Elevated levels of the steroid hormone aldosterone have been reported in populations at risk for cardiovascular disease and are associated with adverse cardiovascular events, such as myocardial infarction, stroke, and death. Clinical trials demonstrate that either inhibition of aldosterone production or antagonism of its receptor, the mineralocorticoid receptor (MR), significantly reduces cardiovascular ischemic events and mortality. Classically, aldosterone regulates blood pressure by binding to the MR in renal epithelial cells and increasing expression and activity of ion channels in the distal nephron resulting in sodium retention and blood pressure elevation. Accordingly, clinical trials of MR antagonists report decreases in blood pressure. However, the modest reductions in BP do not fully account for the cardioprotective effects of MR antagonism nor do renal electrolyte changes fully explain the antihypertensive effects of MR antagonists. For the past 2 decades, it has become clear that aldosterone and MR have extrarenal actions that may contribute to alterations in vascular function leading to the development and progression of cardiovascular disease. Exploration of the precise mechanisms by which aldosterone/MR regulates vascular function and promotes cardiovascular diseases remains a very active area of investigation and a potential avenue for novel pharmacological interventions.

**MR as a Global Regulator of Ion Channels**

Because MR is a critical regulator of renal ion channels, and more recently has been implicated in ion channel regulation in the heart, we propose that MR may also regulate and more recently has been implicated in ion channel regulation, and vascular stiffness (reviewed elsewhere) and generation of vascular tone, vessel contraction and relaxation, and cardiovascular disease. Vascular ion channels are critical contributing to vascular dysfunction associated with diseases, including atherosclerosis, diabetes mellitus, and hypertension. This review focuses on new evidence supporting a role for MR in regulating vascular ion channels and the contribution of these channels to MR-mediated vascular diseases. These findings offer new mechanistic insights into the clinical benefits of MR antagonism. Moreover, elucidation of vascular ion channel expression and function, thereby contributing to aldosterone/MR-induced vascular dysfunction and cardiovascular disease. Vascular ion channels are critical to generation of vascular tone, vessel contraction and relaxation, and vascular stiffness (reviewed elsewhere) and contribute to vascular dysfunction associated with diseases, including atherosclerosis, diabetes mellitus, and hypertension. This review focuses on new evidence supporting a role for MR in regulating vascular ion channels and the contribution of these channels to MR-mediated vascular diseases. These findings offer new mechanistic insights into the clinical benefits of MR antagonism. Moreover, elucidation of vascular ion channel expression and function, thereby contributing to aldosterone/MR-induced vascular dysfunction and cardiovascular disease. Vascular ion channels are critical to generation of vascular tone, vessel contraction and relaxation, and vascular stiffness (reviewed elsewhere) and contribute to vascular dysfunction associated with diseases, including atherosclerosis, diabetes mellitus, and hypertension. This review focuses on new evidence supporting a role for MR in regulating vascular ion channels and the contribution of these channels to MR-mediated vascular diseases. These findings offer new mechanistic insights into the clinical benefits of MR antagonism. Moreover, elucidation of vascular ion channel expression and function, thereby contributing to aldosterone/MR-induced vascular dysfunction and cardiovascular disease. Vascular ion channels are critical to generation of vascular tone, vessel contraction and relaxation, and vascular stiffness (reviewed elsewhere) and contribute to vascular dysfunction associated with diseases, including atherosclerosis, diabetes mellitus, and hypertension. This review focuses on new evidence supporting a role for MR in regulating vascular ion channels and the contribution of these channels to MR-mediated vascular diseases. These findings offer new mechanistic insights into the clinical benefits of MR antagonism. Moreover, elucidation of vascular ion channel expression and function, thereby contributing to aldosterone/MR-induced vascular dysfunction and cardiovascular disease.

**Epithelial Sodium Channels**

The epithelial sodium channel (ENaC) is recognized as a major target of aldosterone in the distal nephron and contributes to classical MR regulation of renal sodium reabsorption and blood pressure control. In addition to its crucial role in the kidney, ENaC has more recently been identified in vascular endothelial cells (ECs) and vascular smooth muscle cells (SMCs) where it mediates Na+ transport into the endothelium and contributes to myogenic tone in SMC. The mechanisms of ENaC regulation by MR in the kidney have been reviewed elsewhere. Here, we focus on recent studies highlighting the role of MR in regulating endothelial ENaC and how this may contribute to vascular endothelial function. A role for MR in regulating SMC ENaC has yet to be determined and warrants further exploration.

