Brief Reviews

Cell Membrane–Associated Mineralocorticoid Receptors? New Evidence

Alexander W. Krug, Luminita H. Pojoga, Gordon H. Williams, Gail K. Adler

Abstract—The purpose of the present article is to provide an overview of plasma membrane steroid hormone receptors and their implications in nongenomic signaling. We especially focus on recent evidence supporting the notion of a possible membrane-associated aldosterone receptor, whether this receptor is different from the classic nuclear receptor, and the possible implications of such a receptor for nongenomic and genomic aldosterone effects in physiological and pathophysiological processes. (Hypertension. 2011;57:1019-1025.)

Key Words: mineralocorticoid receptor • aldosterone • nongenomic effects • plasma membrane

Aldosterone is the major physiological regulator of Na+ and electrolyte balance and, hence, blood pressure homeostasis. In the kidneys, aldosterone mediates Na+ retention and K+ excretion via genomic effects through the cytosolic mineralocorticoid receptor (MR), which acts as a transcription factor. In 1984, Moura and Worcel described rapid (within minutes) effects of aldosterone on Na+ efflux from smooth muscle cells, and this effect could not be blocked by the transcription inhibitor actinomycin D. Later it was shown that aldosterone can elicit activation of Na+/H+ exchanger and signaling cascades, such as mitogen activated protein kinases (MAPKs) or protein kinase C, through nongenomic mechanisms, and these effects may impact cellular functions, such as growth and differentiation. Several excellent recent reviews have extensively described the physiology and biology of aldosterone action.5–7

Evidence for a Nonclassic Aldosterone Receptor in the Plasma Membrane and MR-Independent Rapid Signaling

Initially, the classic cytosolic MR protein was suggested to be involved in mediating both nongenomic and genomic aldosterone signaling; however, some studies reported that these nongenomic effects could not be blocked by MR antagonists such as spironolactone. In addition, although rapid, nongenomic effects were elicited by the mineralocorticoids aldosterone, deoxycorticosterone, and 9α-fludrocortisone, these rapid effects could not be elicited by the glucocorticoids cortisol and corticosterone.5 In analogy to other steroid hormones, such as estrogens, Wehling et al hypothesized an aldosterone receptor that is distinct from the classic MR protein, located at the membrane, and responsible for nongenomic aldosterone effects. This view was initially supported by several studies demonstrating functional evidence for the possible existence of a plasma membrane–bound MR. For example, presumably membrane-impermeable, BSA-coupled aldosterone showed the same rapid effects as aldosterone, supporting the idea of a plasma membrane–bound receptor; however, aldosterone-BSA conjugates may undergo dissociation into hormone and BSA requiring critical and careful handling as shown for estrogen-BSA. Additional support for a distinct membrane MR comes from recent work by Wildling et al. Using endothelial cell swelling as an index of an aldosterone effect, it was shown that spironolactone could only block this effect 5 minutes but not 1 minute after the application of aldosterone. These investigators provided additional data supporting their hypothesis using atomic force microscopy technology. A single aldosterone molecule covalently bound to an atomic force microscopy tip was used to detect unbinding forces between aldosterone and plasma membrane–associated molecules. These studies suggested the existence of an aldosterone-binding protein in the plasma membrane. As neither dexamethasone nor spironolactone influenced the binding of aldosterone to its plasma membrane interaction site, the authors concluded that the classic glucocorticoid receptor (GR) and MR were not involved in these observations. These results, however, need to be interpreted with caution, because the delay in spironolactone action might relate to the kinetics of spironolactone binding to the receptor and/or the relatively poor water solubility of spironolactone. Moreover, the technology used cannot determine whether the aldosterone binding sites are located in the plasma membrane or in the several 100 nm spanning glycocalyx on the cell surface that contains molecules that have been secreted into the extracellular space and have then been adsorbed onto the cell surface. Valid conclusions about the quantity of these aldosterone-binding sites could not be made.

