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Rapid aldosterone actions: from the membrane to signaling cascades to gene transcription and physiological effects☆

Brigitte Boldyreff, Martin Wehling*

Department of Clinical Pharmacology, Faculty of Clinical Medicine Mannheim, Heidelberg University, Theodor-Kutzer Ufer 1-3, D-68167 Mannheim, Germany

Abstract

Nongenomic actions of aldosterone have been described in a number of cell culture and in vivo systems. They occur, in contrast to the classical genomic effects on gene transcription, rapidly within seconds to minutes after aldosterone administration. The primary effector is still unknown. Whether it is a so far unidentified membrane bound aldosterone receptor or the classical mineralocorticoid receptor or both is under debate. The downstream signaling cascade involved in such rapid actions begins to be elucidated. In this work, we discuss the nature of the putative membrane receptor for aldosterone and summarize observed rapid aldosterone effects in different in vitro and in vivo systems.

Keywords: Aldosterone; Signaling; Rapid actions; Membrane receptor; Mineralocorticoid receptor; MAPK

1. Introduction

Steroid hormones have a dual mechanism of action. They affect the transcriptional modulation of target genes via intracellular receptors, but also elicit rapid effects on second messenger signaling cascades similar to those initiated by peptide hormones or catecholamines. The Mannheim classification of nongenomically initiated rapid steroid actions has classified the possible mechanisms involved [1]. Several scenarios are discussed how these rapid effects are specifically initiated [2]: Binding of the steroid hormone to an as-yet uncharacterized membrane receptor and thereby mediated rapid signaling have been postulated [3]. Alternatively, the cognate intracellular steroid receptor could, in addition to its role as a transcription factor, activate second messenger systems. As a third option, other heterologous membrane receptors might be utilized. Obviously, nature has realized all three possibilities.

Membrane steroid binding sites have been identified. Their properties to bind agonists and antagonists are not always compatible with being identical to the classical intracellular receptors. However, none of the postulated membrane steroid receptors has yet been purified to homogeneity and cloned.

For most steroid hormones experimental data exist which prove that the classical intracellular receptor is involved in rapid signaling, e.g. a small population of estrogen receptors (ERs) is located at the cell surface, binds estradiol and activates G-proteins [4] and consequently protein kinase cascades of signal transduction including the Src/MAPK and phosphatidylinositol 3-kinase (PI3-K)/Akt pathway. Similarly, binding of corticosteroids to the glucocorticoid receptor stimulates PI3-K [5], rapid vitamin D3 effects involve vitamin D receptor–Src interaction and increase in Src-kinase activity [6], the progesterone receptor upon hormone binding interacts with SH3 domains and activates Src family tyrosine kinases [7].

In a few cases, it has also been shown that steroid hormones are capable to interact with peptide hormone signaling. The interactions of progesterone with oxytocin signaling [8], of estradiol with growth factor and angiotensin II signaling [9] and of aldosterone with epidermal growth factor receptor (EGFR) signaling [10] have been described. In addition, modulation of cell function by a steroid via the $GABA_A$ receptor has been shown for neuroactive steroids that can rapidly alter the excitability of $GABA_A$ receptor [11]. However, direct binding of a steroid hormone to a heterologous receptor has been described in only one case so far, namely progesterone binding to oxytocin [8].

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* Corresponding author. Tel.: +49-621-383-4058; fax: +49-621-383-2024.

E-mail address: martin.wehling@kpha.ma.uni-heidelberg.de (M. Wehling).

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Rapid downstream signaling cascades have extensively been characterized for estrogen [4,12,13]. In a cell context specific manner cascades involving phospholipase C, protein kinase C, MAPK, PI3-K or p38MAPKAP-2 are activated. Activation of the MAPK cascade has also been described for androgens [14], activation of the PI3-K pathway has been described for vitamin D3 [15], progesterone [16] and corticosteroids [5]. A common denominator for the initiation of the signaling pathways seems to be the interaction of the steroid hormone receptors with signaling molecules such as Src kinase with subsequent activation.

The final biological effects caused by the rapid steroid hormone actions are still largely unclear. For estrogens and androgens, an antiapoptotic effect on osteoblasts and osteocytes via the MAPK pathway has been shown. In this case, the rapid effect was demonstrated to be dissociable from the transcriptional activity of the classical receptor, since it is mediated by the ligand binding domain alone and eliminated by nuclear targeting of the receptor protein [14]. It has been speculated that ovarian hormone-induced MAPK activation enhances transcriptional activation of ER, which is a potential mechanism by which growth of breast cancer cells is stimulated [17]. This can be seen as a feed-forward system where cross-talk between the nongenomic hormone action and the genomic response occurs. In addition, evidence exists that estrogen through membrane initiated steroid signaling activates discrete programs of gene expression, different from the one caused by nuclear ER action [4].

