Aldosterone-stimulated PKC signalling cascades: from receptor to effector

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Abstract
Aldosterone plays an important role in the regulation of blood pressure. The effects of this hormone have classically been described in terms of the transcriptional regulation of genes that facilitate electrolyte transport, particularly across high-resistance epithelia. The protein kinase signalling cascades that are rapidly activated in response to aldosterone are emerging as important modulators of the transcriptional response, and may serve to prime cells for the subsequent transcriptional changes. The activation of protein kinase D through an epidermal growth factor receptor transactivation pathway by aldosterone in renal cells has the potential to impact on cell trafficking events that regulate transporter activity.

The mineralocorticoid hormone, aldosterone, is released into the circulation by the adrenal cortex as the final stage in the rennin/angiotensin pathway. Aldosterone binds to the cytoplasmic MR (mineralocorticoid receptor), which is expressed by aldosterone-responsive tissues. The MR–aldosterone complex translocates to the cell nucleus where it acts as a ligand-dependent transcription factor. Genes transcriptionally regulated by aldosterone include (i) the membrane transporters ENaC (epithelial sodium channel), ROMK1 (renal outer medullar potassium 1 channel) and Na+/K+-ATPase, which facilitate aldosterone-stimulated electrolyte transport, and (ii) signalling intermediates such as SGK1 (serum- and glucocorticoid-induced protein kinase 1), which regulate these transporters. Transcriptional changes elicited by aldosterone become apparent 30–60 min after hormone treatment; however, this response cannot account for the earliest physiological effects stimulated by aldosterone. The first report of rapid physiological responses to aldosterone described the hormone’s effects on Na+ and K+ excretion into the urine within 5 min following intra-arterial administration [1]. It is now established that aldosterone stimulates rapid signalling and physiological responses in cardiac tissue, the vascular endothelium and vascular smooth muscle and in high-resistance epithelia such as the distal colon and distal nephron. The principal actions of aldosterone in such epithelia are the promotion of Na+ absorption and K+ secretion. The osmotic movement of water concurrent with Na+ absorption across these epithelia means that the net effect of aldosterone release on the whole body is to increase extracellular fluid volume and consequently raise blood pressure. Pathophysiological consequences of inappropriate aldosterone action include the development of hypertension and associated conditions such as cardiovascular disease.

Rapid signalling responses have been observed following aldosterone treatment, including the activation of protein kinase signalling cascades and a rise in secondary messengers such as [Ca2++]i, (intracellular Ca2++ concentration) [2]. The identity of the receptor responsible for initiating the rapid signalling responses to aldosterone has been the subject of debate. The existence of a membrane-associated receptor distinct from MR has been proposed on the basis of (i) detectable, aldosterone-binding sites on the cell surface, (ii) the potency of a membrane-impermeable aldosterone–BSA complex in initiating signalling events [3] and (iii) the observation that some of the rapid responses stimulated by aldosterone are detectable in MR-knockout cells (reviewed in [4]). The insensitivity of some aldosterone-stimulated effects in renal cell lines to treatment with MR antagonists such as spironolactone has also been proposed as evidence for the involvement of a novel receptor in transducing the rapid effects of aldosterone [3,5]. Recent work has established that MR antagonists with more flexible structures such as eplerenone are more effective in suppressing non-genomic action than spironolactone, strengthening the counter argument that MR is indeed the sole aldosterone receptor (reviewed in [6]). Differences in the efficacy of MR antagonists may depend on changes in the tertiary structure of MR modulated through its interaction with a complex of accessory molecules such as the HSPs (heat-shock proteins). The liberation of HSP90 from its complex with MR following aldosterone binding to the receptor has been implicated in initiating some of the earliest aldosterone-stimulated signalling events, such as the rapid stimulation of c-Src tyrosine kinase activity [7,8].

