010a).

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The rapid physiological actions of aldosterone and other steroid hormones have been termed "non-genomic" because the observed effects occur within a time frame after treatment that cannot be accounted for by changes in gene expression at the level of transcription. The rapid responses are observed, for the most part, well in advance of the more latent pronounced effects of the hormones and as a result an artificial dichotomy has arisen with the rapid and transcriptional responses being regarded as separate independent actions of the hormone. In fact the different facets of aldosterone action ultimately act through common effectors, so contributing to the physiological outcomes of maintaining whole body electrolyte balance and regulating blood pressure. The close inter-connection between rapid and transcriptional responses is observed at multiple levels of regulation. For example, the activity and localization of aldosterone-responsive transcription factors is influenced by their phosphorylation state which can be modulated by rapidly-induced kinases; while the products of aldosterone-induced transcription may include signalling intermediates that contribute to the aldosterone sensitivity of the target tissues. This review aims to examine the mechanisms which underpin the rapid actions of aldosterone and to show how these rapid actions synergize with the later transcriptional responses that aldosterone elicits in diverse target tissues.

2. Aldosterone-induced signalling cascades

2.1. Mitogen activated protein kinases

The activation of protein kinase signalling cascades is the most extensively documented facet of rapid aldosterone responses. The mechanisms by which these signalling cascades impact upon cell physiology are now being elucidated. The activation of the different members of the mitogen activated protein (MAP) kinase family has been described in various aldosterone-responsive tissues. The sometimes antagonistic downstream signalling processes that are coupled to the different MAP kinases leads to subtle, tissue-specific effects that impact upon whole organism physiology. The activation of ERK1/2 has been investigated by many groups using experimental models of diverse tissues including Madin–Darby canine kidney (MDCK) cells (Gekle et al., 2001), a model for the intercalating cells of the renal cortical collecting duct (CCD); M1-CCD cells (Markos et al., 2005; McEneaney et al., 2010a) a model for the CCD principal cells; vascular smooth muscle cells (VSMCs) (Manegold et al., 1999); cardiac myocytes (Okoshi et al., 2004) and the mesangial cells of the glomerulus (Nishiyama et al., 2005). ERK1/2 activation is most often associated with the modulation of cell growth, either through the promotion of proliferation (McEneaney et al., 2010a; Nishiyama et al., 2005; Stockand and Meszaros, 2003) or hypertrophy (Okoshi et al., 2004). The kinetics of ERK1/2 activation shows some variation and is influenced by the concurrent activation of other signalling cascades. For example in MDCK cells, ERK1/2 activation occurs within 5 min and is sustained over a period of hours (Gekle et al., 2001). In M1-CCD cells the early phase of ERK1/2 activation is coupled to EGFR trans-activation, and the activation of protein kinase D1 (PKD1) is required to maintain ERK1/2 activation beyond 2–5 min (McEneaney et al., 2010a). The contribution of PKD1 to stabilizing ERK1/2 activation has also been described, where ERK1/2 activation occurs in response to growth factors; however, this does not involve direct phosphorylation of ERK1/2 by PKD1 (Sinnett-Smith et al., 2004). The prolonged phase of ERK1/2 activation stimulated by aldosterone in A6 renal cells is coupled to the stimulation of Ki-RasA expression, while aldosterone also stimulates Ki-RasA GTPase activity within 15 min of treatment (Tong et al., 2004).

The p38 MAP kinase sub-family, another signalling target of aldosterone, has four identified isoforms (α, β, γ and δ) which have different and often antagonistic roles in cell growth. The p38-α isoform is implicated in differentiation (Lovett et al., 2010) and the promotion of apoptosis through p53 phosphorylation (Liu et al., 2011), while p38-γ is implicated in advancing cell cycle progression and stimulating DNA repair to promote cell survival (Wu et al., 2010). Aldosterone promotes biphasic p38 activation in VSMCs within 1 min of treatment (Callera et al., 2005), followed by a second phase of activation detectable after 30 min. The VSMC p38 response was dependent on MR and c-Src co-activation and the authors further implicated p38 in the profibrotic effects of aldosterone on VSMCs through NADPH regulation. The connection between aldosterone-induced p38 activation and cardiovascular disease progression is emphasized by the observation that p38 antagonism with the novel inhibitor GSK-AHAB, counteracted the deleterious effects of high fat and high salt diet in a spontaneously hypertensive rat model (Willette et al., 2009). The MR-dependent activation of p38 in glomerular podocytes is also stimulated by aldosterone and this contributes to the induction of apoptosis in these cells (Chen et al., 2009).

The members of the JNK family of MAP kinases are also activated by aldosterone. Aldosterone treatment promotes dopamine synthesis by adrenal pheochromocytoma PC12 cells via the transcriptional regulation of tyrosine hydroxylase (TH) expression. TH abundance is interlinked with the transcription-independent stimulation of SAP kinase by aldosterone. Aldosterone-induced SAP kinase activation was mediated via a rapid, Rho small GTPase-dependent pathway and aldosterone-induced RhoA activation was enhanced by bone morphogenetic protein (BMP-4) (Goto et al., 2009).

2.2. Protein kinase C

The PKC family regulate cellular processes as diverse as proliferation, apoptosis, trafficking and tight-junction formation. Aldosterone promotes the MR-independent activation of PKCα in renal CCD cells within 2–5 min (Le Moellic et al., 2004; Markos et al., 2005). Here PKCα activation relies upon the direct binding of aldosterone to the kinase (Alzamora et al., 2007) and a concurrent rise in $[Ca_i^{2+}]$. PKCδ and PKCε can also be activated rapidly in response to aldosterone, but this does not rely upon direct binding of the hormone to the kinases but instead is coupled to MR through EGFR trans-activation (McEneaney et al., 2008). Protein kinase D isoform 1 (PKD1) is rapidly activated in response to aldosterone and is a substrate for the non-classical, Ca^{2+}-independent PKC isoforms (nPKCs) such as PKCδ and PKCε. The aldosterone-induced activation of PKD1 in M1-CCD cells follows the same kinetics as does aldosterone-induced nPKC isoform activation and is coupled to MR through EGFR transactivation (McEneaney et al., 2007, 2008). Rapid activation of PKD1 has been implicated in aldosterone-induced proliferation in M1-CCD cells (McEneaney et al., 2010a) and in the stimulation of hypertrophy in cardiac myocytes following aldosterone treatment (Tsybouleva et al., 2004).

In addition to steroid receptor-dependent and -independent activation of protein kinases, several studies have demonstrated direct activation of different PKC isoforms by a wide variety of steroid hormones. The first evidence of direct activation of specific PKC isoforms (PCKα, PKCγ and the novel PKCε) by a steroid hormone was demonstrated for 1,25(OH)2-vitamin D3 by Slater et al. (1995). This direct 'in vitro' stimulatory effect on protein kinases has been shown for other hormones such as aldosterone, estrogen (Alzamora et al., 2007; Doolan et al., 2000) and glucocorticoids (for review see (Alzamora and Harvey, 2008)). These direct effects appear additive to the stimulatory effects of diacylglycerol and phorbol esters and require an intact C2 binding domain. These findings raise the interesting and controversial possibility that PKC isoforms may act as receptors for non-genomic transduction of

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certain rapid responses to steroid hormones additional to the activation of PKC isoforms by DAG and phospholipase C via membrane receptors.

2.3. Secondary messengers: calcium and cAMP

Aldosterone promotes the activation of multiple secondary messenger responses including a rise in $[Ca_i^{2+}]$, cyclic adenosine monophosphate (cAMP) biosynthesis and nitric oxide (NO) release. Aldosterone raised $[Ca_i^{2+}]$ in renal CCD (Harvey and Higgins, 2000), in isolated colonic crypts (Maguire et al., 1999), VSMCs (Wehling et al., 1994) and in the brain, preferentially in the ventral hippocampus over the dorsal hippocampus (Maggio and Segal, 2010). The regulatory mechanism and route of the $[Ca_i^{2+}]$ increase in the nephron and colon is not defined; however, the Ca^{2+} response was insensitive to spironolactone in CCD cells and sustained by Ca^{2+} entry from outside of the cell and PKC-dependent in colonic crypts (Doolan et al., 1998). Aldosterone enhanced a tetanic stress response in hippocampal cells by stimulating Ca^{2+} entry through nifedipine-sensitive, L-type calcium channels (Maggio and Segal, 2010). The dorsal and ventral hippocampus express MR but the nature of the initiating receptor for the Ca^{2+} response is not yet confirmed. The PLC/PKC-dependent activation of L-type calcium channels is required to elicit vasoconstriction within 5 min of aldosterone treatment in the afferent arterioles of the renal micro-circulation, while stimulation of vasoconstriction in efferent arterioles is mediated by aldosterone-induced activation of T-type Ca^{2+} channels (Hayashi et al., 2003).

The interplay between rapid aldosterone effects and cAMP signalling as expressed through cAMP response element binding protein (CREB)-dependent transcription differs between tissues. Aldosterone stimulated an increase in intracellular cAMP within 1 min and CREB phosphorylation within 5 min in VSMCs (Christ et al., 1999). In HEK-293 cells, aldosterone treatment suppressed CREB-dependent transcription through the stimulation of calcineurin/protein phosphatase 2B (PP2B) activity (Grossmann et al., 2010b). It is unclear whether aldosterone had a rapid effect on basal CREB phosphorylation in the HEK-293 cells; however, pre-incubation with aldosterone for 20 min was sufficient to suppress the CREB induction by forskolin.

2.4. Secondary messenger: nitric oxide

Nitric oxide (NO), a gaseous molecule synthesized in the vasculature by the endothelial nitric oxide synthase (eNOS) is a key regulator of vascular tone. In smooth muscle cells, NO activates soluble guanylyl cyclase which via cGMP, phosphorylates the myosin light chain kinase and Ca^{2+}-ATPase, thereby inducing vasodilation. Vascular endothelium exposed to aldosterone shows a decreased synthesis and release of NO (Hashikabe et al., 2006; Nagata et al., 2006; Nishizaka et al., 2004). However, other reports show that aldosterone induces an acute increase in NO bioavailability in endothelial cells. Short-term treatment with aldosterone enhanced ATP-induced NO production in endothelial cells, along with an increase in the phosphorylation of eNOS, in an MR- and phosphoinositol 3-kinase (PI3K)-dependent manner (Mutoh et al., 2008).