For aldosterone, knowledge of the mechanisms of rapid actions is limited. A large body of phenomenological observations exists showing rapid increases in the concentrations of second messengers as cAMP, DAG, IP_3 and Ca^{2+}, as well as activation of protein kinases PKC and MAPK. Several clinical studies underscored the physiological significance of rapid aldosterone actions in vivo. However, we are only beginning to understand the underlying mechanisms.

2. Primary targets for rapid aldosterone actions: novel membrane receptors, classical intracellular receptors or heterologous receptors?

Specific high affinity binding sites for aldosterone have been characterized in membranes of different cells or tissues by us and others (Table 1). Binding properties as affinities or specificities of these sites for a series of agonists and antagonists were in most cases different from the ones of the classical mineralocorticoid receptor (MR). An identified 50 kDa membrane protein from human mononuclear leukocytes had, in comparison to the classical MR, a 10-fold higher affinity to radiolabeled aldosterone; in contrast to the classical MR this protein could not bind cortisol [24]. Therefore, it has been supposed that the membrane binding sites represent novel membrane bound aldosterone receptors different from the intracellular MR. This hypothesis is supported by several other lines of evidence. Rapid effects have been described which were insensitive towards classical MR antagonists, e.g. hydrocortisone, even at a 1000-fold higher concentrations did not block in vitro the rapid aldosterone effect on the Na^+/H^+ exchange (NHE) [27]. Similarly, canrenone failed to block rapid aldosterone-induced elevation of IP_3 levels in human mononuclear leukocytes [28]. Also spironolactone was uneffective to interfere with aldosterone-induced increase in intracellular pH in strips of human vascular vessels [29]. We found recently that short-term aldosterone treatment of the cortical collecting duct cell line M-1-induced MAPK phosphorylation. This phosphorylation could also not be blocked by any of the available MR antagonists (Rossol-Haseroth et al., in preparation).

The strongest argument in favor of a novel aldosterone receptor probably comes from experiments with cells of a MR knockout mouse. In the MR gene of this mouse, most of exon 3 containing the first zink finger of the MR DNA binding domain was replaced by a *LacZ* reporter gene and a neo selection marker [30]. There is no evidence that there is any residual MR activity in these mice. A splicing between the intact exons 2 and 4 would lead to a reading frame

Table 1
Membrane binding sites for aldosterone

Aldo analogue	Kd (nM)	Agonist/antagonist properties	Cell system	Reference
^{3}H-aldosterone	100		Rat kidney	[18]
^{3}H-aldosterone	3–13	Spironolactone effective	Rat kidney	[19]
^{3}H-aldosterone	2.7		HML	[20]
^{125}I-aldosterone	0.1	Cortisol; dexamethasone, RU26988, corticosterone, oubain, amiloride, 18-hydroxyprogesterone, canrenone inactive as ligands	HML	[21–24]
^{125}I-aldosterone	0.1	Hydrocortisone Ki >100 nM	Pig kidney	[25]
^{3}H-aldosterone	$Kd_1 \leq 11$ nM, $Kd_2 = 118$ nM	Cortisol ineffective	Pig liver	[26]

Membrane binding sites for aldosterone were characterized using radiolabeled aldosterone analogues by determination of dissociation constants (Kd) and binding properties of agonists/antagonists. HML, human mononuclear leukocytes.

shift, a putative protein would contain MR sequences encoded by the second exon, i.e. 585 amino acids of the variable N-terminal region followed by unrelated amino acids, but no DNA or ligand binding domain. However, RT-PCR analysis of the knockout mice using different primers lying in exons downstream or upstream of the deleted exon 3 did not give any products. Also, staining of tissue sections of the MR-knockout mice with a MR specific antibody did not yield any signals (Berger, S., personal communication). We used skin fibroblasts from newborn $MR^{-/-}$ mice for aldosterone stimulation experiments. Aldosterone increased intracellular levels of calcium and cAMP within 1–2 min [31]. Since no residual MR is present in these cells, the primary aldosterone target has to be a protein different from MR.

Although rapid steroid actions initiated by the classical receptor have been shown for the ERα and other steroid hormones (see Section 1), no convincing evidence yet exists for aldosterone. Alzamora et al. demonstrated that RU 28318, a weak competitor for cytosolic MR, completely blocked the rapid effect of aldosterone on intracellular pH increase in rings from chorionic arteries [29]. However, little is known about the mechanism of action of RU 28318.

