Aldosterone: Refreshing a Slow Hormone by Swift Action

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Aldosterone elicits not only genomic effects with physiological consequences within hours or days but also elicits rapid nongenomic effects, such as activation of sodium transport in target cells, within seconds or minutes. Rapid aldosterone effects, which have also been shown in several in vivo studies in humans (e.g., increase in peripheral vascular resistance and blood pressure), are of potential clinical importance.

As for all steroid hormones, the classical model of aldosterone action involves the following steps. Due to its lipophilic nature, aldosterone enters the cell by diffusion through the cellular membrane. In the cytoplasm, it binds to an intracellular receptor, the mineralocorticoid receptor (MR), which is complexed to several other proteins including heat shock proteins and immunophilins. On binding of aldosterone to the MR, heat shock proteins are released. The aldosterone-MR complex then translocates to the nucleus, binds to specific DNA sequences (so-called mineralocorticoid-responsive elements), and acts as a transcription factor, i.e., together with various cofactors it transactivates or represses the transcription of target genes. Thus the protein content and thereby the metabolism and functioning of target cells is altered. The earliest effects evoked by such a mechanism, involving transcription of genes and therefore termed “genomic,” can be observed 30 min after hormone release or administration. However, numerous steroid hormone effects in diverse cell types cannot be explained by the action via the intracellular receptor and transcription, simply because of the narrow time frame in which they are taking place. It should also be noted that in humans plasma levels of aldosterone rapidly increase when the body moves from a horizontal to an upright position. This increase would be meaningless if there were not a corresponding rapid cellular response. Such rapid responses were termed “nongenomic,” because no transcription of genes takes place and inhibitors or transcription and translation are ineffective in suppressing these rapid effects; rather, modification of preexisting proteins (i.e., activation or repression of their activities) is involved.

Rapid, nongenomic aldosterone action: historical overview

The first report on rapid steroid hormone action dates back to 1941. Selye noted an anesthetic effect of steroids in rats when they were administered intraperitoneally or intravenously. One of the first rapid effects of aldosterone was reported in 1963 by Klein and Henk, who noticed an increase in peripheric vascular resistance and blood pressure as well as a decrease in cardiac output within 5 min of intravenous aldosterone administration. One year later, in 1964, Spach and Streeten demonstrated retarded sodium exchange in dog erythrocytes within minutes after administration of physiological aldosterone concentrations. Their experiments ruled out the classical mechanism of action, not only because of the rapid mode of action but also because erythrocytes lack a nucleus; hence the observed effects cannot be explained by modulation of transcription and translation. The number of reports on rapid effects of steroids that are incompatible with the classical model of genomic action has increased dramatically within the past decade.

Aldosterone causes rapid changes at the cellular level

Rapid influence of aldosterone on various cellular parameters has been described in different target tissues and cells in vivo and in vitro by us and by others (1). In the major target organs, i.e., kidney and colon, aldosterone is responsible for sodium absorption across epithelia. This is achieved by delayed mechanisms of onset as well as by a rapid activation of sodium channels. In principal cells from renal collecting duct, aldosterone leads to increased sodium current within <2 min via the amiloride-sensitive epithelial sodium channel (ENaC) (19). In Madin-Darby canine kidney (MDCK) cells, a cell culture system modeling renal cortical collecting duct epithelium, rapid activation of the Na+/H+ exchanger (NHE) was observed (8, 11). Also, in human distal colon, aldosterone-induced rapid activation of Na+/H+ exchange and of K+ recycling was demonstrated (4). Additionally, aldosterone rapidly stimulated sodium transport in nonclassical target cells such as mononuclear leukocytes and vascular smooth muscle cells (2).

Rapid aldosterone-induced in vivo effects

Rapid aldosterone action was not only shown at the cellular level and in vitro. Several clinical studies demonstrated rapid systemic effects of aldosterone in vivo, which indicates the clinical significance of such effects. Aldosterone might be involved in acute stress adaptation of cellular oxidative metabolism in human muscle physiology.
The results of Christ et al. (3) point in this direction. After isometric contraction of the calf muscle, phosphocreatine recovered to significantly higher levels after application of aldosterone compared with placebo. Effects appeared immediately after isometric contraction and occurred within 8 min of aldosterone administration. Hypoxia completely blocked this aldosterone-induced effect. This result suggests that aldosterone probably reduces ATP turnover by causing an oxygen-dependent imbalance of ATP utilization and ATP synthesis rate.