Kusche-Vihrog et al demonstrated that aldosterone increases expression of ENaC in human ECs in an MR-dependent manner. Endothelial ENaC contributes to mechanical cellular stiffening, and aldosterone increases both endothelial ENaC protein expression in the plasma membrane and EC stiffness. The molecular mechanism by which ENaC alters endothelial stiffness likely involves increases in Na+ influx and colocalization with F-actin. Both of these mechanisms increase the F-actin:G-actin ratio in the cortical cytoskeleton, thereby increasing cortical stiffness of the EC (Figure). Shear forces on the endothelium also promote nitric oxide (NO) release. MR antagonism prevented the increase in ENaC expression and EC stiffening and also enhanced NO release. It is unclear whether the reversal of mechanical stiffening by spironolactone contributed to the increase in NO release or whether MR inhibition influences other processes involved in NO synthesis.

Mutations in the ENaC β or γ subunits have been identified in patients with Liddle syndrome, a genetic form of hypertension. These mutations result in increased channel density/activity, enhanced sodium retention, and hypertension.
ENaC surface expression, thereby regulating the mechanistic regulation of vascular ENaC by MR in ECs and potentially also in SMCs. Furthermore, the mechanisms by which aldosterone/MR regulates vascular ENaC may be altered in different pathologies and should also be examined in future studies.

**Voltage-Gated L-Type Ca\(^{2+}\) Channels**

In the heart, aldosterone and MR have previously been found to regulate expression of voltage-gated L-type Ca\(^{2+}\) channels (LTCC) with potential implications for arrhythmia generation.\(^{13}\) LTCCs are also expressed on the plasma membrane of vascular SMCs and contribute substantially to vascular function. These channels are composed of 4 subunits: \(\alpha_1\), \(\beta\), \(\gamma\), and \(\delta\). The \(\alpha_1\) subunits are primarily involved in Ca\(^{2+}\) permeability, Ca\(^{2+}\)-dependent inactivation, and are inhibited by commonly used antihypertensive Ca\(^{2+}\)-channel blocking drugs.\(^{43-45}\) LTCCs are activated in response to SMC membrane depolarization, allowing the influx of Ca\(^{2+}\) to initiate SMC contraction and to mediate myogenic tone in arterioles. Inactivation of LTCCs occurs by SMC membrane hyperpolarization, partly because of activation of Ca\(^{2+}\)-activated K\(^+\) channels (BKCa), thereby limiting contraction and promoting vessel relaxation (Figure). The role of LTCCs in vascular function\(^{46}\) and of aldosterone/MR in the regulation of LTCCs in the heart\(^{11}\) has been reviewed elsewhere. Here, we focus on recent findings implicating vascular MR in regulation of LTCCs in vascular function and blood pressure regulation.

Several studies have observed effects of aldosterone on SMC calcium currents and channels, but the mechanisms are unclear.\(^{47-49}\) Our laboratory recently generated mice with MR specifically deleted from SMC and established a direct role for SMC-MR in the regulation of blood pressure.\(^{50}\) In this study, SMC-MR-knockout mice had a decrease in the age-associated elevation in blood pressure without impairments in renal sodium handling. Contractile responses of mesenteric arterioles to an LTCC agonist were attenuated in the aged SMC-MR-knockout mice. In addition, expression of the pore-forming LTCC \(\alpha_1C\) subunit (Ca\(_{1.2}\)) mRNA was decreased in the aortas of SMC-MR-knockout mice, suggesting that SMC-MR may regulate Ca\(_{1.2}\) expression. These data support the conclusion that SMC-MR directly participates in the age-associated rise in blood pressure, in part, by regulating the expression and activity of resistance vessel LTCC.\(^{50}\) The detailed molecular mechanism by which SMC-MR regulates LTCC expression and function is being actively explored. The elucidation of this mechanism is critical to the current understanding of blood pressure regulation by MR, which likely involves the vessel in addition to the kidney and may offer novel insight into age-associated changes in vascular function and blood pressure regulation that could be exploited therapeutically. The potential role of MR in regulating LTCCs in different physiological states other than aging, as well as in common disease states associated with MR activation...
including obesity, diabetes mellitus, and atherosclerosis, remains to be determined.