Aldosterone induces the rapid induction of either vasoconstriction or vasodilation, depending on the bioavailability of endogenous nitric oxide (NO) (Arima et al., 2004; Schmidt et al., 2003, 2006; Uhrenholt et al., 2003). Aldosterone infused into the brachial artery of healthy male volunteers decreased blood flow significantly within 4 min compared with the contralateral forearm, indicating rapid vasoconstrictor responses; this effect was not sustained and flow returned to baseline after 30 min (Romagni et al., 2003). Similarly, aldosterone induced vasoconstriction in microperfused rabbit afferent arterioles through the activation of PLC and Ca^{2+} mobilization, and this response was spironolactone insensitive (Arima et al., 2003), and was modulated by NO (Arima et al., 2004). On the other hand, aldosterone-induced vasodilation has also been described in both rodents and humans (Liu et al., 2003; Uhrenholt et al., 2003). Aldosterone counteracted K^+-induced vasoconstriction within 2–5 min in microperfused rabbit renal afferent arterioles, an effect which was dependent on MR, and inhibition of NO formation by L-NAME restored K^+-induced vasoreactivity (Uhrenholt et al., 2003). Similarly, aldosterone counteracted phenylephrine-induced vasoconstriction in rat aortic rings, while a dose-dependent enhancement of the vasoconstriction response was induced by aldosterone in endothelial-denuded vessels (Liu et al., 2003). In the same study, the authors demonstrated that in cultured endothelial cells, aldosterone induced a PI3K-dependent increase in nitric oxide synthase activity as well as a PI3K-dependent activation of ERK1/2 and p70/S6 kinase (Liu et al., 2003). NO can modulate intracellular signalling cascades by acting on a variety of kinases and G protein-coupled receptors (Iwakiri et al., 2006; Rizzo and Piston, 2003; Ushio-Fukai, 2009). For example, shear stress-induced NO release leads to an S-nitrosylation of several proteins including ER-ATPase, Hsp90, and tubulin-β chain (Huang et al., 2009).

In chronic diseases such as hypertension and diabetes mellitus, reactive oxygen species (ROS) are generated, which uncouple eNOS from NO production and divert eNOS to superoxide generation (Forstermann and Li, 2010). Aldosterone-induced renal injury is mediated by ROS generation through NADPH oxidase-dependent mechanisms (Nishiyama and Abe, 2006). Aldosterone exerts negative effects on the cardiovascular system through the production of ROS. Aldosterone increased the expression of the NADPH oxidase subunits p22phox and gp91phox in the aorta, leading to an increase in ROS (Calo et al., 2004; Hirono et al., 2007). Aldosterone also induced a rapid non-genomic activation of NADPH oxidase, resulting in an induction of apoptosis in neonatal rat cardiac myocytes (Hayashi et al., 2008). Fig. 2 depicts a summary of aldosterone-induced actions in the vasculature.

3. Crosstalk between rapid and genomic responses

3.1. Post-translational modulation of receptors and coactivators

Aldosterone-induced transcription is subject to modulation and potentiation by rapidly activated signalling cascades. Aldosterone stimulates the expression of type-I, -III and -IV collagens after 36 h in renal fibroblasts; an effect that is inhibited by MR and ERK1/2 antagonism, even though ERK1/2 activation is detected within 5 min (Nagai et al., 2005). The impact of rapid signalling events may be through direct phosphorylation of MR, phosphorylation of co-factors required for transcription initiation by MR or phosphorylation of factors that initiate transcription at nuclear receptor-independent promoters. Steroid receptors have multiple phosphorylation sites; Ser118 of ERα is phosphorylated in response to ERK1/2 activation in breast carcinoma and stabilizes ERα in the nucleus (Kato et al., 1995). The progesterone receptor is also phosphorylated by ERK1/2 after 5 min progestin treatment and this leads to the recruitment of factors involved in chromatin remodelling (Vicent et al., 2006). The glucocorticoid receptor GRα is phosphorylated by MAPKs, cyclin-dependent kinases and GSK-3 (glycogen synthase kinase 3) (Oakley and Cidlowski, 2011) and phosphorylation-deficient GRα mutants were compromised in their ability to activate reporter genes in a promoter-dependent fashion (Webster et al., 1997). Phosphorylation also modulates the subcellular trafficking of GRα; phosphorylation at Ser-203 promotes the cytoplasmic retention of the receptor and thus results

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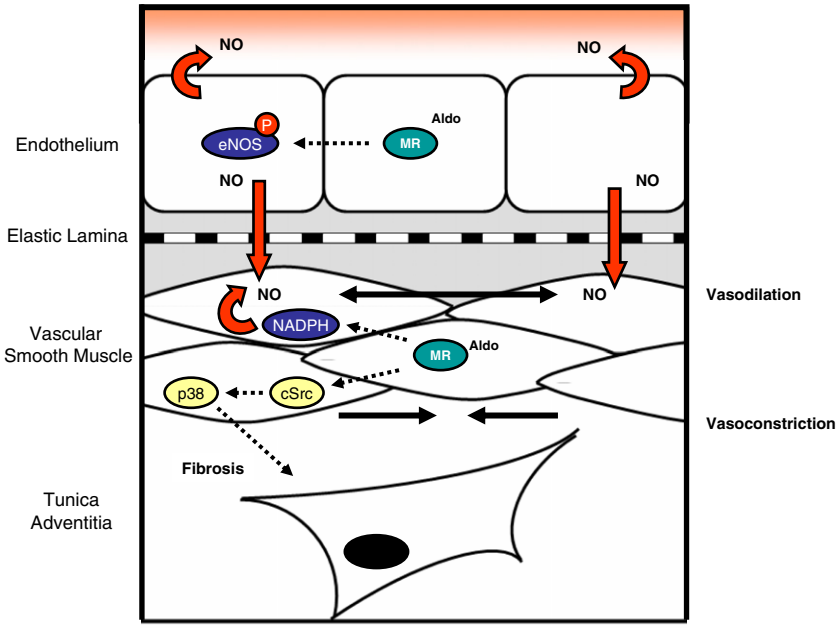

Fig. 2. Rapid aldosterone actions and vascular tone. Aldosterone elicits multiple rapid actions on the vascular endothelium and vascular smooth muscle cell (VSMC) layer to regulate vascular tone. MR-coupled phosphorylation of endothelial nitric oxide synthase (eNOS) by an as yet unidentified kinase stimulates nitric oxide (NO) release that acts on the VSMCs to promote vasodilation in synergy with the delayed transcriptional up-regulation of NADPH oxidase. Endothelial denudation followed by aldosterone treatment promotes vasoconstriction, suggesting that aldosterone may act to restrict blood flow in damaged vessels. Aldosterone treatment promotes rapid activation of c-Src and p38 mitogen activated kinase in VSMCs which chronically results in fibrosis of the vessel wall.

in a diminished recruitment to glucocorticoid-responsive target genes (Blind and Garabedian, 2008). The rapid phosphorylation of MR following aldosterone treatment has been described; however, the role of receptor phosphorylation in regulating MR localization and transcriptional activity is undetermined (Le Moellic et al., 2004). PKA inhibition blocks the dissociation of Hsp90 from MR that precedes nuclear accumulation of the receptor (Massaad et al., 1999) and p21 activated kinase activation augments MR nuclear-association (Shibata et al., 2008).

The p160 family of steroid receptor co-activators (SRCs) SRC1, SRC2 (TIF2) and SRC3 (AIB1) are selectively recruited to sites of transcription initiation by nuclear receptors. The phosphorylation state of these co-activators at multiple amino acid residues influences their association with nuclear receptors, the recruitment of other co-factors and co-activator resistance to degradation. Estradiol-induced SRC-3 phosphorylation is dependent on a direct interaction between SRC-3 and ERα (Zheng et al., 2005), and ERK1/2 phosphorylation was implicated in regulating the localization of SRC-3 and its interaction with ERα (Amazit et al., 2007). Aldosterone-stimulated kinases may also phosphorylate the SRCs. PKA phosphorylates SRC2, while SRC3 is a substrate for p38; the effect of these specific phosphorylation events is to promote ubiquitination and turnover of the SRCs (Gianni et al., 2006; Hoang et al., 2004).

3.2. Genomic induction of rapid signalling intermediates

The expression of crucial signalling intermediates, including some of those that are integral to the cascades rapidly activated by aldosterone are subject to modulation by MR. Aldosterone treatment promoted the expression of EGFR in aorta smooth muscle cells, rendering the cells more sensitive to EGF (Grossmann et al., 2007). EGFR is also a signalling hub for cascades rapidly induced by aldosterone (Grossmann et al., 2005; McEneaney et al., 2007) and enhanced expression of EGFR may serve to amplify these rapid responses. Aldosterone also induces the expression of the serum and glucocorticoid-induced kinase (SGK-1) (Naray-Fejes-Toth and Fejes-Toth, 2000). SGK-1 regulates the cell surface expression of the epithelial sodium channel, ENaC by phosphorylating the E3 ubiquitin ligase Nedd4-2, thus preventing the ubiquitination and degradation of the ENaC channel (Debonneville et al., 2001; Snyder et al., 2002). PDK1 phosphorylates SGK1 in the activation loop (Biondi et al., 2001) and the fully activated kinase is then recruited by glucocorticoid-induced leucine zipper (GILZ) to substrates that are associated with ENaC, such as Nedd 4-2 (Soundararajan et al., 2009). The convergence between the rapid signalling and transcriptional responses coupled to the interaction of aldosterone with MR thus occurs at multiple levels, and contributes to the precise regulation of mineralocorticoid-sensitive physiology.

3.3. Aldosterone and microRNAs

microRNAs (miRNAs) are endogenous small non-coding RNA molecules with the ability to repress gene expression and are believed to play an important role in development, differentiation, proliferation, survival and oncogenesis (Inui et al., 2010). Pre-miRNA precursor transcript and mature miRNA can be modulated within minutes by transcription factors such as CREB, which are known targets of rapid responses to steroid hormones. Although this research is in its infancy, miRNAs represent a novel class of molecules rapidly activated by steroid hormones. microRNA expression in the kidney has been shown to be modulated by aldosterone, in particular miR-192 which regulates WNK1 (with no lysine kinase 1) expression, was down-regulated by aldosterone, sodium depletion or potassium loading (Elvira-Matelot et al., 2010). Moreover, the post-transcriptional regulation of MR gene expression was shown to be modulated by miR-124 and miR-135a (Sober et al., 2010). Taken together, these results suggest a miRNA-driven mechanism of gene modulation by aldosterone, involved in the control of sodium and potassium balance by the kidney, and therefore in blood pressure regulation.

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4. Membrane targets of aldosterone and mechanisms of regulation by rapid signalling events

4.1. Na^+/H^+ exchanger (NHE)

The nine isoforms of the Na^+/H^+ exchanger family (NHE1-9) regulate intracellular pH (pHi) via electroneutral exchange of intracellular H^+ for extracellular Na^+ and play central roles in cell volume regulation, initiation of cell growth and proliferation (Aronson, 1985; Frelin et al., 1990; Little et al., 1986). In polarized epithelia, NHE1 is expressed basolaterally and is mainly involved in cytoplasmic pH and volume regulation, whereas NHE3 is expressed apically and mediates $NaHCO_3$ and NaCl reabsorption. Aldosterone regulates the activity of NHE isoforms through various mechanisms (Fig. 3). In cells of the amphibian kidney, aldosterone rapidly activated Na^+/H^+ exchange to promote cytoplasmic alkalinization within 20 min (Oberleithner et al., 1987). The aldosterone-dependent rise in intracellular pH (pHi) associated with activation of NHE in MDCK cells is dependent upon ERK1/2 activation and a rapid 3-fold increase in $[Ca_i^{2+}]$, within 1 min of aldosterone treatment (Gekle et al., 2001, 1996). In another study, aldosterone induced a concentration-dependent increase in pHi recovery from an acid load within 5 min in M1-CCD cells, and this effect was attenuated by inhibiting PKCα or MAPK activity (Markos et al., 2005).