Lately, Gekle et al. reported that aldosterone interacts with EGFR signaling in the renal epithelial MDCK cell line by inducing a rapid increase in EGFR phosphorylation [32,33]. This was further confirmed by transfection of EGFR into CHO cells which do not express EGFR and are not aldosterone responsive [10]. In this system aldosterone led to EGFR phosphorylation and activation of the MEK1/2-ERK1/2 signaling cascade. Yet, a direct interaction between aldosterone and EGFR seems to be ruled out, since at least one upstream factor involved, namely c-Src kinase, was identified.

The existence of a novel membrane aldosterone receptor is still an open question. In case of rapid aldosterone effects, which were insensitive towards the classical MR antagonist, it is hard to imagine that the classical MR when located in the membrane exhibits different binding properties towards antagonists. This has also not been observed for other membrane bound classical steroid hormone receptors. It therefore seems likely that a MR unrelated receptor is involved. In case of rapid aldosterone action in $MR^{-/-}$ cells the assumption of a novel receptor for aldosterone distinct from MR is the only option to explain the observed effects.

3. Signaling cascades

Numerous rapid aldosterone-induced changes of the concentration of intracellular second messengers have been described. Table 2 gives an overview. Among the cellular systems analyzed were classical target cells as renal and colonic cells, but also nonclassical cell types as vascular smooth muscle cells and lymphocytes.

cAMP increase caused by aldosterone has been described in vascular smooth muscle cells and the inner medullary collecting duct [34,35]. Christ et al. [34] correlated the cAMP level with CREB phosphorylation, which led to the assumption that aldosterone via cAMP activates PKA which in turn phosphorylates the transcriptional coactivator CREB. Thereby MR interaction with the basal transcriptional machinery and consequently genomic aldosterone effects may be influenced.

The phosphatidylinositol second messenger system is obviously affected in a greater variety of cell types than the cAMP second messenger system and the calcium increase seems to be a ubiquitously induced aldosterone signal. Rapid increases in DAG and IP_3 levels have been observed in vascular smooth muscle cells and in several other cell systems. The usual consequences, rise of intracellular concentrations Ca^{2+}, which is known to be released from IP_3-sensitive

Table 2
Rapid aldosterone-induced changes in second messenger concentrations

Rapid effect	Time	Aldosterone concentration	Cell system	Reference
cAMP ↑	1 min	EC_{50} 0.01–0.1 nM	VSMC (porcine coronary artery)	[34]
cAMP ↑	4 min	EC_{50} 0.2 nM	Inner medullary collecting duct	[35]
DAG ↑	30 s	1–10 nM	VSMC	[36]
IP_3 ↑	1 min	EC_{50} 0.1 nM	HML	[28]
IP_3 ↑	30 s	EC_{50} 0.5–1 nM	VSMC	[37]
IP_3 ↑	20–60 min	2.7 μM	A6 cells	[38]
IP_3 ↑	45 s	10–100 nM	Skeletal muscle cells	[39]
Ca^{2+} ↑	5 min	EC_{50} 0.1 nM	VSMC, EC	[40]
Ca^{2+} ↑	3–5 min	EC_{50} 0.1–0.5 nM	VSMC (rat and rabbit aortic)	[41]
Ca^{2+} ↑	2 min	0.1 nM	T84 colonic epithelial cells	[42]
Ca^{2+} ↑	1–5 min	EC_{50} 1 pM	EC	[43]
Ca^{2+} ↑	2– min	10 nM	Skin fibroblasts from $MR^{-/-}$ mice	[31]
Ca^{2+} ↑	1–2 min, 30 min	10–100 nM	Skeletal muscle cells	[39]
Ca^{2+} ↑	5 min	1 nM	CCDC (M-1)	[44]

VSCM: vascular smooth muscle cells; HML: human mononuclear leukocytes; EC, endothelial cells; CCDC, cortical collecting duct cells. Rapid aldosterone effects were observed within the time indicated and at the aldosterone concentrations given.

stores, on the one hand, and PKC activation, commonly thought to be stimulated by DAG, on the other hand have also been demonstrated [36,45].

Inhibitors of phospholipase C and G proteins prevented aldosterone-induced PKC activation in vascular smooth muscle cells and cultured kidney cells [36,45] suggesting a G protein-dependent mechanism involving phospholipase C. However, in another system, in neonatal rat cardiomyocytes, aldosterone led to PKC inhibition [46] which might be an effect specific for cardiomyocytes. In colonic cells, PKC α isoform has been suggested as the primary target for rapid aldosterone responses, since direct stimulation in a cell free assay was demonstrated [47].