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From the Brigham and Women’s Hospital/Harvard Medical School, Department of Endocrinology, Diabetes, and Hypertension, Boston, MA.
Correspondence to Alexander W. Krug, Brigham and Women’s Hospital/Harvard Medical School, Division of Endocrinology, Diabetes, and Hypertension, 221 Longwood Ave, Boston, MA 02115. E-mail awkrug@partners.org
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Functional evidence for MR-independent rapid aldosterone effects comes from studies using MR-deficient human embryonic kidney cells. Aldosterone-induced Ca\(^{2+}\) signaling was similar in MR-transfected and untransfected cells and could not be prevented by MR blockade.\(^{16}\) Similarly, aldosterone-induced Ca\(^{2+}\) signaling in fibroblasts from MR knockout mice is unchanged compared with the wild type, indicating MR-independent mechanisms.\(^{17}\) Taken together, results from these studies provided indirect evidence for a unique membrane-bound MR, making conclusive and definitive statements difficult.\(^{6}\)

However, one could speculate that the described aldosterone interaction site on the plasma membrane represents an already known molecule with a distinct physiological function. For example, the \(\alpha\beta\delta\) integrin is believed to act as a cell surface thyroid hormone receptor mediating T4-induced MAPK activation\(^{18}\) and affecting nuclear thyroid hormone receptor and estrogen receptor (ER) function.\(^{19,20}\) A very recent article by Gros et al,\(^{21}\) published in Hypertension, builds on this concept, providing for the first time more direct and convincing evidence for the existence of a plasma membrane aldosterone receptor. At low picomolar concentrations, aldosterone increased extracellular signal–regulated kinase (ERK) 1/2 phosphorylation in vascular smooth muscle cells, which was inhibited both by an antagonist to the orphan G protein–coupled receptor (GPR) 30 and the MR blocker eplerenone. Functional data suggest that this effect seems to be specific for aldosterone versus glucocorticoids, with an EC\(_{50}\) in the low picomolar range. In rat aortic endothelial cells with no detectable MR expression levels, aldosterone also induced ERK1/2 activation, and GPR30 blockade as well as adenosivirus-mediated downregulation of GPR30 almost completely prevented aldosterone-mediated ERK1/2 phosphorylation. In conclusion, both MR- and GPR30-mediated mechanisms seem to be involved in rapid aldosterone signaling, such as ERK1/2 and phosphatidylinositol 3-kinase activation. Although this study did not include binding assays of aldosterone and GPR30, it has established the membrane protein GPR30 as a crucial factor in MR-independent rapid aldosterone signaling. GPR30 has also been described as a high-affinity/low-capacity estrogen-responsive protein and a linker in the membrane ER signaling complex. However, it does not seem to function as an independent ER in the absence of classic ER protein.\(^{12}\) Future investigations addressing the physiological and pathophysiological significance of aldosterone-GPR30 interaction, including aldosterone-GPR30 binding studies, should further characterize the nature of aldosterone-GPR30 interaction.

**Evidence That the Classic MR Mediates Both Nongenomic and Genomic Aldosterone Signaling and a Small Fraction May Be Found in the Plasma Membrane**

