Rapid Responses to Steroid Hormones in the Kidney

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expressed by target tissues such as the renal nephron to positively or negatively regulate the expression of a large repertoire of responsive genes. Hormone-responsive genes include signalling intermediates and membrane transport targets. The interaction of the hormones with their nuclear receptors allows the receptors to act as ligand-dependent transcription factors. The gene products that are hormonally regulated have a pronounced effect on electrolyte transport in the kidney, which is generally detectable within a few hours of treatment. The increased expression of the serum glucocorticoid-activated kinase (SGK)-1 is the earliest detectable genomic event occurring within 30 min of aldosterone treatment [1]. The transcriptional regulation of this kinase has been linked to the early physiological effects of aldosterone on ion transport through the activation of signalling cascades that activate membrane targets directly or promote their subcellular relocalization [2, 3]. SGK-1 is undoubtedly the pre-eminent and best-characterized signalling intermediate coupled to mineralocorticoid and glucocorticoid action; however, the delayed increase in its activity fails to account for the earliest physiological responses detected in response to these hormones [4].

Increasingly, evidence points to the activation of other, more rapid signalling responses that impact on cellular physiology within a few minutes of hormone administration. The physiological relevance of these rapid steroid-induced responses in the context of the pronounced but

Key Words
Aldosterone • Mineralocorticoid • Glucocorticoid • Oestrogen • Rapid responses • Steroid

Abstract
Rapid signalling responses stimulated by steroid hormones have been detected in various tissues including the nephron. The significance of these responses in modulating the physiological effects elicited by mineralocorticoids, glucocorticoids and the reproductive hormones in the kidney is now becoming more evident. This review outlines how rapid signalling responses stimulated by these hormones are coupled to the regulation of membrane transport targets that impact upon the reabsptive and excretory functions of the kidney.
delayed effects of the hormones on gene expression is the subject of debate. It seems most likely that these early responses serve either to potentiate the transcriptional effects of the steroids and so maximizing their effect or else allow the body to respond rapidly to deleterious changes in its internal environment, to maintain a ‘holding position’ until the transcriptional changes become effective. Weight is lent to this view with the recent data obtained for oestrogen that shows the cyclical recruitment of transcription factors to the promoter region of oestrogen-responsive genes following hormone treatment [5]. In a sense, cells are continually sampling their environment to detect fluctuations in hormone receptor interactions, and the signalling cascades that are activated by steroids may be important facets of this sampling process.

The aim of this review is to summarize current knowledge of the rapid responses to steroids detected in the kidney and to highlight areas for further investigation.

**Aldosterone**

The distal nephron is the principal site for regulating the rate of Na⁺ efflux from the body and the mineralocorticoid hormone aldosterone plays a crucial role in regulating this process (fig. 1). Aldosterone promotes Na⁺ reabsorption and net K⁺ secretion through the regulation of membrane transporter activity. The overall systemic effect of aldosterone action is to raise blood pressure through the osmotic movement of water from the renal ultrafiltrate that occurs in conjunction with Na⁺ reab-

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Fig. 1. Summary of the interactions between aldosterone-stimulated signalling pathways and membrane targets in cells of the distal nephron. Evidence from different researchers implicates the binding of aldosterone to MR and a distinct non-genomic receptor in initiating rapid signalling events. The activation of Src family tyrosine kinases is coupled to the transactivation of EGFR and the ERK1/2 MAP kinase cascade. EGFR is also coupled to PKD and the stimulation of NHE3 activation by aldosterone. The activation of PKC family isoforms is central to the rapid stimulation of membrane ion transport by aldosterone in the distal nephron. ENaC activity is the rate-limiting factor in Na⁺ reabsorption, H⁺-ATPase activity contributes to blood pH homeostasis and Na⁺/K⁺-ATPase activity exchanges reabsorbed Na⁺ for K⁺ at the basolateral membrane of the epithelial cells. Trafficking and stability of pre-expressed ENaC and Na⁺/K⁺-ATPase are also subjected to delayed regulation through aldosterone-induced changes in SGK-1 expression. The mobilization of intracellular Ca²⁺, the rise in intracellular pH and activation of the ERK1/2 cascade by aldosterone are also PKC-dependent and promote basolateral K⁺ recycling through the activation of Ca²⁺- and ATP-dependent K⁺ channels.
The development of hypertension that can result from excessive Na⁺ conservation has major pathophysiological consequences for the kidney and the vasculature making hypertension-linked events such as stroke and myocardial infarction, major causes of mortality in the developed world [7, 8]. The aldosterone-sensitive distal nphron is comprised of the thick ascending limb (TAL) of the loop of Henle, the distal convoluted tubule, the connecting tubule, and the cortical collecting duct (CCD). Na⁺ is reabsorbed at the apical surface of the principal epithelial cells in the CCD from the renal ultrafiltrate through the epithelial sodium channel (ENaC). Na⁺ is transported out of the epithelium at the basolateral membrane by the Na⁺/K⁺-ATPase pump and into the blood, which in turn maintains a gradient for apical Na⁺ uptake. These Na⁺ membrane transporters and others such as the Na⁺/H⁺ exchangers (NHE) are regulated by aldosterone at the transcriptional level over a number of hours after hormone treatment; however, rapid stimulation of cell-signalling cascades by aldosterone have been described by a number of groups since the first description of rapid aldosterone-dependent physiological effects some 50 years ago [4]. The rapid responses stimulated by aldosterone in the kidney have been the subject of most intensive investigation and the body of literature relating to aldosterone in this field is more extensive than for the other hormones reviewed here.