Aldosterone induced activation of NHE1 in rat distal colonic crypts, independently of MR but dependent on activation of a G protein-coupled receptor (Winter et al., 1999).

Aldosterone regulates NHE activity in VSMC through both rapid and genomic actions (Ebata et al., 1999). In cultured VSMCs, long-term exposure to aldosterone resulted in a 3-fold increase in NHE1 mRNA levels, whereas short-term aldosterone treatment resulted in a significant increase in NHE activity, which was insensitive to inhibitors of transcription/translation. Aldosterone also rapidly activated PKC within 5 min and this contributed to both the rapid and transcriptional effects of aldosterone on NHE activity (Ebata et al., 1999). The rapid activation of NHE was inhibited by disruptors of microtubules and filamentous actin, outlining the crucial role of cytoskeletal components in the induction of NHE activity and pointing towards a trafficking-based regulatory mechanism.

In the renal proximal tubule, 60–70% of filtered NaCl is reabsorbed; the main transporters involved are the apically expressed NHE3 and basolateral Na^+/K^+ATPase. The regulation of NHE3 is crucial for the maintenance of Na^+ balance, extracellular fluid volume, blood pressure, and acid–base homeostasis. Early studies discovered that aldosterone enhanced proximal tubule NaCl and fluid reabsorption in rats, in a spironolactone-sensitive manner (Stolte et al., 1969). Subsequent studies in adrenalectomized rats found that this was due to elevated NHE3 abundance in brush border membranes, which occurred without increasing gene expression (Krug et al., 2003). A similar response in primary human renal proximal tubule epithelial cells was dependent on EGFR activity (Drumm et al., 2006). In contrast, aldosterone-mediated inhibition of NHE3 has also been demonstrated. Aldosterone exposure for 15 min resulted in a 30% decrease in apical NHE3 activity in renal medullary thick ascending limb (MTAL), resulting in decreased transepithelial HCO_3^- absorption (Good et al., 2002, 2006). The aldosterone-mediated inhibition of NHE3 was mediated via MR-independent ERK1/2 signalling (Watts et al., 2006). This contrasts with other experimental systems where ERK1/2 activation by aldosterone is MR-dependent. How ERK1/2 signalling regulates NHE activity is unclear. Regulation of NHE3 in other cell systems involves trafficking between the plasma membrane and intracellular vesicles (Moe, 1999) and a role for ERK1/2 signalling in regulating intracellular trafficking of membrane proteins has been described (Giovannardi et al., 2002; Huang et al., 2003). However, ERK1/2 may also regulate NHE activity through direct phosphory-

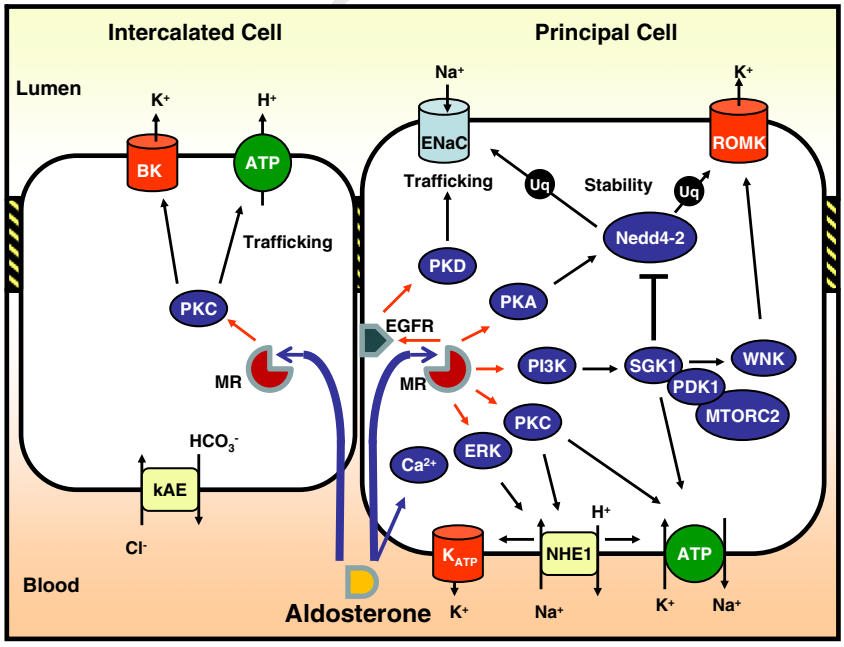

Fig. 3. Schema of rapid actions of aldosterone on ion transporters in intercalated and principal cells of the renal collecting duct. Aldosterone activates the trafficking of H^+ATPase pumps in intercalated cells and ENaC subunits in principal cells via rapid protein kinase signalling which is transduced by the mineralocorticoid receptor (MR). H^+ATPase and anion exchanger (kAE) activity and expression levels are also modulated by whole animal acid/base status. K^+ secretion is mediated via large conductance calcium-activated K^+ channels (BK) in intercalated cells and via inwardly-rectifying small conductance K^+ channels (ROMK) in principal cells. ROMK is regulated via aldosterone-mediated SGK-1 activity. Ubiquitination of ENaC via SGK-1 inhibition of Nedd4-2 stabilizes ENaC in the apical membrane. SGK-1 activation requires the combined association with, and phosphorylation by, mammalian target of rapamycin with rictor (mTORC2) and PDK1. Aldosterone-MR transactivation of EGFR activates protein kinase D to stimulate rapid trafficking of ENaC subunits to the membrane. The rapid stimulation by aldosterone of basolateral membrane Na^+/K^+ ATPase, Na^+/H^+ exchange and K_{ATP} channels ensures covariant 'cross-talk' regulation of all transporters required to sustain the transepithelial reabsorption of Na^+.

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lation of the exchanger or the phosphorylation of interacting proteins such as Na^+/H^+ exchange regulatory factor (NHERF)-1/2.

In another study on the effects of aldosterone on NHE3 activity, the authors described an overlap between long-term genomic responses and acute rapid responses. They showed increases in cell surface expression of NHE and Na^+/K^+ATPase α-subunit after 1 nM aldosterone treatment in human intestinal Caco-2BBE monolayers, with overall expression levels increasing after 4 h (Musch et al., 2008). Serum and glucocorticoid regulated kinase (SGK)-1 and PI3K were rapidly activated by aldosterone and aldosterone-induced NHE3 gene promoter activity was inhibited by PI3K inhibition or SGK-1 silencing (Musch et al., 2008). This study elegantly outlines the synergism between aldosterone-mediated long-term genomic effects and the preceding rapid signalling effects and that both levels of effects cannot easily be separated. More recently, acute stimulation of placental tissue with 10 nM aldosterone resulted in a spironolactone-sensitive rapid regulation of NHE activity as seen by an increased rate of pHi recovery from an acid load (Speake et al., 2010). Interestingly, this effect was only present in placental tissue derived from female infants and was absent in that of male infants, introducing a gender-specific difference in aldosterone-mediated rapid responses.

4.2. H^+-ATPase

Aldosterone stimulates urinary acidification through stimulation of H^+ flux through H^+-ATPase pumps. These responses were first described in detail in turtle urinary bladder (Al-Awqati et al., 1976) and frog skin (Ehrenfeld and Garcia-Romeu, 1977). Proton pumps were shown to be localised to apical cell membranes of mitochondria-rich cells whose number and morphology were altered by aldosterone treatment. Whole-cell patch-clamp recordings in these cells revealed that aldosterone produced a rapid exocytotic insertion of H^+ pumps into luminal membranes within 10 min, which was sensitive to PKC inhibitors and disruptors of the cytoskeleton (Harvey, 1992). In the kidney, acid–base regulation is controlled in the distal nephron through the reabsorption of bicarbonate and the release of H^+ into the renal ultrafiltrate. The vacuolar H^+-ATPase, expressed apically in type A intercalated cells of the collecting duct, actively mediates H^+ secretion. Aldosterone plays a key role in the regulation of the renal H^+-ATPase pump and many facets of this regulation are governed by rapid signalling events (Fig. 3). For example, in outer medullary collecting ducts of mouse kidney, exposure to 10 nM aldosterone for 15 min resulted in an MR-dependent increase in H^+ extrusion from acid-loaded type A intercalated cells (Winter et al., 2004). Interestingly, similar to the response in frog skin, the increase in H^+-ATPase activity was dependent on Ca^{2+}-induced PKC activity and blocked by colchicine, indicating an involvement of the microtubule network (Winter et al., 2004). Furthermore, aldosterone-injected mice showed increased apical expression of H^+-ATPase in type A intercalated cells (Winter et al., 2004), supporting the idea of aldosterone-regulated trafficking of the H^+-ATPase as a means to control acid–base homeostasis.

A recent study demonstrated that aldosterone invoked both rapid and genomic stimulatory effects on the H^+-ATPase in isolated proximal tubules of rat kidney (Leite-Dellova et al., 2010). Here, after 2 min of aldosterone pre-incubation, a significant increase was observed in the intracellular pH recovery rate from an acid load, and a transient increase in $[Ca_i^{2+}]$ was observed after 1 min aldosterone. These effects were MR-independent as shown by their insensitivity to spironolactone and were also not dependent on transcription/translation. After 6 min aldosterone, a further increase in $[Ca_i^{2+}]$ occurred and this persisted after 1 h. This later effect was MR- and transcription/translation-dependent (Leite-Dellova et al., 2010).