Currently, a large body of evidence supports the view that both nongenomic and genomic aldosterone effects are mediated via the classic cytosolic MR protein in various cell types,\(^{22-24}\) and at least a small fraction of classic MR is located in the membrane. In contrast to spironolactone, more water-soluble, open-ring form MR blockers, such as RU28318, inhibited the same rapid aldosterone effects,\(^{5,23}\) supporting a role for the classic MR in nongenomic aldosterone signaling. Moreover, a study from Alzamora et al\(^{22}\) has demonstrated in strips of human vascular vessels that inhibition of 11\(\beta\)-hydroxysteroid dehydrogenase, an enzyme that metabolizes glucocorticoids to their inactive \(\alpha\)-keto forms, caused cortisol to induce similar nongenomic effects as aldosterone. Cortisol has similar affinity to MR as aldosterone, suggesting that classic MR is involved in these nongenomic effects. Using MR-deficient human embryonic kidney cells, studies from Grossmann et al\(^{16}\) have shown rapid aldosterone activation of a variety of signaling cascades, including ERK1/2, p38, and c-Jun N-terminal kinase, only when cells were transfected with a gene encoding the classic human MR. Support for the idea that at least a fraction of the classic MR protein is localized at the plasma membrane comes from another recent study; Grossmann et al\(^{26}\) used green fluorescent protein–tagged MR in a heterologous expression system, with coimmunoprecipitation and fluorescence energy transfer imaging techniques, to demonstrate that a small fraction of MR is colocalized with epidermal growth factor receptor (EGFR) at the plasma membrane. Similar to other classic steroid hormone receptors,\(^{12}\) MR-mediated MAPK activation depends on src-mediated EGFR transactivation.\(^{27,28}\) Interestingly, in cells transfected with human MR, membrane colocalization of EGFR and MR was not dependent on the presence of MR ligand; however, the presence of hormone seemed to be necessary to activate EGFR and to induce ERK1/2 phosphorylation. Long-term aldosterone stimulation resulted in the disappearance of MR-EGFR colocalization and translocation of the receptor into the nucleus. Disruption of cholesterol-rich domains reduced MR-EGFR interaction at the membrane, further supporting the notion of an MR/EGFR signaling complex located in the plasma membrane.\(^{26}\) In an earlier study, the group demonstrated that the MR-green fluorescent protein construct shows similar functional behavior with respect to nongenomic and genomic signaling compared with the untagged human MR.\(^{16}\) However, it should be noted that a heterologous expression system represents an artificial situation, and it is possible that aldosterone binds to supramolecular complexes of MR associated with membrane-bound proteins. ER, GR, progesterone receptor (PR), and androgen receptor (AR) all have a palmitoylation sequence, which is considered to be required for membrane localization and rapid steroid signaling but not nuclear localization.\(^{29,30}\) Palmitoylation is the reversible addition of a palmitate group to the sulfhydryl group of a cysteine, providing a protein with weak membrane affinity with a hydrophobic anchor.\(^{31}\) In contrast, MR does not seem to have a perfect palmitoylation sequence, but it contains a FYQLTLLK or FPAMLVEI sequence, which might serve as a palmitoylation sequence. Additional studies are needed to determine whether these or other MR sequences are relevant for subcellular MR localization and possible binding of the classic MR to the plasma membrane.\(^{26}\)

Coimmunoprecipitation studies from our laboratory demonstrated an association between MR and caveolin (Cav) 1, as has been noted for Cav-1 and ER.\(^{32}\) Cav-1 is the major component of caveolae, which contain a variety of signaling
molecules, including steroid hormone receptors, growth factor receptors, and molecules involved in rapid steroid signaling (see Table for a selection of Cav-1–binding proteins). In addition, Cav-1 knockout mice have decreased cardiac and vascular MR expression. In mice infused with \( \text{L-arginine methyl ester} \) and angiotensin II, lack of Cav-1 reduces biventricular myocardial damage and reduces vascular expression of proinflammatory factors despite similar circulating levels of aldosterone in wild-type and Cav-1 knockout mice. Loss of Cav-1 blunted the association between inflammation and aldosterone levels. Interestingly, \( \text{N'-nitro-L-arginine methyl ester} \)/angiotensin II infusion resulted in an increase in cardiac MR expression in wild-type but not in knockout animals, suggesting that Cav-1 is an important factor determining cardiovascular MR expression and signaling in vivo; however, because Cav-1 is likely involved in a variety of transduction pathways related to inflammation but not associated with MR activation, these findings need to be interpreted with caution until confirmative data are available.