Membrane Transporters

The physiological effects of aldosterone on the distal nphron are expressed through the regulation of specific ion transporters. The electroneutral exchange of Na⁺ for H⁺ across the cell membrane is mediated by several NHE isoforms. In polarized renal epithelia, NHE1 at the basolateral membrane contributes to homeostatic processes such as cell volume regulation and cytoplasmic pH modulation. Early changes in NHE activity following aldosterone treatment has been coupled to the rapid activation of signalling cascades with Ca²⁺ as a secondary messenger. Aldosterone promotes a Ca²⁺-dependent increase in NHE activity in Madin-Darby canine kidney (MDCK) cells within 1 min of treatment [9, 10]. An aldosterone-stimulated rise in NHE1 activity in the murine M1-CCD cell line was also preceded by a Ca²⁺ influx [11]. PKC and ERK1/2 MAP kinase were also implicated; however, the MR antagonist spironolactone had no effect [11]. It has recently been proposed that aldosterone inhibits the activity of apical NHE3 in the medullary TAL through a non-genomic, MR-independent mechanism to block HCO₃⁻ re-absorption [12, 13]. The stimulation of NHE1 by aldosterone may modulate the hormone’s transcriptional effects through pH-sensitive signalling processes, while NHE3 activity contributes to the aldosterone-sensitive absorption of HCO₃⁻ from the renal ultrafiltrate.

The pH shift generated by NHE1 stimulation contributes to the activation of other membrane transporters that promote K⁺ recycling in the distal tubule such as K⁺ATP channels [14].

ENaC activation by aldosterone stimulates electrogenic Na⁺ transport in the principal cells of the distal nphron. Aldosterone promotes the trafficking of pre-expressed ENaC subunits to the cell membrane and stimulates MR-dependent ENaC gene expression. Tong et al. [15] described an aldosterone-stimulated cross-talk between ERK1/2 and PI3-K signalling, where PI3-K promotes ENaC activity through SGK-1, whereas ERK1/2 suppresses ENaC activity. Aldosterone-dependent ERK1/2 activation is coupled to K-Ras [15]. K-RasA becomes methylated after aldosterone treatment in A6 cells and activation of ENaC is protein methylation-sensitive [16, 17]. The increased ENaC current detected within 2 min of aldosterone (100 nM) treatment in isolated rabbit principal CDD cells cannot be accounted for by the transcriptional changes [18]. However, PKC has been implicated in the phosphorylation of each of the ENaC subunits and subunit phosphorylation leads to increased channel activity in insulin-treated A6 amphibian renal principal cells [19].

The basolateral Na⁺/K⁺-ATPase pump provides the main electrochemical driving force for the luminal influx of Na⁺ and the basolateral efflux of K⁺ in the distal nphron. Aldosterone increases Na⁺/K⁺-ATPase activity through the recruitment of pre-existing pump subunits to the cell membrane and later through the transcriptional effects of MR on Na⁺/K⁺-ATPase expression. Aldosterone also stimulates Na⁺/K⁺-ATPase activity within 30 min in isolated CCD tubules [20]. SGK-1 is the only kinase identified which directly regulates Na⁺/K⁺-ATPase in response to aldosterone in the kidney [21]. This kinase is regulated at the transcriptional level and its effects on Na⁺/K⁺-ATPase activity are only observed after an extended period [21]. The Na⁺/K⁺-ATPase α subunit has phosphorylation sites for PKC (Ser-23) [22] and PKA (Ser-943) [23] that have been implicated in Na⁺ transport [24]. Na⁺/K⁺-ATPase activity is also pH-sensitive [25, 26] and intracellular pH affects the cation-binding specificity of the transporter [27]. Since PKC activation and changes in intracellular pH are early events stimulated by aldoste-
rone in the renal epithelium, they may contribute to the early phase in aldosterone-induced Na⁺/K⁺-ATPase activity.