4.3. K^+ channels

In the principal cells of the collecting duct, K^+ enters the cell via the basolateral Na^+/K^+ATPase and is secreted into the lumen through apical K^+ channels, along a favourable electrochemical gradient (O'Neil and Sansom, 1984). The renal outer medullary K^+ channel (ROMK) is the principal K^+ secreting channel in the kidney and is expressed apically along the aldosterone-sensitive distal nephron (ASDN) (Kohda et al., 1998). ROMK mediates apical K^+ recycling in the thick ascending limb (TAL) and net K^+ secretion by ASDN cells in the connecting segment and CCD (Aguilar-Bryan et al., 1998; Hebert et al., 2005). Aldosterone regulates ROMK function mainly through the actions of SGK-1 activity (Fig. 3). Cell surface expression of ROMK was found to be regulated by aldosterone-induced SGK-1 activity (Yoo et al., 2003). Co-expression of SGK-1 and the scaffolding protein NHERF2 with ROMK1 increased K^+ channel activity through an increase in membrane abundance (Yun et al., 2002). NHERF-1 and NHERF-2 each contain 2 PDZ (protein–protein interaction) domains; ROMK preferentially associates with the second PDZ domain of NHERF-1 and the first PDZ domain of NHERF-2 (Yoo et al., 2004). The association with NHERF scaffolding proteins increases surface abundance of ROMK and also increases the interaction between ROMK and CFTR (Yoo et al., 2004). CFTR was found to be required for the PKA-regulated ATP sensitivity of ROMK in murine TAL (Lu et al., 2006). SGK-1 can also stimulate ROMK activity by the phosphorylation of WNK4 (with no lysine (K)) kinase (Ring et al., 2007). Mutations in WNK4 cause pseudohypoaldosteronism type II (PHAII), a disease featuring increased renal NaCl reabsorption and impaired K^+ secretion. PKC-induced phosphorylation of ROMK was required for trafficking of ROMK1 to the cell membrane in HEK293 cells (Lin et al., 2002). PKC was also shown to inhibit ROMK activity, through a PIP2-dependent mechanism (Zeng et al., 2003). Here, the interaction between PIP2 and ROMK was required for channel opening and a reduction in membrane PIP2 levels contributed to the inhibition of ROMK1 by PKC.

In the CCD, K^+ can also enter the cell via basolateral K^+ channels, if the basolateral membrane hyperpolarizes to exceed the K^+ equilibrium potential (Wang and Giebisch, 2009). This may occur as a consequence of mineralocorticoid-induced stimulation of the Na^+/K^+ATPase (Sansom and O'Neil, 1986). Aldosterone rapidly (within 15 min) stimulated the activity of ATP-dependent K^+ channel (K^+_{ATP}) activity in A6 amphibian renal principal cells, by modulating the open probability of the channel (Urbach et al., 1996). The mammalian colon is a major target of aldosterone action, with levels of MR expression observed at even higher levels than in the kidney (Fuller and Verity, 1990; Will et al., 1980). In the distal colon, aldosterone induces the apical expression of ENaC and the basolateral expression of Na^+/K^+ATPase, thus inducing a switch from electroneutral NaCl absorption to stimulated electrogenic Na^+ absorption (Binder et al., 1989; Kunzelmann and Mall, 2002). Here, aldosterone also induces apical K^+ channels, resulting in a switch from net K^+ absorption to net K^+ secretion (Sweiry and Binder, 1989). Aldosterone mediated the non-genomic inhibition of Ca^{2+}-dependent intermediate conductance K^+ channels (IK_{Ca}) in the basolateral membranes of human colonic crypt cells, and this involved stimulation of Na^+/H^+ exchange (Bowley et al., 2003). This effect was later found to be dependent on PKC activity, whereby the inhibition of IK_{Ca} was blocked using PKC inhibitors (chelerythrine chloride and Go 6976) and IK_{Ca} activity was rapidly decreased within 10 min of addition of PMA (a PKC activator) (Bowley et al., 2007). Aldosterone activated basolateral Na^+/H^+ exchange via a PKC- and Ca^{2+}-dependent signalling pathway; the resultant intracellular alkalinization up-regulated K^+_{ATP} channel and inhibited a K^+_{Ca} channel (Maguire et al., 1999). These effects

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were MR-independent and were insensitive to inhibitors of transcription/translation.

4.4. ENaC

The ENaC channel, thought to be a heterotrimer composed of 1α, 1β and 1γ subunit (Jasti et al., 2007), is expressed apically in absorptive epithelia, including the ASDN. Here the basolateral Na^+/K^+ATPase provides the main electrochemical driving force for ENaC-mediated Na^+ reabsorption and the rate of Na^+ reabsorption is determined by ENaC cell surface abundance and open probability. Aldosterone is a central regulator of Na^+ reabsorption in the ASDN, through the stimulation of both ENaC and Na^+/K^+ATPase activities. Aldosterone induces ENaCα expression in the distal nephron and ENaCβ and ENaCγ in the colon, through MR-dependent transcription. Aldosterone also has many indirect effects on the expression, stability and trafficking of the channel (Fig. 3). Cell surface ENaC can be targeted for degradation by the proteasome by the action of the E3 ubiquitin ligase Nedd4-2 (Goulet et al., 1998), which interacts with ENaC via a C-terminal PY internalization motif. Interestingly, an inherited form of hypertension, Liddle Syndrome, is defined by a defect in the interaction between Nedd4-2 and ENaC caused by a mutation/deletion in the PY motifs of ENaCβ or ENaCγ (Shimkets et al., 1994). The result is the increased membrane abundance of ENaC. Nedd4-2 is phosphorylated by SGK-1, both *in vitro* and *in vivo* and this phosphorylation leads to a disruption in the interaction between Nedd4-2 and ENaC, thereby increasing the surface residency time of ENaC, resulting in increased Na^+ transport (Debonneville et al., 2001; Snyder et al., 2004, 2002). Aldosterone induces the MR-mediated upregulation of SGK-1 mRNA expression in the distal nephron of rat kidney within 30 min (Bhargava et al., 2001) the earliest transcriptional response of aldosterone. In this way aldosterone indirectly regulates the rate of Na^+ absorption by modulating the transcription of the rapidly-acting kinase SGK-1. Moreover, SGK-1 is also required for the aldosterone-mediated upregulation in activity of the Na^+/K^+ATPase, which provides the electrochemical driving force for Na^+ reabsorption.

SGK1 activity, as is the case for the related serine–threonine kinase PKB/Akt, is dependent on phosphorylation at two serine residues by phosphoinositide-dependent protein kinase (PDK1), an effector of PI3K signalling (Kobayashi and Cohen, 1999). SGK-1 activity is therefore blocked by PI3K inhibitors and is dependent on PIP3, (a phosphoinositide generated when PI3K phosphorylates PIP2 at the 3′ position), for complete activation (Kobayashi and Cohen, 1999; Park et al., 1999). Interestingly, in a mouse CCD cell line, aldosterone induced PIP3 production in the plasma membrane and PIP3 was found to mediate aldosterone stimulation of ENaC (Helms et al., 2005), suggesting an interplay between the activation of SGK-1 via PI3K-mediated PDK-1 activation and the lipid product of PI3K activity, PIP3, which could be involved in SGK-1 membrane recruitment. Recently, the phosphorylation-induced activation of the hydrophobic motif domain of SGK1 has been shown to be dependent upon association with mTOR including rictor (mTORC2), which then permits interaction and phosphorylation with PDK1 and the activation of ENaC (Lu et al., 2010).

Work from our own laboratory has shown that the novel protein kinase D1 (PKD1) plays a crucial role in the regulation of ENaC. Aldosterone rapidly activated PKD1 within 5 min in a murine CCD cell line, in an MR- and EGFR-dependent manner (McEneaney et al., 2007). This activation was found to be required for the aldosterone-mediated rapid trafficking of CFP-tagged ENaC subunits (McEneaney et al., 2008) and for the apical membrane expression and activity of endogenous ENaC subunits, an effect observed after chronic aldosterone treatment (McEneaney et al., 2010b). PKD1 is a member of a family of proteins (PKD1, 2 and 3) with a multitude of functions, including the regulation of post-Golgi trafficking events (Rykx et al., 2003; Van Lint et al., 2002). PKD1 phosphorylates phosphatidylinositol 4-kinase (PI4K) at the Golgi complex, resulting in the upregulation of vesicle fission from the *trans* Golgi network to the plasma membrane (Hausser et al., 2005). Therefore, aldosterone may regulate fission events at the Golgi complex, so up-regulating the rate of ENaC translocation to the plasma membrane.

Members of the Ras superfamily of small GTPases have emerged as key regulators of vesicular transport. These molecular switches cycle between GDP- and GTP-bound forms, as regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Aldosterone induces K-RasA expression and activity, promoting ENaC open probability via a PI3K signalling pathway (Staruschenko et al., 2004). Aldosterone promotes the interaction between K-RasA and PI3K, and K-RasA interacts with ENaC (Staruschenko et al., 2005), highlighting the dual role of K-RasA acting as both a molecular scaffold, bringing PI3K in close proximity to ENaC, and as an activator of PI3K. Another member of the Ras superfamily, RhoA, also plays a central role in ENaC regulation. RhoA rapidly increases ENaC membrane levels via Rho-kinase and PI(4)P5-kinase activation, and the resulting increases in PIP2 levels likely promote ENaC plasma membrane insertion (Pochynyuk et al., 2006). Aldosterone promotes the rapid activation of Rho kinase within 10 min in mesangial cells, resulting in hypertrophy and increased actin polymerization (Diah et al., 2008). Moreover, VSMC remodelling induced by aldosterone was mediated via Rho kinase activation (Miyata et al., 2008). Rho GTPases and their associated kinases are well known to be important regulators of cytoskeleton structure, and consequently play an important role in subcellular vesicle trafficking. Total internal reflection (TIRF) microscopy and fluorescence recovery after photobleaching (FRAP) analysis showed that RhoA accelerates the rate of ENaC trafficking to the plasma membrane, through effects on microtubules (Pochynyuk et al., 2007). Aldosterone increased the expression and phosphorylation of the Rab-GAP, AS160, in CCD epithelia, and these phosphorylation sites were found to overlap with SGK-1 substrate sites (Liang et al., 2010). Aldosterone induced an increase in apical ENaC localization in AS160-over-expressing epithelia, and in the absence of aldosterone, AS160 over-expression increased total ENaC expression without affecting surface abundance or activity. AS160 thus stabilizes ENaC in intracellular compartments under basal conditions, while aldosterone-dependent AS160 phosphorylation facilitates ENaC forward trafficking (Liang et al., 2010).

Aldosterone stimulates the expression of the small chaperone protein, GILZ (glucocorticoid-induced leucine zipper protein 1) in renal CCDs (Robert-Nicoud et al., 2001). GILZ is a component of the ENaC regulatory signalling complex found to selectively modulate the cell surface expression of ENaC (Soundararajan et al., 2009). The inhibitory components of this complex, Raf-1 and Nedd4-2, interact with ENaC and decrease the cell surface abundance of this channel. The aldosterone-stimulated components of the ENaC regulatory complex, SGK-1 and GILZ, cooperatively inhibit the activities of Raf-1 and Nedd4-2 and therefore synergistically increase ENaC cell surface expression (Soundararajan et al., 2009). Moreover, GILZ1 inhibits the ubiquitinylation of SGK-1 and its subsequent proteasome-mediated degradation, thereby prolonging its half-life and increasing its steady-state expression (Soundararajan et al., 2010).

Rho family members and their regulatory proteins are involved in the trans-activation of several steroid receptors (Kino et al., 2006; Rubino et al., 1998; Su et al., 2001). Constitutive over-expression of Rac1, a member of the Rho family GTPases, induced an up-regulation in MR nuclear translocation and MR-dependent transcription, whereas constitutively active RhoA suppressed

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aldosterone-stimulated reporter activity (Shibata et al., 2008). This study provides a clear example of one of the possible routes of cross-talk between intracellular signalling cascades and MR-mediated transcription.