The activation of PKC (protein kinase C) family isoforms is a prominent aspect of the aldosterone-induced early signalling responses in epithelia. The release of Ca2++ from intracellular stores or the activation of Ca2++ channels in the cell membrane results in a transient rise in [Ca2++]i, which activates Ca2+-dependent protein kinases such as PKCa. A transient,
Figure 1 | Summary of rapid aldosterone-induced signalling in renal collecting duct cells

Aldosterone (Aldo) binds to its specific receptor MR, which translocates to the nucleus where it modulates the expression of target genes. The interaction between aldosterone and MR results in the displacement of accessory proteins such as HSP90 from the complex and the induction of signalling cascades through c-Src activation. Activated c-Src phosphorylates EGFR, resulting in its transactivation, and also stimulates the p42/p44 MAPK cascade. EGFR activation is coupled with an nPKC (novel PKC) isoform signalling cascade that activates PKD. PKD activation has been implicated in stabilization of p42/p44 MAPK activation and the regulation of subcellular trafficking. The activation of p42/p44 MAPK has been implicated in NHE regulation in aldosterone-treated CCD cells to modulate intracellular pH, whereas PKD activation may contribute to the trafficking of pre-expressed transporters following aldosterone treatment. DAG, diacylglycerol; IP3, inositol trisphosphate.

3-fold rise in $[Ca^{2+}]_i$ has been observed in M1-CCD cells (M1 renal cortical collecting duct cells) within 2 min of treatment with 1 nM aldosterone [9]. The stimulation of NHE1 (Na$^+$/H$^+$ exchanger 1) activity by aldosterone as measured through enhanced pH recovery following acid load of M1-CCD cells could be blocked by PKCa inhibition, implicating this kinase in physiological responses to aldosterone [5]. The activation of PKCa is also detectable in rat RCCD2 cells after 5 min treatment with aldosterone. In this model, PKC activation contributed to the stimulation of an amiloride-sensitive, trans-epithelial short circuit current ($I_{SC}$) by aldosterone [10]. Non-genomic PKC activation was consequently linked to the early stages of ENaC stimulation by aldosterone that yielded the $I_{SC}$ effect. The activation of p42/p44 MAPK (mitogen-activated protein kinase) in response to aldosterone has been reported and may contribute to aldosterone-stimulated cell proliferation. The rapid activation of MAPK in aldosterone-treated M1-CCD cells is PKC-dependent [5,11]. Here, the non-genomic effect of aldosterone on Na$^+$/K$^+$/2Cl$^-$ co-transporter activity was rapidly detectable within 15 min, but was also prolonged with a duration of 7 days after hormone treatment. PKD (protein kinase D)/PKC$\mu$ is regulated by novel PKC isoforms such as PKC$\epsilon$ and PKC$\eta$. It has recently been described that PKD1 is rapidly activated in response to aldosterone in the M1-CCD cell line through a signalling pathway initiated at the MR [7].

The phosphorylation of EGFR (epidermal growth factor receptor) in response to aldosterone has been observed in MDCK (Madin–Darby canine kidney) cells and treatment with EGFR antagonists blocked aldosterone-induced p42/p44 MAPK activation and the aldosterone-induced rise in $[Ca^{2+}]_i$ in these cells [14]. In M1-CCD cells, the EGFR antagonist tyrphostin AG 1478 also blocked the rapid aldosterone-induced activation of PKD1 [7]. In this case, the phosphorylation of EGFR in response to aldosterone was at least in part due to direct phosphorylation by c-Src at residue Tyr$^{845}$. The transactivation of EGFR through the liberation of HSP90 from MR to activate c-Src represents a novel coupling of MR–aldosterone interaction with the EGFR signalling hub (Figure 1). PKD family isoforms are important modulators of subcellular trafficking through the regulation of vesicle fission from the Golgi organelle (reviewed in [15]). The subcellular
redistribution of pre-expressed membrane transporters is emerging as a significant facet of the earliest physiological responses to aldosterone. It remains to be established whether PKD1 plays a role in the aldosterone-induced translocation of pre-expressed transporters such as the ENaC subunits, which are subject to post-translational modification and maturation in the Golgi in advance of membrane insertion.

References

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