**Table. Membrane-Associated Caveolin 1–Binding Proteins, Including Steroid Hormone Receptors, Growth Factor Receptors, and Signaling Molecules, Involved in Rapid Steroid Signaling**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Receptors</strong></td>
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<tr>
<td>Endothelin type A, B receptors</td>
<td>Chun et al(^{23})</td>
</tr>
<tr>
<td></td>
<td>Okamoto et al(^{34})</td>
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<tr>
<td></td>
<td>Yamaguchi et al(^{35})</td>
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<tr>
<td>Insulin receptor</td>
<td>Yamamoto et al(^{36})</td>
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<tr>
<td></td>
<td>Nystrom et al(^{37})</td>
</tr>
<tr>
<td>Epidermal growth factor receptor</td>
<td>Couet et al(^{38})</td>
</tr>
<tr>
<td><strong>Steroid hormone receptors</strong></td>
<td></td>
</tr>
<tr>
<td>Estrogen receptors ( \alpha ) and ( \beta )</td>
<td>Schlegel et al(^{39})</td>
</tr>
<tr>
<td></td>
<td>Chambless et al(^{40})</td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>Lu et al(^{41})</td>
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<tr>
<td></td>
<td>Pedram et al(^{29})</td>
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<tr>
<td>Glucocorticoid receptor</td>
<td>Jain et al(^{42})</td>
</tr>
<tr>
<td>Progesterone receptors A and B</td>
<td>Pedram et al(^{29})</td>
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<tr>
<td>Mineralocorticoid receptor</td>
<td>Own observations (2010, unpublished)</td>
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<tr>
<td><strong>Enzymes</strong></td>
<td></td>
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<tr>
<td>Src</td>
<td>Sargiacomo et al 1993(^{43})</td>
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<tr>
<td></td>
<td>Li et al(^{44})</td>
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<tr>
<td>Protein kinase A</td>
<td>Razani et al(^{45})</td>
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<td>Mitogen activated protein kinase kinase</td>
<td>Engelman et al(^{46})</td>
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<td>Zundel et al(^{47})</td>
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<td>Adenylyl cyclase</td>
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<td>Endothelial NO synthase</td>
<td>Garcia-Cardenas et al(^{51})</td>
</tr>
<tr>
<td></td>
<td>Liu et al(^{52})</td>
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<td></td>
<td>Shaul et al(^{53})</td>
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**Estrogen, Glucocorticoids, Androgens, and Progesterone**

The nature and function of plasma membrane steroid receptors have been characterized best for estrogen. Szego and Davis\(^{56}\) demonstrated rapid effects of 17\( \beta \)-estradiol on cAMP and Ca\(^{2+} \) signaling in cell and animal models and identified an estrogen-binding protein in the plasma membrane.\(^{57,58}\) Subsequently, both the 46-kDa and full-length 66-kDa forms of ER\( \alpha \) were found in the plasma membrane, with the 66-kDa protein probably being the functionally relevant receptor.\(^{12}\) Approximately 5% to 10% of the endogenous cellular ER\( \alpha \) pool is found in the plasma membrane. The membrane and nuclear ER have similar and high affinity for ligand. This membrane-localized ER is responsible for rapid estrogen signaling, with the ligand-binding domain of ER\( \alpha \) being necessary for both rapid signaling and membrane binding.\(^{59}\)

Two endogenous ER\( \beta \) variants of \( \approx \)54 kDa and 60 kDa have also been described at the membrane, but less is known about their function compared with that of ER\( \alpha \).\(^{12,60}\)