5. Conclusion

Steroid hormones such as aldosterone induce rapid effects independent of *de novo* protein synthesis in numerous target tissues and these effects play a crucial role in the fine-tuning of latent genomic responses to the hormone. Aldosterone-mediated rapid signalling effects such as the activation of multiple kinase cascades allows for the dynamic regulation of transcriptional events through the phosphorylation of the mineralocorticoid receptor itself, of coactivators or direct phosphorylation of the target proteins themselves such as the various ion channels/transporters discussed.

The rapid responses to aldosterone are mediated either via the classical nuclear MR or through an as yet unidentified membrane MR. Interestingly, as opposed to the ER, AR and PR, MR lacks the conserved palmitoylation motif involved in the membrane anchoring of these receptors. Controversy still abounds on the identity of a membrane MR and its functional role in physiology, and further work is required to examine the membrane targeting of classical MR, which could occur either through a lipid modification of the receptor or through direct interactions with membrane scaffolding proteins. Some rapid non-genomic effects of aldosterone do not appear to require MR such as intracellular Ca^{2+} mobilization and specific protein kinase isoform activation. The activation of Ca^{2+} entry can occur within seconds and represents one of the earliest non-genomic responses to a wide range of steroid hormones. Some steroid hormones such as vitamin D, estrogen, glucocorticoids and aldosterone have been shown to directly activate specific protein kinase isoforms (PKCα, PKCζ, PKCδ) in cell-free systems raising the possibility that under certain conditions these kinases can act as receptors for steroid hormones. The question is still open if this type of 'in vitro' non-genomic signalling can occur in an intact cell, how its specificity to cell types can be conferred given the ubiquitous expression of these kinases and its importance to the physiological response to steroid hormones. A fast PKCα–Ca^{2+} response has been demonstrated for aldosterone and estrogen in CCD and colonic crypts. One possibility is the direct activation of PKCα as the missing-link receptor to produce the near instantaneous entry of Ca^{2+} through a microdomain localization and activation of the kinase and another as yet unidentified co-regulator (e.g. calmodulin kinase).

Aldosterone-induced rapid signalling effects modulate multiple membrane targets, either by directly affecting their activity, or indirectly through the modulation of MR-dependent transcription. A complex network of cross-talk exists between rapid and latent-induced effects and synergism between both pathways results in the ultimate fine-tuning of the physiological response to aldosterone.

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References

Acconcia, F., Ascenzi, P., Bocedi, A., Spisni, E., Tomasi, V., Trentalance, A., Visca, P., Marino, M., 2005. Palmitoylation-dependent estrogen receptor alpha membrane localization: regulation by 17beta-estradiol. Mol. Biol. Cell 16, 231–237.

Aguilar-Bryan, L., Clement, J.P.T., Gonzalez, G., Kunjilwar, K., Babenko, A., Bryan, J., 1998. Toward understanding the assembly and structure of KATP channels. Physiol. Rev. 78, 227–245.

Al-Awqati, Q., Norby, L.H., Mueller, A., Steinmetz, P.R., 1976. Characteristics of stimulation of H^+ transport by aldosterone in turtle urinary bladder. J. Clin. Invest. 58, 351–358.

Alzamora, R., Harvey, B.J., 2008. Direct binding and activation of protein kinase C isoforms by steroid hormones. Steroids 73, 885–888.

Alzamora, R., Brown, L.R., Harvey, B.J., 2007. Direct binding and activation of protein kinase C isoforms by aldosterone and 17beta-estradiol. Mol. Endocrinol. 21, 2637–2650.

Amazit, L., Pasini, L., Szafran, A.T., Berno, V., Wu, R.C., Mielke, M., Jones, E.D., Mancini, M.G., Hinojos, C.A., O'Malley, B.W., Mancini, M.A., 2007. Regulation of SRC-3 intercompartmental dynamics by estrogen receptor and phosphorylation. Mol. Cell. Biol. 27, 6913–6932.

Arima, S., Kohagura, K., Xu, H.L., Sugawara, A., Abe, T., Satoh, F., Takeuchi, K., Ito, S., 2003. Nongenomic vascular action of aldosterone in the glomerular microcirculation. J. Am. Soc. Nephrol. 14, 2255–2263.

Arima, S., Kohagura, K., Xu, H.L., Sugawara, A., Uruno, A., Satoh, F., Takeuchi, K., Ito, S., 2004. Endothelium-derived nitric oxide modulates vascular action of aldosterone in renal arteriole. Hypertension 43, 352–357.

Aronson, P.S., 1985. Properties of the renal Na^+–H^+ exchanger. Ann. NY Acad. Sci. 456, 220–228.

Asher, C., Wald, H., Rossier, B.C., Garty, H., 1996. Aldosterone-induced increase in the abundance of Na^+ channel subunits. Am. J. Physiol. 271, C605–C611.

Beesley, A.H., Hornby, D., White, S.J., 1998. Regulation of distal nephron K+ channels (ROMK) mRNA expression by aldosterone in rat kidney. J. Physiol. 509 (Pt 3), 629–634.

Bhargava, A., Fullerton, M.J., Myles, K., Purdy, T.M., Funder, J.W., Pearce, D., Cole, T.J., 2001. The serum- and glucocorticoid-induced kinase is a physiological mediator of aldosterone action. Endocrinology 142, 1587–1594.

Binder, H.J., McGlone, F., Sandle, G.I., 1989. Effects of corticosteroid hormones on the electrophysiology of rat distal colon: implications for Na^+ and K^+ transport. J. Physiol. 410, 425–441.

Biondi, R.M., Kieloch, A., Currie, R.A., Deak, M., Alessi, D.R., 2001. The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB. EMBO J. 20, 4380–4390.

Blind, R.D., Garabedian, M.J., 2008. Differential recruitment of glucocorticoid receptor phospho-isoforms to glucocorticoid-induced genes. J. Steroid Biochem. Mol. Biol. 109, 150–157.

Bowley, K.A., Morton, M.J., Hunter, M., Sandle, G.I., 2003. Non-genomic regulation of intermediate conductance potassium channels by aldosterone in human colonic crypt cells. Gut 52, 854–860.

Bowley, K.A., Linley, J.E., Robins, G.G., Kopanati, S., Hunter, M., Sandle, G.I., 2007. Role of protein kinase C in aldosterone-induced non-genomic inhibition of basolateral potassium channels in human colonic crypts. J. Steroid Biochem. Mol. Biol. 104, 45–52.

Callera, G.E., Montezano, A.C., Yogi, A., Tostes, R.C., He, Y., Schiffrin, E.L., Touyz, R.M., 2005. C-Src-dependent nongenomic signaling responses to aldosterone are increased in vascular myocytes from spontaneously hypertensive rats. Hypertension 46, 1032–1038.

Calo, L.A., Zaghetto, F., Pagnin, E., Davis, P.A., De Mozzi, P., Sartorato, P., Martire, G., Fiore, C., Armanini, D., 2004. Effect of aldosterone and glycyrrhetinic acid on the protein expression of PAI-1 and p22(phox) in human mononuclear leukocytes. J. Clin. Endocrinol. Metab. 89, 1973–1976.

Chen, C., Liang, W., Jia, J., van Goor, H., Singhal, P.C., Ding, G., 2009. Aldosterone induces apoptosis in rat podocytes: role of PI3-K/Akt and p38MAPK signaling pathways. Nephron Exp. Nephrol. 113, e26–e34.

Christ, M., Gunther, A., Heck, M., Schmidt, B.M., Falkenstein, E., Wehling, M., 1999. Aldosterone, not estradiol, is the physiological agonist for rapid increases in cAMP in vascular smooth muscle cells. Circulation 99, 1485–1491.

Debonneville, C., Flores, S.Y., Kamynina, E., Plant, P.J., Tauxe, C., Thomas, M.A., Munster, C., Chraibi, A., Pratt, J.H., Horisberger, J.D., Pearce, D., Loffing, J., Staub, O., 2001. Phosphorylation of Nedd4-2 by SGK1 regulates epithelial Na(+) channel cell surface expression. EMBO J. 20, 7052–7059.

Diah, S., Zhang, G.X., Nagai, Y., Zhang, W., Gang, L., Kimura, S., Hamid, M.R., Tamiya, T., Nishiyama, A., Hitomi, H., 2008. Aldosterone induces myofibroblastic transdifferentiation and collagen gene expression through the Rho-kinase dependent signaling pathway in rat mesangial cells. Exp. Cell Res. 314, 3654–3662.

Doolan, C.M., O'Sullivan, G.C., Harvey, B.J., 1998. Rapid effects of corticosteroids on cytosolic protein kinase C and intracellular calcium concentration in human distal colon. Mol. Cell. Endocrinol. 138, 71–79.

Doolan, C.M., Condliffe, S.B., Harvey, B.J., 2000. Rapid non-genomic activation of cytosolic cyclic AMP-dependent protein kinase activity and [Ca(2+)](i) by 17beta-oestradiol in female rat distal colon. Br. J. Pharmacol. 129, 1375–1386.

Drumm, K., Kress, T.R., Gassner, B., Krug, A.W., Gekle, M., 2006. Aldosterone stimulates activity and surface expression of NHE3 in human primary proximal tubule epithelial cells (RPTEC). Cell. Physiol. Biochem. 17, 21–28.

Ebata, S., Muto, S., Okada, K., Nemoto, J., Amemiya, M., Saito, T., Asano, Y., 1999. Aldosterone activates Na^+/H^+ exchange in vascular smooth muscle cells by nongenomic and genomic mechanisms. Kidney Int. 56, 1400–1412.

Ehrenfeld, J., Garcia-Romeu, F., 1977. Active hydrogen excretion and sodium absorption through isolated frog skin. Am. J. Physiol. 233, F46–F54.

Elvira-Matelot, E., Zhou, X.O., Farman, N., Beaurain, G., Henrion-Caude, A., Hadchouel, J., Jeunemaitre, X., 2010. Regulation of WNK1 expression by miR-192 and aldosterone. J. Am. Soc. Nephrol. 21, 1724–1731.

Forstermann, U., Li, H., 2010. Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing eNOS uncoupling. Br. J. Pharmacol..

Frelin, C., Vigne, P., Lazdunski, M., 1990. The Na^+/H^+ exchange system in vascular smooth muscle cells. Adv. Nephrol. Necker Hosp. 19, 17–29.

Fuller, P.J., Verity, K., 1990. Mineralocorticoid receptor gene expression in the gastrointestinal tract: distribution and ontogeny. J. Steroid Biochem. 36, 263–267.

Gekle, M., Golenhofen, N., Oberleithner, H., Silbernagl, S., 1996. Rapid activation of Na^+/H^+ exchange by aldosterone in renal epithelial cells requires Ca^{2+} and stimulation of a plasma membrane proton conductance. Proc. Natl. Acad. Sci. USA 93, 10500–10504.