The ATP-sensitive K⁺ channels facilitate K⁺ recycling across the basolateral membrane to balance the activity of the Na⁺/K⁺-ATPase pump. Aldosterone treatment of frog skin principal cells stimulated pH-sensitive ḰATP channel activation within 2 min [4]. More recent work proposes that the aldosterone-induced upregulation of the Kir1.1/ROMK K⁺ channel activity in murine TAL cells relies upon cystic fibrosis trans-membrane conductance regulator (CFTR) Cl⁻ channel activation [28] and is also dependent on ENaC expression [29]. Aldosterone-responsive membrane transporters are thus under the control of integrated regulatory mechanisms. CFTR has multiple potential PKA phosphorylation sites and so may act as a PKA-dependent switch for the regulation of K⁺ secretion by the distal nephron through CFTR coupling to Kir1.1 [28]. Rapid PKC but not PKA activation has been identified as a consequence of aldosterone treatment in colonic epithelium [30]. However, increased cAMP production has been detected in isolated inner medullary collecting duct cells within 4 min of aldosterone treatment that could potentiate PKA activity [31].

The kidneys contribute to homeostatic acid-base regulation through the reabsorption of HCO₃⁻ and the release of H⁺ into the renal ultrafiltrate in the distal nephron. The release of H⁺ takes place in the CCD and outer medullary collecting duct (OMCD) at the apical surface of type A intercalated cells through the vacuolar H⁺-ATPase pump. H⁺-ATPase activity can be regulated through systemic electrolyte balance [32], angiotensin II release [33] and by aldosterone [34]. In the CCD, aldosterone stimulates H⁺ secretion directly through increased insertion of H⁺-ATPase pumps into the apical membrane and indirectly through increased absorption of Na⁺ largely through ENaC in the principal cells, which renders the tubule lumen electronegative and so promotes H⁺ diffusion [35, 36]. The aldosterone-induced increase in H⁺-ATPase activity resulting in urine acidification has been observed over prolonged exposure [34]; however, a rapid activation of H⁺-ATPase has also been described. Aldosterone stimulated a 2- to 3-fold increase in H⁺-ATPase activity in isolated outer MCD after 15 min treatment [37]. This activity was spironolactone sensitive but insensitive to inhibition of transcription or translation. The increase in H⁺-ATPase activity was blocked by disruption of the subcellular microtubule network using colchicine and also sensitive to PKC inhibition. The rapid activation of H⁺-ATPase activity in the intercalated cells of the OMCD is therefore dependent on the trafficking of already pre-expressed H⁺-ATPase pumps to the apical membrane. This trafficking event occurs independently of de novo protein synthesis and is coupled to PKC activation. The stimulation of H⁺-ATPase activity in angiotensin II-treated rat proximal tubule cells was also PKC-dependent and sensitive to PKA inhibition [33].

**Signalling Intermediates**

Multiple signalling pathways are activated rapidly following aldosterone treatment of primary renal tissue and defined cell lines. Research has focused on how these pathways modulate the activation of membrane transporters through direct phosphorylation by protein kinases, the availability of free Ca²⁺ or changes in intracellular pH. The activation of the PKC family of serine/threonine protein kinases is an important component in the rapid responses to aldosterone in the kidney and was first described in isolated renal cells [38]. The direct activation of the Ca²⁺-dependent PKC isofrom PKCα by aldosterone in a cell-free system has been proposed [30] akin to the direct activation of PKC achieved by other lipids such as arachidonic acid [39]. The stimulation of NHE activity by aldosterone in the M1-CCD cells could be blocked by PKCα inhibition [30] and the activation of PKCα in RCCD2 cells is detectable within 5 min of treatment and peaks at 10–15 min [40]. PKCe is emerging as a probable regulator of Na⁺/K⁺-ATPase activity in aldosterone-treated cardiomyocytes [41, 42]. Protein kinase D (PKD) is regulated by novel PKC isofroms and PKD1 is rapidly activated in response to aldosterone in the M1-CCD cell line [43]. PKD family isofroms are emerging as major modulators of subcellular trafficking through the regulation of vesicle fission from the Golgi organelle reviewed in Yeaman et al. [44]. It remains to be established whether PKD1 plays a role in the aldosterone-induced translocation of pre-expressed transporters.