In endothelial and other cells, membrane-bound ER\( \alpha \) and ER\( \beta \) are found primarily in caveolae and noncaveolae rafts. Cav-1 modifies ER and enzyme activity in vascular tissue, facilitating rapid activation of endothelial NO synthase and NO production.\(^{61}\) Recruitment of ER\( \alpha \) to the plasma membrane is mediated by palmitoylation within the E domain of the ER,\(^{30,62}\) which enhances physical association of the receptor A/B domain with Cav-1, followed by ER\( \alpha \) translocation to the membrane. Striatin, which was originally purified from rat brain synaptosomes, binds via its conserved N-terminal motif to Cav-1 in a Ca\(^{2+}\)-dependent way,\(^{63}\) promoting binding of ER\( \alpha \) to the membrane. Striatin contains a Ca\(^{2+}\)-calmodulin–binding domain and a large WD (tryptophan-aspartate)-repeat domain,\(^{64}\) which is commonly found in cytoskeletal and signaling proteins and allows multiple protein-protein interactions. For example, striatin is found in a complex with protein phosphatase 2A, which directly binds to and regulates ER\( \alpha \) function.\(^{65}\) Because rapid estrogen signaling is characterized by membrane, but not nuclear, localization of ER,\(^{59}\) disruption of the ER/striatin complex prevents nongenomic, but not genomic, estrogen signaling; thus, this indicates a crucial role for striatin in the regulation of nongenomic versus genomic ER signaling.\(^{66}\)

The scaffold protein GPR30 has been described as a high-affinity/low-capacity estrogen-responsive protein, which is likely to serve as a linker in the ER signaling complex in a variety of cells, including endothelial and vascular smooth muscle cells. The exact role of GPR30 in membrane-mediated estrogen signaling is not clear yet, but GPR30 has been shown to collaborate with ER\( \alpha \) at the membrane to mediate rapid estrogen-mediated kinase signaling in a variety of cells, such as endometrial and ovarian cells resulting in proliferation.\(^{12}\)

Estrogen stimulation induces dissociation of the Cav-1/ER\( \alpha \) complex, allowing interaction of the receptor with signaling intermediates, such as c-src.\(^{67}\) In the nonactivated state, ER\( \alpha \) is bridged via its E domain to c-src by a scaffold protein that has been named MNAR (modulator of nongenomic action of ER).\(^{68,69}\) which contains multiple LXXLL protein-protein interaction motifs. LXXLL modules (where L
is leucine and X is any amino acid) are specific motifs contained within a nuclear receptor coregulatory protein that mediate the binding of the protein to this nuclear receptor.\(^\text{70}\) Other ER domains interact with additional scaffold proteins, such as she, and the ER/she interaction mediates downstream kinase activation. Depending on the cellular context and stimulus, c-src activation initiates downstream activation of ras, raf, MAPK, ERK1/2, or phosphatidylinositol 3-kinase, AKT,\(^\text{71}\) resulting in different physiological cellular outcomes. In the case of estrogens, rapid steroid signaling may persist for several hours,\(^\text{72}\) leading to activation of protooncogenes such as raf and ras.\(^\text{73}\)

The first evidence for plasma membrane–bound GRs (mGR) was described in neuronal cells, as well as human and rodent leukemia cell lines.\(^\text{74,75}\) Overexpression studies of a classic GR gene in a transfection model did not show enhanced cell-surface GR expression, suggesting that mGR may be a splice variant or product of posttranslational modification of the classic GR gene.\(^\text{76}\) The exact structure and importance of mGRs in rapid glucocorticoid signaling has not been elucidated. Several reports suggest the involvement of mGR in the pathogenesis of chronic inflammatory diseases.\(^\text{77}\) For example, in patients with rheumatoid arthritis, increased numbers of mGR-positive monocytes and B-lymphocytes are directly associated with parameters of disease activity,\(^\text{78}\) and nongenomic glucocorticoid signaling is believed to mediate immunosuppressive effects in T cells.\(^\text{79}\) Rapid extranuclear glucocorticoid signaling also seems to be involved in suppression of adrenocorticotropic hormone secretion from the pituitary gland; this effect likely involves the classic GR, because it can be blocked by the GR antagonist mifepristone.\(^\text{80}\)

The AR is even less well characterized. AR-mediated signaling also seems to originate from caveolae located in the plasma membrane.\(^\text{81}\) No specific membrane AR, which is different from the classic AR protein, has been described. Thus, both rapid and nuclear AR signaling seem to be mediated by the same AR protein, with a fraction of AR being located to the membrane. For example, in prostate cancer cells, rapid AR signaling includes EGF-R-mediated c-src activation, which may involve an androgen-dependent AR/MNAR interaction.\(^\text{82}\) Similarly, testosterone rapidly activates src in Sertoli cells, resulting in EGFR transactivation and MAPK signaling,\(^\text{83}\) possibly affecting androgen-dependent and -independent prostate cell growth.