Gekle, M., Freudinger, R., Mildenberger, S., Schenk, K., Marschitz, I., Schramek, H., 2001. Rapid activation of Na^+/H^+-exchange in MDCK cells by aldosterone involves MAP-kinase ERK1/2. Pflugers Arch. 441, 781–786.

Gianni, M., Parrella, E., Raska Jr., I., Gaillard, E., Nigro, E.A., Gaudon, C., Garattini, E., Rochette-Egly, C., 2006. P38MAPK-dependent phosphorylation and degradation of SRC-3/AIB1 and RARalpha-mediated transcription. EMBO J. 25, 739–751.

Giovannardi, S., Forlani, G., Balestrini, M., Bossi, E., Tonini, R., Sturani, E., Peres, A., Zippel, R., 2002. Modulation of the inward rectifier potassium channel IRK1 by the Ras signaling pathway. J. Biol. Chem. 277, 12158–12163.

Good, D.W., George, T., Watts 3rd, B.A., 2002. Aldosterone inhibits HCO absorption via a nongenomic pathway in medullary thick ascending limb. Am. J. Physiol. Renal Physiol. 283, F699–F706.

Good, D.W., George, T., Watts 3rd, B.A., 2006. Nongenomic regulation by aldosterone of the epithelial NHE3 Na(+)/H(+) exchanger. Am. J. Physiol. Cell Physiol. 290, C757–C763.

Goto, J., Otsuka, F., Yamashita, M., Suzuki, J., Otani, H., Takahashi, H., Miyoshi, T., Mimura, Y., Ogura, T., Makino, H., 2009. Enhancement of aldosterone-induced catecholamine production by bone morphogenetic protein-4 through activating Rho and SAPK/JNK pathway in adrenomedullar cells. Am. J. Physiol. Endocrinol. Metab. 296, E904–E916.

Goulet, C.C., Volk, K.A., Adams, C.M., Prince, L.S., Stokes, J.B., Snyder, P.M., 1998. Inhibition of the epithelial Na^+ channel by interaction of Nedd4 with a PY motif deleted in Liddle's syndrome. J. Biol. Chem. 273, 30012–30017.

Gros, R., Ding, Q., Sklar, L.A., Prossnitz, E.E., Arterburn, J.B., Chorazyczewski, J., Feldman, R.D., 2011. GPR30 expression is required for the mineralocorticoid receptor-independent rapid vascular effects of aldosterone. Hypertension.

Grossmann, C., Benesic, A., Krug, A.W., Freudinger, R., Mildenberger, S., Gassner, B., Gekle, M., 2005. Human mineralocorticoid receptor expression renders cells responsive for nongenotropic aldosterone actions. Mol. Endocrinol. 19, 1697–1710.

Grossmann, C., Krug, A.W., Freudinger, R., Mildenberger, S., Voelker, K., Gekle, M., 2007. Aldosterone-induced EGFR expression: interaction between the human mineralocorticoid receptor and the human EGFR promoter. Am. J. Physiol. Endocrinol. Metab. 292, E1790–E1800.

Grossmann, C., Husse, B., Mildenberger, S., Schreier, B., Schuman, K., Gekle, M., 2010a. Colocalization of mineralocorticoid and EGF receptor at the plasma membrane. Biochim. Biophys. Acta 1803, 584–590.

Grossmann, C., Wuttke, M., Ruhs, S., Seiferth, A., Mildenberger, S., Rabe, S., Schwerdt, G., Gekle, M., 2010b. Mineralocorticoid receptor inhibits CREB signaling by calcineurin activation. FASEB J. 24, 2010–2019.

Harvey, B.J., 1992. Energization of sodium absorption by the H(+)-ATPase pump in mitochondria-rich cells of frog skin. J. Exp. Biol. 172, 289–309.

Harvey, B.J., Higgins, M., 2000. Nongenomic effects of aldosterone on Ca^{2+} in M-1 cortical collecting duct cells. Kidney Int. 57, 1395–1403.

Hashikabe, Y., Suzuki, K., Jojima, T., Uchida, K., Hattori, Y., 2006. Aldosterone impairs vascular endothelial cell function. J. Cardiovasc. Pharmacol. 47, 609–613.

Hausser, A., Storz, P., Martens, S., Link, G., Toker, A., Pfizenmaier, K., 2005. Protein kinase D regulates vesicular transport by phosphorylating and activating phosphatidylinositol-4 kinase IIIbeta at the Golgi complex. Nat. Cell Biol. 7, 880–886.

Hayashi, K., Ozawa, Y., Fujiwara, K., Wakino, S., Kumagai, H., Saruta, T., 2003. Role of actions of calcium antagonists on efferent arterioles – with special references to glomerular hypertension. Am. J. Nephrol. 23, 229–244.

Hayashi, H., Kobara, M., Abe, M., Tanaka, N., Gouda, E., Toba, H., Yamada, H., Tatsumi, T., Nakata, T., Matsubara, H., 2008. Aldosterone nongenomically produces NADPH oxidase-dependent reactive oxygen species and induces myocyte apoptosis. Hypertens. Res. 31, 363–375.

Hebert, S.C., Desir, G., Giebisch, G., Wang, W., 2005. Molecular diversity and regulation of renal potassium channels. Physiol. Rev. 85, 319–371.

Helms, M.N., Liu, L., Liang, Y.Y., Al-Khalili, O., Vandewalle, A., Saxena, S., Eaton, D.C., Ma, H.P., 2005. Phosphatidylinositol 3,4,5-trisphosphate mediates aldosterone stimulation of epithelial sodium channel (ENaC) and interacts with gamma-ENaC. J. Biol. Chem. 280, 40885–40891.

Hirono, Y., Yoshimoto, T., Suzuki, N., Sugiyama, T., Sakurada, M., Takai, S., Kobayashi, N., Shichiri, M., Hirata, Y., 2007. Angiotensin II receptor type 1-mediated vascular oxidative stress and proinflammatory gene expression in aldosterone-induced hypertension: the possible role of local renin-angiotensin system. Endocrinology 148, 1688–1696.

Hoang, T., Fenne, I.S., Cook, C., Borud, B., Bakke, M., Lien, E.A., Mellgren, G., 2004. CAMP-dependent protein kinase regulates ubiquitin–proteasome-mediated degradation and subcellular localization of the nuclear receptor coactivator GRIP1. J. Biol. Chem. 279, 49120–49130.

Huang, Y., Kim, S.O., Jiang, J., Frank, S.J., 2003. Growth hormone-induced phosphorylation of epidermal growth factor (EGF) receptor in 3T3-F442A cells. Modulation of EGF-induced trafficking and signaling. J. Biol. Chem. 278, 18902–18913.

Huang, B., Chen, S.C., Wang, D.L., 2009. Shear flow increases S-nitrosylation of proteins in endothelial cells. Cardiovasc. Res. 83, 536–546.

Inui, M., Martello, G., Piccolo, S., 2010. MicroRNA control of signal transduction. Nat. Rev. Mol. Cell Biol. 11, 252–263.

Iwakiri, Y., Satoh, A., Chatterjee, S., Toomre, D.K., Chalouni, C.M., Fulton, D., Groszmann, R.J., Shah, V.H., Sessa, W.C., 2006. Nitric oxide synthase generates nitric oxide locally to regulate compartmentalized protein S-nitrosylation and protein trafficking. Proc. Natl. Acad. Sci. USA 103, 19777–19782.

Jasti, J., Furukawa, H., Gonzales, E.B., Gouaux, E., 2007. Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. Nature 449, 316–323.

Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotoh, Y., Nishida, E., Kawashima, H., Metzger, D., Chambon, P., 1995. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270, 1491–1494.

Kim, H.P., Lee, J.Y., Jeong, J.K., Bae, S.W., Lee, H.K., Jo, I., 1999. Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor alpha localized in caveolae. Biochem. Biophys. Res. Commun. 263, 257–262.

Kino, T., Souvatzoglou, E., Charmandari, E., Ichijo, T., Driggers, P., Mayers, C., Alatsatianos, A., Manoli, I., Westphal, H., Chrousos, G.P., Segars, J.H., 2006. Rho family guanine nucleotide exchange factor Brx couples extracellular signals to the glucocorticoid signaling system. J. Biol. Chem. 281, 9118–9126.

Kobayashi, T., Cohen, P., 1999. Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. Biochem. J. 339 (Pt 2), 319–328.

Kohda, Y., Ding, W., Phan, E., Housini, I., Wang, J., Star, R.A., Huang, C.L., 1998. Localization of the ROMK potassium channel to the apical membrane of distal nephron in rat kidney. Kidney Int. 54, 1214–1223.

Kolla, V., Litwack, G., 2000. Transcriptional regulation of the human Na/K ATPase via the human mineralocorticoid receptor. Mol. Cell. Biochem. 204, 35–40.

Krug, A.W., Papavassiliou, F., Hopfer, U., Ullrich, K.J., Gekle, M., 2003. Aldosterone stimulates surface expression of NHE3 in renal proximal brush borders. Pflugers Arch. 446, 492–496.

Kunzelmann, K., Mall, M., 2002. Electrolyte transport in the mammalian colon: mechanisms and implications for disease. Physiol. Rev. 82, 245–289.

Le Moellic, C., Ouvrard-Pascaud, A., Capurro, C., Cluzeaud, F., Fay, M., Jaisser, F., Farman, N., Blot-Chabaud, M., 2004. Early nongenomic events in aldosterone action in renal collecting duct cells: PKCalpha activation, mineralocorticoid receptor phosphorylation, and cross-talk with the genomic response. J. Am. Soc. Nephrol. 15, 1145–1160.

Leite-Dellova, D.C., Malnic, G., Mello-Aires, M.D., 2010. Genomic and nongenomic stimulatory effect of aldosterone on H^+-ATPase in proximal S3 segment. Am. J. Physiol. Renal Physiol..

Liang, X., Butterworth, M.B., Peters, K.W., Frizzell, R.A., 2010. AS160 modulates aldosterone-stimulated epithelial sodium channel forward trafficking. Mol. Biol. Cell 21, 2024–2033.

Lin, D., Sterling, H., Lerea, K.M., Giebisch, G., Wang, W.H., 2002. Protein kinase C (PKC)-induced phosphorylation of ROMK1 is essential for the surface expression of ROMK1 channels. J. Biol. Chem. 277, 44278–44284.

Little, P.J., Cragoe Jr., E.J., Bobik, A., 1986. Na–H exchange is a major pathway for Na influx in rat vascular smooth muscle. Am. J. Physiol. 251, C707–C712.

Liu, S.L., Schmuck, S., Chorazcyzewski, J.Z., Gros, R., Feldman, R.D., 2003. Aldosterone regulates vascular reactivity: short-term effects mediated by phosphatidylinositol 3-kinase-dependent nitric oxide synthase activation. Circulation 108, 2400–2406.