The majority of clinical studies have focused on the cardiovascular action of aldosterone. The already-mentioned study by Klein and Henk, which showed a rapid increase in peripheral vascular resistance and blood pressure as well as a rapid decrease in cardiac output after aldosterone administration, was recently confirmed by our group (16). In a double-blind placebo-controlled randomized parallel trial on 17 patients with suspected coronary heart disease, we assessed the effects of aldosterone on cardiovascular function by using cardiac catheterization. We found an aldosterone effect 3 min after administration that dissipated within 10 min. The systemic vascular resistance increased and cardiac output decreased significantly.

Acute effects of aldosterone on cardiac electrophysiological properties have also been described. In a study with six patients suffering from supraventricular arrhythmia, aldosterone injection caused an increase in monophasic action potential within minutes after application (15). Because elevated plasma aldosterone levels represent an independent risk factor for increased mortality in congestive heart failure and because sudden cardiac death and atrial fibrillation substantially contribute to excessive mortality, one might hypothesize that aldosterone partly exerts its unfavorable effect via altering myocardial repolarization.

Data from animal models suggest that aldosterone has direct influence on the autonomic nervous system and the baroreflex. This was confirmed in humans by Yee and Struthers (17), who showed that aldosterone blunts the baroreceptor sensitivity. It has been proposed that this blunting of baroreceptor activity is mediated nongenomically and that nongenomic aldosterone action itself is modulated by the adrenergic system. This interaction between the autonomic nervous system and rapid, nongenomic aldosterone effects was proven by Schmidt et al. (14). The authors showed that aldosterone has diverse effects on the cardiovascular system depending on the preexisting adrenergic state. For example, during β-agonist activation aldosterone decreases mean arterial blood pressure, whereas during β-antagonist treatment an increase occurred. Thus rapid aldosterone actions may antagonize intended clinical effects of β-agonists and antagonists that might be of importance during the treatment of hypertension and heart failure.

In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) (12), the magnitude of the effect of aldosterone blockade by eplerenone was smaller than in the Randomized Aldactone Evaluation Study (RALES), a trial of aldosterone blockade by spironolactone. These differences may be attributed, among other factors, to the different use of beta blockers. Both aldosterone antagonists, eplerenone and spironolactone, block the classical action via the mineralocorticoid receptor, but in all studies performed, so far no blockade of nongenomic aldosterone actions by these two compounds could be detected. Thus it might be that in the EPHESUS study, where more patients were treated with beta blockers, aldosterone exerts a more negative effect via the nongenomic mechanism than in the RALES study.

**Mechanisms of rapid aldosterone action**

What are the mechanisms leading to rapid aldosterone actions? Changes in the concentrations and/or activity of components of intracellular second messenger systems and signal transduction cascades are involved (reviewed in Ref. 1, Fig. 1). cAMP increase caused by aldosterone has been described in vascular smooth muscle cells and the inner medullary collecting duct. The phosphatidylinositol second messenger system plays a role in rapidly transmitting the aldosterone signal in various cell types. Rapid rise in diacylglycerol (DAG) and inositol triphosphate (IP3) concentrations were observed, for example, in vascular smooth and skeletal muscle cells, lymphocytes, endothelial cells, different renal cells, and colonic epithelial cells. Consequently, an increase in intracellular calcium, which is usually released by IP3-sensitive stores, and activation of PKC, commonly stimulated by DAG, were observed to be rapidly induced by aldosterone.