Beside other second messenger systems, it has also been shown that the MAPK cascade plays a role in rapid aldosterone signaling. In MDCK-C11 cells, resembling the intercalated cells of the cortical collecting duct, rapid activation of the NHE by aldosterone involves MAP kinases ERK1/2 [48]. Phosphorylation of ERK1/2 was rapidly induced by aldosterone, whereas inhibitors of ERK1/2 activation prevented NHE activation. Thus, the MAPK pathway may mediate the rapid aldosterone signal in order to activate the NHE. Furthermore the same group showed, that aldosterone-induced rapid increases in EGFR–Tyr phosphorylation, and inhibition of EGFR kinase abolished aldosterone-induced signaling [33]. Thus, they demonstrated that aldosterone uses the EGFR-ERK1/2 signaling cascade in MDCK cells. In a reconstituted cell system, aldosterone potentiated the action of EGF within minutes. In addition to EGFR–Tyr phosphorylation also c-Src phosphorylation was enhanced by aldosterone [10]. Consequently, a hypothetical model of the aldosterone-EGF interaction was developed: Aldosterone leads to phosphorylation of c-Src, which then co-stimulates EGFR when EGF is present. Subsequently, the MAPK cascade is activated leading to cellular responses as NHE activation.

We could demonstrate, as already mentioned, induction of MAPK ERK1/2 phosphorylation in another cortical collecting duct cell line, M-1, 5 min after administration of 1 nM aldosterone. Also phosphorylation of Raf and MEK, two upstream protein kinases, was seen under the same conditions. ERK1/2 phosphorylation was inhibited by using MEK inhibitors (Haseroth et al., in preparation). In addition, preliminary data were obtained indicating that Src-kinase activity is stimulated in this system. Hence, Src kinase activation seems to be the most upstream rapid aldosterone effect demonstrated so far. It remains to be seen how aldosterone leads to the activaton of Src.

It should be noted that in renal A6 cells and cardiac fibroblasts the maximum of MAPK activation was observed 2–4 h after aldosterone administration [49,50]. This activity peak could be correlated with an MR-dependent activation of Ki-RasA. In A6 cells also, a calcium signal was observed 60 min after aldosterone administration, which was dependent on de novo transcription and translation [51]. This suggests that aldosterone also via the delayed genomic process uses intracellular signaling cascades to regulate target cell function.

A complete picture with integration of all the observed effects has only begun to be assembled. It is neither clear how aldosterone induces second messenger elevations or signaling cascades nor how this leads to the cellular downstream effects.

4. Cross-talk between rapid aldosterone signaling and gene transcription

It has been suggested that modulation of intracellular signaling influences genomic steroid action. A two-step model of steroid action was developed integrating both genomic and nongenomic aspects and their possible interaction [52]. First, aldosterone elicits rapid responses which then influence and co-stimulate the subsequently occuring genomic responses. The major players in this game might be the rapidly activated protein kinases which could affect genomic aldosterone actions through phosphorylation of transcriptional (co)factors. Modulation of transcription rates has been observed after stimulation with mineralocorticoids in cells transfected with MRs during coincubation with 8-bromo-cAMP [53,54]. A current model assumes that MR could be maintained in an inactive state through interaction with a protein or a complex of proteins. Through a phosphorylation step, PKA could release the MR from the complex and thus allow it to interact with DNA and activate transcription. Possible candidates for proteins complexed with MR are the nuclear corepressors NcoR and SMRT [54].

According to our own data, CREB might be another transcriptional factor influenced by rapid aldosterone action. In porcine coronary vascular smooth muscle cells, both increases in intracellular cAMP levels and CREB phosphorylation were observed [34].

In search of early aldosterone-regulated genes in renal cells, we recently identified the *gadd153* (Kellner et al., in press) gene. The induction of this gene could not be blunted by either GR and MR antagonists. This indicates an effect correlated to the classic GR- and MR-mediated pathways. Interestingly, gadd153 expression was reported to be controlled by a CRE/ATF promotor [55], suggesting that upregulation occurs by the active transcription factor CREB. The putative pathway might involve rapid aldosterone-induced cAMP generation, PKA activation and CREB phosphorylation.

Beside PKA, which could play a role in linking rapid aldosterone signaling and aldosterone-induced gene transcription, other kinases might be involved as well. Among the substrates of MAPKs are several transcription factors and kinases which phosphorylate transcription factors, e.g. MAPK phosphorylates $p90^{RSK}$ which in turn can phosphorylate CREB. Thus, CREB might also be activated upon rapid aldosterone-induced MAPK activation.