Liu, H., Pedram, A., Kim, J.K., 2011. Oestrogen prevents cardiomyocyte apoptosis by suppressing p38alpha-mediated activation of p53 and by down-regulating p53 inhibition on p38beta. Cardiovasc. Res. 89, 119–128.

Lovett, F.A., Cosgrove, R.A., Gonzalez, I., Pell, J.M., 2010. Essential role for p38alpha MAPK but not p38gamma MAPK in Igf2 expression and myoblast differentiation. Endocrinology 151, 4368–4380.

Lu, M.L., Schneider, M.C., Zheng, Y., Zhang, X., Richie, J.P., 2001. Caveolin-1 interacts with androgen receptor. A positive modulator of androgen receptor mediated transactivation. J. Biol. Chem. 276, 13442–13451.

Lu, M., Leng, Q., Egan, M.E., Caplan, M.J., Boulpaep, E.L., Giebisch, G.H., Hebert, S.C., 2006. CFTR is required for PKA-regulated ATP sensitivity of Kir1.1 potassium channels in mouse kidney. J. Clin. Invest. 116, 797–807.

Lu, M., Wang, J., Jones, K.T., Ives, H.E., Feldman, M.E., Yao, L.J., Shokat, K.M., Ashrafi, K., Pearce, D., 2010. MTOR complex-2 activates ENaC by phosphorylating SGK1. J. Am. Soc. Nephrol. 21, 811–818.

Maggio, N., Segal, M., 2010. Cellular basis of a rapid effect of mineralocorticosteroid receptors activation on LTP in ventral hippocampal slices. Hippocampus.

Maguire, D., MacNamara, B., Cuffe, J.E., Winter, D., Doolan, C.M., Urbach, V., O'Sullivan, G.C., Harvey, B.J., 1999. Rapid responses to aldosterone in human distal colon. Steroids 64, 51–63.

Manegold, J.C., Falkenstein, E., Wehling, M., Christ, M., 1999. Rapid aldosterone effects on tyrosine phosphorylation in vascular smooth muscle cells. Cell Mol. Biol. (Noisy-le-grand) 45, 805–813.

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Markos, F., Healy, V., Harvey, B.J., 2005. Aldosterone rapidly activates Na^+/H^+ exchange in M-1 cortical collecting duct cells via a PKC–MAPK pathway. Nephron Physiol. 99, p1–p9.

Massaad, C., Houard, N., Lombes, M., Barouki, R., 1999. Modulation of human mineralocorticoid receptor function by protein kinase A. Mol. Endocrinol. 13, 57–65.

Matthews, L., Berry, A., Ohanian, V., Ohanian, J., Garside, H., Ray, D., 2008. Caveolin mediates rapid glucocorticoid effects and couples glucocorticoid action to the antiproliferative program. Mol. Endocrinol. 22, 1320–1330.

McEneaney, V., Harvey, B.J., Thomas, W., 2007. Aldosterone rapidly activates protein kinase D via a mineralocorticoid receptor/EGFR trans-activation pathway in the M1 kidney CCD cell line. J. Steroid Biochem. Mol. Biol. 107, 180–190.

McEneaney, V., Harvey, B.J., Thomas, W., 2008. Aldosterone regulates rapid trafficking of epithelial sodium channel subunits in renal cortical collecting duct cells via protein kinase D activation. Mol. Endocrinol. 22, 881–892.

McEneaney, V., Dooley, R., Harvey, B.J., Thomas, W., 2010a. Protein kinase D stabilizes aldosterone-induced ERK1/2 MAP kinase activation in M1 renal cortical collecting duct cells to promote cell proliferation. J. Steroid Biochem. Mol. Biol. 118, 18–28.

McEneaney, V., Dooley, R., Yusef, Y.R., Keating, N., Quinn, U., Harvey, B.J., Thomas, W., 2010b. Protein kinase D1 modulates aldosterone-induced ENaC activity in a renal cortical collecting duct cell line. Mol. Cell. Endocrinol. 325, 8–17.

Miyata, K., Hitomi, H., Guo, P., Zhang, G.X., Kimura, S., Kiyomoto, H., Hosomi, N., Kagami, S., Kohno, M., Nishiyama, A., 2008. Possible involvement of Rho-kinase in aldosterone-induced vascular smooth muscle cell remodeling. Hypertens. Res. 31, 1407–1413.

Moe, O.W., 1999. Acute regulation of proximal tubule apical membrane Na/H exchanger NHE-3: role of phosphorylation, protein trafficking, and regulatory factors. J. Am. Soc. Nephrol. 10, 2412–2425.

Musch, M.W., Lucioni, A., Chang, E.B., 2008. Aldosterone regulation of intestinal Na absorption involves SGK-mediated changes in NHE3 and Na^+ pump activity. Am. J. Physiol. Gastrointest. Liver Physiol. 295, G909–G919.

Mutoh, A., Isshiki, M., Fujita, T., 2008. Aldosterone enhances ligand-stimulated nitric oxide production in endothelial cells. Hypertens. Res. 31, 1811–1820.

Nagai, Y., Miyata, K., Sun, G.P., Rahman, M., Kimura, S., Miyatake, A., Kiyomoto, H., Kohno, M., Abe, Y., Yoshizumi, M., Nishiyama, A., 2005. Aldosterone stimulates collagen gene expression and synthesis via activation of ERK1/2 in rat renal fibroblasts. Hypertension 46, 1039–1045.

Nagata, D., Takahashi, M., Sawai, K., Tagami, T., Usui, T., Shimatsu, A., Hirata, Y., Naruse, M., 2006. Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. Hypertension 48, 165–171.

Naray-Fejes-Toth, A., Fejes-Toth, G., 2000. The SGK, an aldosterone-induced gene in mineralocorticoid target cells, regulates the epithelial sodium channel. Kidney Int. 57, 1290–1294.

Nishiyama, A., Abe, Y., 2006. Molecular mechanisms and therapeutic strategies of chronic renal injury: renoprotective effects of aldosterone blockade. J. Pharmacol. Sci. 100, 9–16.

Nishiyama, A., Yao, L., Fan, Y., Kyaw, M., Kataoka, N., Hashimoto, K., Nagai, Y., Nakamura, E., Yoshizumi, M., Shokoji, T., Kimura, S., Kiyomoto, H., Tsujioka, K., Kohno, M., Tamaki, T., Kajiya, F., Abe, Y., 2005. Involvement of aldosterone and mineralocorticoid receptors in rat mesangial cell proliferation and deformability. Hypertension 45, 710–716.

Nishizaka, M.K., Zaman, M.A., Green, S.A., Renfroe, K.Y., Calhoun, D.A., 2004. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. Circulation 109, 2857–2861.

Oakley, R.H., Cidlowski, J.A., 2011. Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. J. Biol. Chem. 286, 3177–3184.

Oberleithner, H., Weigt, M., Westphale, H.J., Wang, W., 1987. Aldosterone activates Na^+/H^+ exchange and raises cytoplasmic pH in target cells of the amphibian kidney. Proc. Natl. Acad. Sci. USA 84, 1464–1468.

Okamoto, T., Schlegel, A., Scherer, P.E., Lisanti, M.P., 1998. Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. J. Biol. Chem. 273, 5419–5422.

Okoshi, M.P., Yan, X., Okoshi, K., Nakayama, M., Schuldt, A.J., O'Connell, T.D., Simpson, P.C., Lorell, B.H., 2004. Aldosterone directly stimulates cardiac myocyte hypertrophy. J. Card. Fail. 10, 511–518.

O'Neil, R.G., Sansom, S.C., 1984. Characterization of apical cell membrane Na^+ and K^+ conductances of cortical collecting duct using microelectrode techniques. Am. J. Physiol. 247, F14–F24.

Park, J., Leong, M.L., Buse, P., Maiyar, A.C., Firestone, G.L., Hemmings, B.A., 1999. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. EMBO J. 18, 3024–3033.

Pedram, A., Razandi, M., Sainson, R.C., Kim, J.K., Hughes, C.C., Levin, E.R., 2007. A conserved mechanism for steroid receptor translocation to the plasma membrane. J. Biol. Chem. 282, 22278–22288.

Pochynyuk, O., Medina, J., Gamper, N., Genth, H., Stockand, J.D., Staruschenko, A., 2006. Rapid translocation and insertion of the epithelial Na^+ channel in response to RhoA signaling. J. Biol. Chem. 281, 26520–26527.

Pochynyuk, O., Staruschenko, A., Bugaj, V., Lagrange, L., Stockand, J.D., 2007. Quantifying RhoA facilitated trafficking of the epithelial Na^+ channel toward the plasma membrane with total internal reflection fluorescence-fluorescence recovery after photobleaching. J. Biol. Chem. 282, 14576–14585.

Razandi, M., Oh, P., Pedram, A., Schnitzer, J., Levin, E.R., 2002. ERs associate with and regulate the production of caveolin: implications for signaling and cellular actions. Mol. Endocrinol. 16, 100–115.

Razandi, M., Pedram, A., Levin, E.R., 2010. Heat shock protein 27 is required for sex steroid receptor trafficking to and functioning at the plasma membrane. Mol. Cell. Biol. 30, 3249–3261.

Revankar, C.M., Cimino, D.F., Sklar, L.A., Arterburn, J.B., Prossnitz, E.R., 2005. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307, 1625–1630.

Ring, A.M., Leng, Q., Rinehart, J., Wilson, F.H., Kahle, K.T., Hebert, S.C., Lifton, R.P., 2007. An SGK1 site in WNK4 regulates Na^+ channel and K+ channel activity and has implications for aldosterone signaling and K+ homeostasis. Proc. Natl. Acad. Sci. USA 104, 4025–4029.

Rizzo, M.A., Piston, D.W., 2003. Regulation of beta cell glucokinase by S-nitrosylation and association with nitric oxide synthase. J. Cell Biol. 161, 243–248.

Robert-Nicoud, M., Flahaut, M., Elalouf, J.M., Nicod, M., Salinas, M., Bens, M., Doucet, A., Wincker, P., Artiguenave, F., Horisberger, J.D., Vandewalle, A., Rossier, B.C., Firsov, D., 2001. Transcriptome of a mouse kidney cortical collecting duct cell line: effects of aldosterone and vasopressin. Proc. Natl. Acad. Sci. USA 98, 2712–2716.

Romagni, P., Rossi, F., Guerrini, L., Quirini, C., Santiemma, V., 2003. Aldosterone induces contraction of the resistance arteries in man. Atherosclerosis 166, 345–349.

Rubino, D., Driggers, P., Arbit, D., Kemp, L., Miller, B., Coso, O., Pagliai, K., Gray, K., Gutkind, S., Segars, J., 1998. Characterization of Brx, a novel Dbl family member that modulates estrogen receptor action. Oncogene 16, 2513–2526.

Rykx, A., De Kimpe, L., Mikhalap, S., Vantus, T., Seufferlein, T., Vandenheede, J.R., Van Lint, J., 2003. Protein kinase D: a family affair. FEBS Lett. 546, 81–86.