**Large-Conductance Calcium-Activated Potassium Channels**

Ample data support an important role of calcium-activated potassium channels (KCa) in the regulation of vascular contractile function by hyperpolarizing the SMC, thereby limiting or opposing contraction.12,13,51 These channels are divided into 3 subtypes based on their conductance: small conductance, intermediate conductance, and large conductance (BKCa).52 Small-conductance and intermediate-conductance channels are predominately expressed in ECs and are discussed in the next section. BKCa channels are predominately expressed in vascular SMCs. In response to elevations in intracellular SMC Ca2+, BKCa channels produce transient outward K+ current resulting in SMC hyperpolarization, thereby limiting Ca2+ entry through voltage-gated Ca2+ channels and attenuating arterial contraction (Figure). The role of these channels in the regulation of vascular function has been extensively reviewed elsewhere.12,13,53 Here, we review recent studies identifying a role for aldosterone/MR in the regulation of BKCa channels and their contribution to vascular function.

Previous studies have suggested a link between aldosterone and vascular KCa channels.54 Ambroisine et al55 used a mouse model with cardiomyocyte-specific overexpression of the aldosterone synthase gene (MAS mice), resulting in enhanced MR activation in the heart and coronary circulation. Acetylcholine-mediated relaxation responses in isolated coronary arteries were impaired in MAS mice when compared with wild-type control mice. RNA and protein expression of the BKCa and β1 subunits were decreased in the hearts and coronary arteries of MAS mice. BKCa inhibition with iberiotoxin attenuated acetylcholine-induced relaxation in control mice, and the degree of inhibition was markedly reduced in MAS mice when compared with controls. Moreover, in cultured rat aortic SMCs, aldosterone treatment decreased BKCa subunit expression in a MR-dependent manner. These findings suggest that aldosterone acting on SMC-MR suppresses expression of BKCa. In the MAS model, enhanced cardiomyocyte aldosterone production likely acts in a paracrine fashion to downregulate BKCa channel expression in the adjacent coronary vascular SMC, thereby contributing to impaired endothelium-dependent SMC relaxation. These studies support a direct role for SMC-MR in downregulating the expression of vascular SMC BKCa subunits, contributing to enhanced vasoconstriction (Figure).

Another physiological condition in which vascular function is altered is pregnancy, a state of relative vasodilation. MR has recently been implicated in the blunted vasoconstrictor responses observed during pregnancy.56 Vasconstrictor responses to phenylephrine and KCl are attenuated in pregnant rats, and MR inhibition decreased the vascular contractile response to phenylephrine and KCl during BKCa or Ksyn activation in the pregnant rats but increased the same vasoconstrictor responses in nonpregnant rats.56 Together, these data suggest that MR may regulate vascular BKCa channels and that this regulation may be altered during pregnancy, with MR activation resulting in increased BKCa activity in the vasculature during pregnancy, promoting vasodilation, and decreased BKCa activity in the nonpregnant vasculature contributing to vasoconstriction.

In addition to their role in pregnancy, BKCa channels also contribute to the myogenic tone of resistance vessels, and consequently, to systemic vascular resistance and overall blood pressure regulation.13,51 BKCa subunit knockout mice have higher blood pressure when compared with control mice.57 These mice have increased myogenic tone and attenuated relaxation responses in resistance vessels. Although aged SMC-MR-knockout mice have lower blood pressure than their aged MR intact counterparts, McCurley et al58 found no difference in aortic mRNA expression of BKCa subunits. Moreover, patch clamp electrophysiology studies in mesenteric SMCs revealed no difference in BKCa channel function in mice lacking SMC-MR.