5. Physiological effects of rapid aldosterone signaling: phosphorylation of effector molecules

In addition to influencing MR-mediated gene transcription through phosphorylation, rapid aldosterone signaling may also be responsible for phosphorylation of effector molecules. One example is the epithelial sodium channel (ENaC), which becomes phosphorylated upon aldosterone administration via a transcription independent mechanism [56]. That ENaC is an effector of aldosterone has also been supported by the demonstration of mutations in amiloride-sensitive sodium channels in the autosomal recessive form of pseudohypoaldosteronism (PHA) [57]. Defective sodium channels and, therefore, intracellular sodium may be linked to intracellular calcium by the sodium/calcium exchanger of the cell membrane [41]. Indeed, in PHA patients the nongenomic aldosterone effect on rapid calcium increase is impaired [58]. Thus, the sodium channel seems to be a nongenomic aldosterone effector.

Aldosterone rapidly stimulates the Na^+/H^+ antiporter activity in vascular smooth muscle cells [37] and human mononuclear leukocytes [59–61] resulting in cellular alkalinization and increase in cellular volume. In MDCK cells, it has been shown that the rapid activation of NHE is mediated by the MAPK cascade, since aldosterone-induced activation of NHE was prevented by two specific inhibitors of MAPK EK1/2 activation [48]. It is not known which mechanism links the stimulation of ERK1/2 to NHE activation. Possibly, the MAPK substrate p90 ribosomal S6 kinase ($p90^{RSK}$) is involved [62].

6. Rapid aldosterone effects in vivo: clinical studies

The clinical importance of short-term aldosterone effects has been demonstrated in several clinical studies. To the best of our knowledge, the first study dates back to 1963, when Klein and Henk demonstrated an increase in peripheral vascular resistance and blood pressure as well as a decrease in cardiac output 5 min after intravenous application of 1 mg aldosterone [63]. Our group confirmed this finding in a recent study [64], in which significant changes of systemic vascular resistance, cardiac output and cardiac index were found within 3 min after the application of 1 mg aldosterone as assessed by an invasive method (cardiac catheterization). Further evidence for clinically detectable rapid cardiovascular aldosterone effects was also obtained by a study using noninvasive techniques. A higher systemic vascular resistance was observed within 5 min after intravenous injection of 0.5 mg aldosterone [65]. This effect persisted and was statistically significant again after 30 min. After 30 min, even mean arterial pressure was higher during the 0.5 mg aldosterone period than that during the placebo period. The mechanism behind these in vivo effects remains to be elusive. Yet, given the in vitro data on aldosterone action in vascular smooth muscle cells [41], an increase in intracellular calcium in VSMC likely leads to a vasoconstriction and as a consequence to an increase in systemic vascular resistance.

Rapid in vivo aldosterone effects on muscle energy metabolism were analyzed by Zange et al [66]. After isometric contraction, phosphocreatine recovered to significant higher levels after application of 1 mg aldosterone compared with placebo. The effect appeared immediately after isometric contraction and occurred within 8 min of aldosterone administration. This finding points to an involvement of aldosterone in acute stress adaptation of cellular oxidative metabolism in human muscle physiology. A rapid aldosterone receptor may therefore be present in skeletal muscle fibers. Cellular activation may include a rise in the levels of intracellular calcium, sodium and probably protons, as it has been described for other cells, leading to metabolic activation and resulting in an accelerated regeneration of phosphocreatine stores.

Acute effects of aldosterone have also been described on intracardiac monophasic action potential [67]. 0.5 ng aldosterone increases monophasic action potential duration within 4–6 min after intravenous application. Since elevated plasma aldosterone levels represent an independent risk factor for increased mortality in congestive heart failure, it was hypothesized that aldosterone exerts its unfavorable effect partly by altering myocardial repolarization.

7. Conclusions

There is ample evidence for rapid nongenomic aldosterone effects both in vitro and in vivo. A variety of signaling molecules and intracellular signaling cascades affected by aldosterone has been identified. However, the primary target of nongenomic aldosterone actions has not been found. Yet, data are accumulating excluding the involvement of the classical MR. This is in contrast to the initiation of the rapid action by other steroid hormones. For estrogen, corticoids, progesterone and Vitamin D3, the involvement of the classical or a modified classical receptor in rapid effects has been demonstrated. Given the deleterious effects of elevated plasma aldosterone levels on the cardiovascular system and the fact that aldosterone causes short-term effects on cardiovascular functions, it is of utmost importance to understand the mechanisms of rapid aldosterone effects.

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