Sansom, S.C., O'Neil, R.G., 1986. Effects of mineralocorticoids on transport properties of cortical collecting duct basolateral membrane. Am. J. Physiol. 251, F743–F757.

Schmidt, B.M., Oehmer, S., Delles, C., Bratke, R., Schneider, M.P., Klingbeil, A., Fleischmann, E.H., Schmieder, R.E., 2003. Rapid nongenomic effects of aldosterone on human forearm vasculature. Hypertension 42, 156–160.

Schmidt, B.M., Sammer, U., Fleischmann, I., Schlaich, M., Delles, C., Schmieder, R.E., 2006. Rapid nongenomic effects of aldosterone on the renal vasculature in humans. Hypertension 47, 650–655.

Shibata, S., Nagase, M., Yoshida, S., Kawarazaki, W., Kurihara, H., Tanaka, H., Miyoshi, J., Takai, Y., Fujita, T., 2008. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. Nat. Med. 14, 1370–1376.

Shimkets, R.A., Warnock, D.G., Bositis, C.M., Nelson-Williams, C., Hansson, J.H., Schambelan, M., Gill Jr., J.R., Ulick, S., Milora, R.V., Findling, J.W., et al., 1994. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 79, 407–414.

Sinnett-Smith, J., Zhukova, E., Hsieh, N., Jiang, X., Rozengurt, E., 2004. Protein kinase D potentiates DNA synthesis induced by Gq-coupled receptors by increasing the duration of ERK signaling in swiss 3T3 cells. J. Biol. Chem. 279, 16883–16893.

Slater, S.J., Kelly, M.B., Taddeo, F.J., Larkin, J.D., Yeager, M.D., McLane, J.A., Ho, C., Stubbs, C.D., 1995. Direct activation of protein kinase C by 1 alpha, 25-dihydroxyvitamin D3. J. Biol. Chem. 270, 6639–6643.

Snyder, P.M., Olson, D.R., Thomas, B.C., 2002. Serum and glucocorticoid-regulated kinase modulates Nedd4-2-mediated inhibition of the epithelial Na^+ channel. J. Biol. Chem. 277, 5–8.

Snyder, P.M., Olson, D.R., Kabra, R., Zhou, R., Steines, J.C., 2004. CAMP and serum and glucocorticoid-inducible kinase (SGK) regulate the epithelial Na(+) channel through convergent phosphorylation of Nedd4-2. J. Biol. Chem. 279, 45753–45758.

Sober, S., Laan, M., Annilo, T., 2010. MicroRNAs miR-124 and miR-135a are potential regulators of the mineralocorticoid receptor gene (NR3C2) expression. Biochem. Biophys. Res. Commun. 391, 727–732.

Soundararajan, R., Melters, D., Shih, I.C., Wang, J., Pearce, D., 2009. Epithelial sodium channel regulated by differential composition of a signaling complex. Proc. Natl. Acad. Sci. USA 106, 7804–7809.

Soundararajan, R., Wang, J., Melters, D., Pearce, D., 2010. Glucocorticoid-induced Leucine zipper 1 stimulates the epithelial sodium channel by regulating serum- and glucocorticoid-induced kinase 1 stability and subcellular localization. J. Biol. Chem. 285, 39905–39913.

Speake, P.F., Glazier, J.D., Greenwood, S.L., Sibley, C.P., 2010. Aldosterone and cortisol acutely stimulate Na^+/H^+ exchanger activity in the syncytiotrophoblast of the human placenta: effect of fetal sex. Placenta 31, 289–294.

Staruschenko, A., Patel, P., Tong, Q., Medina, J.L., Stockand, J.D., 2004. Ras activates the epithelial Na(+) channel through phosphoinositide 3-OH kinase signaling. J. Biol. Chem. 279, 37771–37778.

Staruschenko, A., Pochynyuk, O.M., Tong, Q., Stockand, J.D., 2005. Ras couples phosphoinositide 3-OH kinase to the epithelial Na^+ channel. Biochim. Biophys. Acta 1669, 108–115.

Stockand, J.D., Meszaros, J.G., 2003. Aldosterone stimulates proliferation of cardiac fibroblasts by activating Ki-RasA and MAPK1/2 signaling. Am. J. Physiol. Heart Circ. Physiol. 284, H176–H184.

Stolte, H., Wiederholt, M., Fuchs, G., Hierholzer, K., 1969. Time course of development of transtubular sodium concentration differences in proximal surface tubules of the rat kidney. Micropuncture experiments in intact and adrenalectomized rats. Pflugers Arch. 313, 252–270.

Su, L.F., Knoblauch, R., Garabedian, M.J., 2001. Rho GTPases as modulators of the estrogen receptor transcriptional response. J. Biol. Chem. 276, 3231–3237.

Sweiry, J.H., Binder, H.J., 1989. Characterization of aldosterone-induced potassium secretion in rat distal colon. J. Clin. Invest. 83, 844–851.

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Tong, Q., Booth, R.E., Worrell, R.T., Stockand, J.D., 2004. Regulation of Na^+ transport by aldosterone: signaling convergence and cross talk between the PI3-K and MAPK1/2 cascades. Am. J. Physiol. Renal Physiol. 286, F1232–F1238.

Tsybouleva, N., Zhang, L., Chen, S., Patel, R., Lutucuta, S., Nemoto, S., DeFreitas, G., Entman, M., Carabello, B.A., Roberts, R., Marian, A.J., 2004. Aldosterone, through novel signaling proteins, is a fundamental molecular bridge between the genetic defect and the cardiac phenotype of hypertrophic cardiomyopathy. Circulation 109, 1284–1291.

Uhrenholt, T.R., Schjerning, J., Hansen, P.B., Norregaard, R., Jensen, B.L., Sorensen, G.L., Skott, O., 2003. Rapid inhibition of vasoconstriction in renal afferent arterioles by aldosterone. Circ. Res. 93, 1258–1266.

Urbach, V., Van Kerkhove, E., Maguire, D., Harvey, B.J., 1996. Rapid activation of KATP channels by aldosterone in principal cells of frog skin. J. Physiol. 491 (Pt 1), 111–120.

Ushio-Fukai, M., 2009. Vascular signaling through G protein-coupled receptors: new concepts. Curr. Opin. Nephrol. Hypertens. 18, 153–159.

Van Lint, J., Rykx, A., Maeda, Y., Vantus, T., Sturany, S., Malhotra, V., Vandenheede, J.R., Seufferlein, T., 2002. Protein kinase D: an intracellular traffic regulator on the move. Trends Cell Biol. 12, 193–200.

Vicent, G.P., Ballare, C., Nacht, A.S., Clausell, J., Subtil-Rodriguez, A., Quiles, I., Jordan, A., Beato, M., 2006. Induction of progesterone target genes requires activation of Erk and Msk kinases and phosphorylation of histone H3. Mol. Cell 24, 367–381.

Wang, W.H., Giebisch, G., 2009. Regulation of potassium (K) handling in the renal collecting duct. Pflugers Arch. 458, 157–168.

Watts 3rd, B.A., George, T., Good, D.W., 2006. Aldosterone inhibits apical NHE3 and HCO_3^- absorption via a nongenomic ERK-dependent pathway in medullary thick ascending limb. Am. J. Physiol. Renal Physiol. 291, F1005–F1013.

Webster, J.C., Jewell, C.M., Bodwell, J.E., Munck, A., Sar, M., Cidlowski, J.A., 1997. Mouse glucocorticoid receptor phosphorylation status influences multiple functions of the receptor protein. J. Biol. Chem. 272, 9287–9293.

Wehling, M., Ulsenheimer, A., Schneider, M., Neylon, C., Christ, M., 1994. Rapid effects of aldosterone on free intracellular calcium in vascular smooth muscle and endothelial cells: subcellular localization of calcium elevations by single cell imaging. Biochem. Biophys. Res. Commun. 204, 475–481.

Will, P.C., Lebowitz, J.L., Hopfer, U., 1980. Induction of amiloride-sensitive sodium transport in the rat colon by mineralocorticoids. Am. J. Physiol. 238, F261–F268.

Willette, R.N., Eybye, M.E., Olzinski, A.R., Behm, D.J., Aiyar, N., Maniscalco, K., Bentley, R.G., Coatney, R.W., Zhao, S., Westfall, T.D., Doe, C.P., 2009. Differential effects of p38 mitogen-activated protein kinase and cyclooxygenase 2 inhibitors in a model of cardiovascular disease. J. Pharmacol. Exp. Ther. 330, 964–970.

Winter, D.C., Schneider, M.F., O'Sullivan, G.C., Harvey, B.J., Geibel, J.P., 1999. Rapid effects of aldosterone on sodium–hydrogen exchange in isolated colonic crypts. J. Membr. Biol. 170, 17–26.

Winter, C., Schulz, N., Giebisch, G., Geibel, J.P., Wagner, C.A., 2004. Nongenomic stimulation of vacuolar H^+-ATPases in intercalated renal tubule cells by aldosterone. Proc. Natl. Acad. Sci. USA 101, 2636–2641.

Wu, C.C., Wu, X., Han, J., Sun, P., 2010. P38gamma regulates UV-induced checkpoint signaling and repair of UV-induced DNA damage. Protein Cell 1, 573–583.

Yoo, D., Kim, B.Y., Campo, C., Nance, L., King, A., Maouyo, D., Welling, P.A., 2003. Cell surface expression of the ROMK (Kir 1.1) channel is regulated by the aldosterone-induced kinase, SGK-1, and protein kinase A. J. Biol. Chem. 278, 23066–23075.

Yoo, D., Flagg, T.P., Olsen, O., Raghuram, V., Foskett, J.K., Welling, P.A., 2004. Assembly and trafficking of a multiprotein ROMK (Kir 1.1) channel complex by PDZ interactions. J. Biol. Chem. 279, 6863–6873.

Yun, C.C., Palmada, M., Embark, H.M., Fedorenko, O., Feng, Y., Henke, G., Setiawan, I., Boehmer, C., Weinman, E.J., Sandrasagra, S., Korbmacher, C., Cohen, P., Pearce, D., Lang, F., 2002. The serum and glucocorticoid-inducible kinase SGK1 and the Na^+/H^+ exchange regulating factor NHERF2 synergize to stimulate the renal outer medullary K+ channel ROMK1. J. Am. Soc. Nephrol. 13, 2823–2830.

Zeng, W.Z., Li, X.J., Hilgemann, D.W., Huang, C.L., 2003. Protein kinase C inhibits ROMK1 channel activity via a phosphatidylinositol 4,5-bisphosphate-dependent mechanism. J. Biol. Chem. 278, 16852–16856.

Zheng, F.F., Wu, R.C., Smith, C.L., O'Malley, B.W., 2005. Rapid estrogen-induced phosphorylation of the SRC-3 coactivator occurs in an extranuclear complex containing estrogen receptor. Mol. Cell. Biol. 25, 8273–8284.

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