The rapid activation of ERK1/2 mitogen-activated protein kinase (MAPK) in aldosterone-treated M1-CCD cells is PKC dependent [11]. The activation of the PKC/MAPK pathway culminates in enhanced activity of the NHE1 in acid-loaded cells [10, 11]. The coupling of MR activation to downstream-signalling pathways is now being elucidated. Aldosterone stimulates the activation of c-Src tyrosine kinase within 2 min of treatment [45]. Aldosterone not only stimulates EGFR expression at the level of transcription [46], but also rapidly promotes its c-Src-dependent phosphorylation [47]. Additionally there
is a synergism in the rapid activation of ERK1/2 between aldosterone- and EGF-stimulated pathways which confirms EGFR family receptor tyrosine kinases as critical signalling integrators in steroid-induced rapid responses [47]. A physiological relevance is lent to EGFR activation in response to aldosterone by the observation that inhibition of EGFR by tyrphostin AG1478 completely blocked the activation of NHE3 by the hormone [48, 49]. The activation of PKD1 in M1-CCD cells treated with aldosterone is also coupled to the transactivation of EGFR by c-Src [43].

A role for aldosterone in regulating calcium homeostasis through the kidney is uncertain. Clearance studies showed either an increase in calciuria [50] or an absence of significant changes in Ca2+ excretion [51, 52] after aldosterone administration. A rise in [Ca2+]i, as a component of aldosterone-induced rapid signalling is better established. Sequestration of free Ca2+ in organelle stores stably maintains low [Ca2+]i. Release of Ca2+ from these 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 PKCα or membrane transporters such as Ca2+-dependent K+ channels. A transient, 3-fold rise in [Ca2+]i has been observed in M1-CCD cells within 2 min of treatment with 1 nM aldosterone [53]. PKC inhibition with chelerythrine blocked this effect suggesting a possible role for Ca2+-independent PKC activation in the response. The rise in [Ca2+]i, stimulated by aldosterone in MDCK cells was sensitive to inhibition of EGFR and ERK1/2 [10, 49]. The extracellular or store source of the Ca2+ rise observed in these experiments has not yet been established experimentally. The aldosterone-induced activation of inositol-signalling pathways has been observed in other experimental systems including the A6 cell line [54], which may result in the activation of IP3-sensitive Ca2+ channels in the endoplasmic reticulum or mitochondria.

**MR versus Novel Receptors**

It has been suggested that classical steroid nuclear receptors do not mediate rapid responses. The contention is in part supported by evidence that some of the rapid responses to aldosterone are detected in fibroblast cells derived from MR knockout mice [55]. Aldosterone treatment of these cells resulted in a rise in Ca2+, and stimulation of adenylate cyclase activity. MR knockout mice display symptoms of pseudohypoaldosteronism and only survive beyond week 2 with subcutaneous injection of an isotonic NaCl solution [56]. ENaC activity is virtually absent in these animals and coincides with a significant (30%) reduction in ENaC mRNA abundance. The expression of ENaC in the kidney was not diminished in aldosterone synthase null mice that do not produce the hormone; however, NaCl conservation in these animals was also perturbed [57]. It is consequently not clear whether the reduced expression of ENaC subunit can adequately account for the observed attenuation of ENaC activity in the MR knockout mice. The failure to activate signalling pathways normally coupled to MR may also contribute to the loss in ENaC activity through the failure to stimulate trafficking of pre-expressed subunits [58]. Some of the aldosterone-induced signalling pathways such as those associated with subcellular trafficking may be coupled to MR, while the activation of at least some secondary messenger pathways appear to be MR independent [55].

Evidence for the existence of a non-genomic receptor distinct from MR has also been drawn from the observation that not all the rapid effects of aldosterone are blocked by specific pharmacological antagonists of the classical MR [11, 59]. The ERK1/2-dependent activation of NHE1 [11] and the transient rise in intracellular calcium [53] in aldosterone-treated M1-CCD cells were spironolactone-insensitive. A novel membrane-associated aldosterone receptor has been proposed, based on impeded ligand experiments. Aldosterone-BSA stimulated a rapid increase in PKC activity 10 min after treatment in rat RCCD2 cells [60] with a concurrent increase in PKCα-dependent MR phosphorylation. MR has a high affinity for cortisol, but is protected from activation by this serum glucocorticoid through the activity of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) which converts cortisol to cortisone [61]. Inhibition of 11β-HSD2 with carbenoxolone allows cortisol to mimic aldosterone in activating NHE, so implicating MR rather than a distinct membrane receptor in this response [62].