Overall, these studies suggest that aldosterone acting on SMC-MR regulates the expression and function of BKCa channels, thereby contributing to vascular function and blood flow regulation (Table). However, there seem to be differences in the role of MR in BKCa channel regulation in distinct vascular beds (coronary versus mesenteric) and in different physiological states (ie, pregnancy and aging). Furthermore, there are regional differences in the Ca2+ sensitivity of BKCa channels, with a greater Ca2+ sensitivity of cerebral SMC BKCa channels when compared with skeletal muscle.29 In addition, there is a higher ratio of β1-subunits/α-subunit of BKCa channels in the cerebral vasculature versus cremaster SMC.29 Future studies are warranted to clarify the mechanism by which MR

### Table. Effect of Vascular Mineralocorticoid Receptor Activation on Vascular Ion Channels

<table>
<thead>
<tr>
<th>Channel Family</th>
<th>Cell Distribution</th>
<th>Function</th>
<th>Effect of Mineralocorticoid Receptor Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>On Channel Expression</td>
<td>On Vessel Function</td>
</tr>
<tr>
<td>ENaC</td>
<td>Endothelial cell</td>
<td>Maintenance of the mechanical properties of endothelial cells</td>
<td>Increases ENaC mRNA and surface protein expression</td>
</tr>
<tr>
<td>LTCC</td>
<td>SMC</td>
<td>Influx of Ca2+: smooth muscle contraction</td>
<td>Increases Ca1,2 mRNA expression and LTCC activity</td>
</tr>
<tr>
<td>BKCa</td>
<td>SMC (primarily)</td>
<td>Membrane hyperpolarization; smooth muscle relaxation</td>
<td>Decreases BKCa expression and activity</td>
</tr>
<tr>
<td>SKCa</td>
<td>Endothelial cell</td>
<td>Membrane hyperpolarization; endothelial-dependent dilation</td>
<td>Increases SK3 expression</td>
</tr>
</tbody>
</table>

BKCa indicates large-conductance calcium-activated potassium channel; ENaC, Epithelial sodium channel; LTCC, L-type calcium channel; SKCa, small-conductance calcium-activated potassium channel; and SMC, smooth muscle cell.
differentially regulates SMC BKCa channels in specific vascular beds and the role of this regulation in vascular function in healthy and diseased states.

**Small-Conductance Calcium-Activated Potassium Channels**
The small-conductance Ca-activated K channels include 3 channels, SK1 (KCa2), SK2 (KCa2.2), and SK3 (KCa2.3), that are expressed in ECs and play a role in vasorelaxation by contributing to endothelium-derived hyperpolarization of neighboring SMC (Figure). Recent data support a role of endothelial MR in regulation of SK3. SK3 channels are located in interendothelial junctions and colocalized with transient receptor potential channels in the caveolae.59,60 The SK3 channel senses local calcium released in response to mechanical deformation during shear stress and, once activated, causes hyperpolarization of the EC and subsequent SMC hyperpolarization, most likely involving myoendothelial gap junctions. The resultant SMC hyperpolarization inactivates voltage-gated calcium channels promoting vasodilation. Thus, SK3 channels act as part of the endothelium-derived hyperpolarizing factor system to link EC to SMC functionally. The role of SK3 channels in vascular function and hypertension has been reviewed elsewhere.14,15 Here, we focus on recent findings implicating a role of aldosterone/MR in the regulation of SK3 channels in the vasculature.

Zhao et al demonstrated a role of MR in regulating SK3 channels in ECs of the choroidal vasculature in rat and human ocular choriorioretinopathy. Central serous choriorioretinopathy (CSR) is a condition in which there is dilation and leakage of the choroidal vessels of the eye, leading to subretinal serous fluid accumulation and ultimately vision loss. The authors demonstrated that MR activation with aldosterone or high-dose glucocorticoids mimics this condition, which was reversed by cotreatment with MR (but not glucocorticoid receptor) antagonism supporting a specific role of MR in the vasodilation of the eye vasculature in CSR. Furthermore, SK3 expression was confined to the vascular ECs, and aldosterone treatment resulted in a MR-dependent increase in choroidal SK3 protein expression. Inhibition of SK3 (and SK2) with apamin prevented the aldosterone-stimulated choroidal vasodilation and choroidal thickening supporting a role of MR-induction of SK3 in the pathogenesis of CSR. On the basis of this study, a pilot clinical study was performed in 4 patients with CSR. Oral treatment with the MR antagonist eplerenone decreased choroidal fluid accumulation and dramatically improved visual function in all patients. Together, these results suggest that MR in the vascular endothelium of the eye regulates expression of SK3 channels that contribute to vasodilation. Moreover, this mechanism likely contributes to CSR, and MR antagonists may be a viable therapy to restore the vision of these patients.