Recently, Gekle et al. (7) and Krug et al. (9) reported rapid activation of other protein kinases [the MAP kinases ERK1/2, Src kinase, and the epidermal growth factor (EGF) receptor tyrosine kinase] by aldosterone in the kidney cell line MDCK-C11, a cell line that resembles the intercalated cells of the cortical collecting duct. All three kinases are involved in rapid signaling of other steroid hormones as well. Otherwise, MAP
kinases and Src transmit a variety of signals, including those from growth factors, cytokines, and stress, whereas the EGF receptor regulates cell proliferation, differentiation, and ion transport on EGF binding, but it is also involved in signaling by growth hormones and cytokines via transactivation. The aldosterone-induced activation of MAP kinase, Src, and EGF receptor kinase seem to be interrelated. According to a model developed by Krug et al. (9), aldosterone first activates Src kinase, which, together with EGF, in turn co-activates the EGF receptor kinase; subsequently, the MAP kinase cascade is activated. Additionally, PKC, already mentioned to be stimulated by aldosterone, can exert a positive effect on MAP kinases. The physiological role of aldosterone in this context might be to fine-tune or modulate the EGF-induced cellular answers. A direct correlation between the aldosterone-induced MAP kinase and NHE activation was shown in kidney cell cultures, where inhibitors of the MAP kinase cascade led to an inhibition of the Na+/H+ exchange (7). This result indicates that aldosterone-induced MAP kinase activation is a prerequisite for the activation of Na+/H+ exchange. However, both the mechanism that links these two phenomena and how the other observed changes in intracellular signaling systems lead to other aldosterone-induced cellular effects are unclear. Since several protein kinases or activators of protein kinases are involved, changes in the phosphorylation status of effector molecules, such as ENaC and NHE, are most likely.

Additional putative effector molecules, which are substrates of the aldosterone-induced protein kinases/kinase cascades, are transcription factors. cAMP-responsive element binding protein (CREB) might be such a factor (discussed in Ref. 1). In porcine coronary vascular smooth muscle cells, we observed a rapid increase in intracellular cAMP and in parallel enhanced phosphorylation of CREB. This finding suggests that PKA is activated by the enhanced cAMP level and phosphorylates CREB. Because CREB is a known co-activator of genomic steroid action, the rapid effect of aldosterone on CREB may finally modulate long-term genomic actions of aldosterone.

The putative aldosterone membrane receptor

The major question yet to be answered is what the primary target of rapid nongenomic aldosterone action is. Whether steroids in general use the classical, possibly modified intracellular receptor or an unrelated novel membrane receptor to evoke their rapid responses has been discussed for many years. Obviously not all steroid hormones need an extra membrane receptor. Some steroids use the intracellular receptor for both genomic and nongenomic signaling. For example, besides its intracellular localization the estrogen receptor has also been found at the cell surface; estradiol binding to those membrane-bound receptor molecules rapidly activates G proteins and subsequently signal transduction cascades (10). However, different domains of the same receptor are obviously responsible for nongenomic signaling or genomic actions, respectively.

Aldosterone may be an example of a steroid hormone that transmits nongenomic signals through a membrane receptor distinct from the intracellular mineralocorticoid receptor. Several lines of evidence support this assumption (reviewed in Ref. 1). First, specific high-affinity binding sites for aldosterone were found in different cells and tissues. Pharmacological properties of these sites were different from the ones of the classical MR, e.g., the affinity of the membrane sites for aldosterone is one order of magnitude higher compared with the one of the MR and the membrane binding sites are selective for aldosterone, i.e., they bind aldosterone, but not cortisol or other glucocorticoids, whereas the MR has the same binding affinity for aldosterone and glucocorticoids. Second, rapid aldosterone-induced effects were observed in mice in which the MR has been knocked out. Third, MR antagonists such as spironolactone or canrenone usually do not block rapid aldosterone effects, which indicates that these effects are not mediated by the MR but by an unrelated receptor. However, a few cases of rapid MR antagonist-sensitive aldosterone effects have been reported (13, 18). These findings may suggest that at least some rapid effects are mediated via the classical intracellular MR.

The aldosterone membrane receptor responsible for the rapid effects has not been cloned yet. A 50-kDa membrane protein from human mononuclear leukocytes has been identified, which might be a candidate for being the membrane aldosterone receptor (5). However, sequence data for this protein have not yet been obtained owing to limited material and instability of the protein. So far only membrane receptors for progesterone and progesterin have been purified and cloned. We characterized a human membrane protein binding progesterone (mPR) with an apparent molecular weight of 28 kDa and one predicted transmembrane domain (6). Zhu et al. (20) cloned a 40-kDa membrane progesterin receptor from sea trout ovarian tissue, which probably belongs to the family of seven-transmembrane receptors and which has three homologs in humans. Although neither our mPR nor the one from Zhou et al. (19) have other close relatives in the human genome, it remains to be seen whether they belong to a family of membrane steroid receptors that might also include the membrane aldosterone receptor.

References

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