Differences in the efficacy of MR-specific inhibitors to block rapid responses to aldosterone may be interpreted as evidence for an aldosterone receptor distinct from MR [63] or may reflect conformational changes in the aldosterone-binding site of MR [64]. MR resides in a complex with other proteins that affect its conformation and the effect of aldosterone binding is to modulate the interaction of MR with the other proteins in the complex [65]. The release of Hsp84, the murine homolog of human Hsp90 from this complex, has been implicated in the activation of c-Src [45], EGFR and PKD1 by aldosterone in M1-CCD cells [43]. The displacement of accessory pro-
teins following ligand binding results in the exposure of the MR nuclear localization signal that causes the translocation of MR from the cytoplasm to the cell nucleus [reviewed in 66].

**Glucocorticoids**

Glucocorticoids are released in response to physiological stress and stimulate responses in multiple organ systems including the kidneys, the vasculature and the respiratory tract. Chronic glucocorticoid overproduction is a feature of Cushing’s syndrome that leads to hypertension and the increased risk of premature death. Glucocorticoids play an important role in the kidney, increasing 

**Sex Steroids**

Sex steroids play a role in normal renal physiology and also in gender-specific susceptibility to renal injury [72, 73]. The kidney is one of the most oestrogen-responsive organs in the body and has extremely high levels of oestrogen receptor expression [74]. The protective effects of oestrogen on renal tissue were regarded as the principal reason for the gender differences in kidney; however, androgen and progesterone are also now known to affect kidney function, for example through the gender-specific regulation of organic anion transporters [75]. Oestrogen and progesterone regulate ENaC subunit expression in the rat kidney at the level of transcription [76] and it is through modulation of ENaC activity that electrolyte homeostasis and extracellular fluid volume are regulated over the menstrual cycle. In ovariectomized rats, oestrogen promotes Na⁺ reabsorption and K⁺ secretion while progesterone inhibits Na⁺ reabsorption. The oestrogenic effects were believed to be a consequence of ER acting as a transcriptional regulator of angiotensin II type 1 receptor [77, 78].

The rapid responses to oestrogen in the kidney are less well understood, although the potential for cross-talk between rapid signalling responses and the transcriptional effects of ER are evident. The activation of the p160 cofactor glucocorticoid receptor-interacting protein 1 (GRIP1) in response to oestrogen in HEK293 cells is p38 MAPK dependent [79], and rapid activation of p38 MAPK in response to oestrogen has been demonstrated in the colon [80]. The activation of p38 MAP kinase in the colon is ERβ dependent; however, only ERα is expressed, at least in the murine kidney [81]. Oestrogen directly binds to and activates ‘sequence like a calcium-activated potassium channel’ (SLACK) K⁺ channels in HEK293 cells [82]. Oestrogen also activates the plasma membrane Ca²⁺-ATPase (PMCA) at the basolateral membrane of isolated distal tubule cells to promote Ca²⁺ transport back into the blood [83]. This effect was only detectable after 24 h; however, increased expression of PMCA protein was not detected, implying a non-genomic activation. Apical absorption of Ca²⁺ from the renal ultrafiltrate by the epithelial Ca²⁺ channel (ECAC1 or TRPV5) is also subject to transcriptional regulation by oestrogen in the distal nephron [84]. In addition to these transcriptional effects, there is also evidence for rapid activation of Ca²⁺ transport by sex steroids in the kidney. Oestrogen and progesterone treatment both induced a rapid rise in [Ca²⁺], in M1-CCD cells that was PKC and extracellular Ca²⁺ dependent [53].

**Conclusion**

The field of rapid steroid responses has advanced markedly over the last decade and understanding how the signalling responses stimulated by oestrogen and particularly aldosterone impact on ion transport in the kidney may provide completely novel avenues for pharmacological intervention in the treatment of hyperten-
sion. The crucial questions that remain include conclusively identifying the non-genomic receptor and elucidating the components of the rapid signalling cascades in their entirety from the receptor to transporter. It is also vitally important to establish the true physiological relevance of signalling events observed in defined cell lines when compared to the whole organism.

References


Acknowledgements

The authors are supported by programme grant 0608097/2/000 from the Wellcome Trust and by the Higher Education Authority of Ireland under the Programme for Research in Third Level Institutions (PRTLI) Cycle 3.