In addition to the classic PR-A/PR-B, a membrane PR family has been described. Showing high-affinity interactions with progestins, membrane PRs are involved in oocyte maturation. Membrane PR also seem to be involved in progestin-mediated myometrium regulation at the end of pregnancy, involving activation of various kinase cascades and cross-talk with classic PR.\(^\text{84}\) Progesterone-binding sites have been shown on the cell surface of sperm cells. Evidence suggests the involvement of another membrane-localized progesterone-binding protein termed progesterone membrane receptor component 1 in the nongenomic, transcription-independent acrosomal reaction in sperm,\(^\text{85}\) and in progesterone effects in granulosa and luteal cells.\(^\text{86}\) Other than genomic signaling, classic PR-B also initiates MAPK signaling via direct binding between the SH3 domain of src and a proline-rich sequence of the PR.\(^\text{87}\) Although it is not completely clear whether PR-mediated src activation is initiated at the plasma membrane, it is believed to occur outside the nucleus.\(^\text{88}\) One

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**Figure.** Hypothetical model describing membrane-initiated rapid aldosterone signaling. Classic mineralocorticoid receptor (MR) is translocated to and associated with the membrane in a signaling complex including striatin, caveolin (Cav) 1, src, and the epidermal growth factor receptor (EGF-R). Similar associations have been described for estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR). On aldosterone stimulation, MR is released from the membrane-signaling complex and EGF-R is transactivated by aldosterone, initiating c-src-mediated mitogen-activated protein kinase (MAPK) activation. In addition, G protein–coupled receptor (GPR) 30 may function as aldosterone receptor mediating rapid MR-independent signaling.
study suggesting membrane localization of PR demonstrated that the PR contains a palmitoylation sequence characteristic of membrane proteins and a 9-amino acid membrane-anchoring motif. Similar to estrogens, androgens, and aldosterone, rapid PR-initiated src activation mediates EGFR transactivation, resulting in downstream MAPK activation. Conversely, EGFR activation leads to PR-B phosphorylation and ligand-independent accumulation of the receptor in the nucleus. These mechanisms are believed to be involved in breast cell proliferation and differentiation.

Summary
Signaling characteristics of steroid actions share similar patterns for different hormones, such as vitamin D, androgens, progesterone, glucocorticoids, and estrogens, and plasma membrane receptors for these hormones have been described. In the case of estrogen, the plasma membrane ERα is responsible for nongenomic signaling. For other steroid hormones, such as androgens and progesterone, plasma membrane receptors have been described, but rapid signaling is mediated both by the cytosolic and the membrane-associated steroid receptors. The evidence for membrane-bound aldosterone receptors discussed in this article is mainly indirect. Still, the concept of a unique aldosterone plasma membrane receptor, which is different from the classic cytosolic MR protein and exclusively mediates rapid aldosterone signaling, has received new support. A recent study has shown that GPR30 mediates MR-independent rapid aldosterone effects in vascular smooth muscle cells and endothelial cells. In addition, a large body of evidence supports the view that both genomic and rapid aldosterone effects are mediated via the classic MR protein. A small fraction of classic MR protein seems to be present in the plasma membrane and associated with Cav-1 in a signaling complex in caveolae. This suggests that the MR is at least in the appropriate location to initiate membrane signaling (see Figure for a hypothetical model of plasma membrane-initiated aldosterone signaling), but it remains to be determined in more detail whether aldosterone receptors at the membrane play an important (patho)physiological role in mediating rapid aldosterone effects.

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