The role of MR in the regulation of SK3 channels in other vascular beds has yet to be explored. The activity of SK3 channels has been implicated in the development of hypertension because SK3-deficient mice have higher blood pressure than control mice. SK3 channels contribute to blood pressure control and arterial tone by modulating EC membrane hyperpolarization. Thus, if MR also regulates SK3 channels in resistance vessels, this could contribute to vascular tone and blood pressure regulation. Increased expression of SK3 would be expected to promote vasodilation, thereby lowering blood pressure. The direct effects of aldosterone on vascular relaxation are controversial, with some studies demonstrating vasodilation and others vasoconstriction. The ultimate outcome seems to depend on the health of the endothelium. Whether MR regulation of SK3 in the vascular endothelium contributes to aldosterone-induced vasodilation in healthy vessels and whether disruption of this pathway may contribute to cardiovascular disorders associated with endothelial dysfunction awaits further exploration.

**Summary and Perspectives**
This review summarizes emerging evidence that aldosterone and vascular MR regulate ion channel expression and function in the vasculature, in addition to the kidney. Overall, the available data support a role of endothelial MR in upregulation of ENaC, resulting in enhanced endothelial stiffness, and upregulation of SK3, promoting endothelial-dependent vasodilation. In SMCs, MR seems to downregulate BKCa channels in specific vascular beds and physiological conditions and to upregulate LTCCs with aging, both of which would be expected to contribute to enhanced vascular tone and increased blood pressure. These novel mechanisms may be contributing to the extra-renal protective mechanisms of MR antagonism. Future studies are needed to clarify the role of aldosterone/MR in regulation of these ion channels in different cells of the vessel or in distinct vascular beds. The differential effects of MR activation on vascular ion channels under various physiological and disease states, including obesity, diabetes mellitus, heart failure, aging, preeclampsia, and other conditions in which aldosterone levels, MR activity, and vascular function have been found to be altered, remain to be clarified. In addition, the investigation of other vascular ion channels that may be aldosterone/MR regulated is warranted. Some evidence suggests that aldosterone enhances the expression of transient receptor potential channels in rat SMCs. Thus, the area of aldosterone/MR regulation of vascular ion channels awaits further exploration.

Studies of genetic forms of hypertension have led to the identification of genes that cause familial hypertension. These genes, including the MR and ENaC, have later been found to contribute to blood pressure regulation in the much larger population of patients with nongenetic forms of hypertension (essential hypertension). Because every gene identified in such studies has been implicated in regulating renal sodium handling, it has been concluded that hypertension is initiated by a primary defect in renal sodium handling. Although the kidney clearly contributes to the genesis of hypertension, it has subsequently become clear that these same proteins and pathways originally studied in the kidney are also expressed in the vasculature. These new findings support the potential for ENaC, MR, and other genes identified in genetic forms of hypertension to contribute to the initiation of hypertension via direct vascular effects in addition to their role in renal electrolyte regulation. This new paradigm in blood pressure control has important clinical implications that remain to be fully investigated.

Clinical trials, such as the Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS) and RALES (randomized aldactone evaluation study), have demonstrated
that MR antagonism reduces morbidity and mortality in patients with heart failure, despite yielding only small reductions in blood pressure.4,5 Other clinical studies have reported that MR antagonists have cardioprotective effects that cannot be solely accounted for by modest decreases in blood pressure.1,2,6,68 Understanding the extrarenal effects of MR antagonists is limited by renal MR inhibition and the resulting hyperkalemia that occurs in some patients. A detailed understanding of the direct effects of vascular MR on cardiovascular function has the potential to identify treatment targets that achieve the vascular benefits of MR antagonist drugs without the renal side effects. Understanding the specific molecular mechanisms by which aldosterone/MR regulates vascular ion channels may aid in the development of precise and innovative therapeutic approaches to improve cardiovascular outcomes in high-risk populations.

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None.

References


47. Garwitz ET, Jones AW. Aldosterone infusion into the rat and dose-dependent changes in blood pressure and arterial tonic ion transport. Hypertension. 1982;4:374–381.


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Beyond the Kidney
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