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Is the Mineralocorticoid Receptor a Potential Target for Stroke Prevention?

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Abstract

In recent years it has become increasingly clear that the extra-renal effects of aldosterone play an important role in the pathogenesis of cardiovascular disease. Stroke is one of the leading causes of death in the Western world; mineralocorticoid receptor (MR) antagonism is a potential preventative therapy for patients at risk for both ischemic and hemorrhagic strokes. This protective effect of MR antagonism appears to occur at the level of the cerebral vasculature and may be related to the expression and activation of the epidermal growth factor receptor and the degree of vessel wall collagen deposition.

Stroke is a leading cause of morbidity and mortality in the Western world [1]. The majority of strokes that occur in the population are ischemic in nature, however the therapeutic options for the treatment of these strokes are few [2]. Tissue plasminogen activator (TPA) is the only currently approved treatment for ischemic stroke. Yet, only 3% of stroke patients receive this therapy [3] and 10% of these patients die before leaving the hospital [4]. Until our understanding of the factors affecting the outcome of stroke increases, the paucity of useful therapies to be administered post-stroke is unlikely to change. It would therefore seem prudent to also study the factors that increase an individual's risk of having a stroke with a view of developing preventative therapies. Recently, it has become increasingly clear that aldosterone has deleterious extra-renal effects that play an important role in the pathogenesis of cardiovascular disease and its associated end-organ damage. Several studies have suggested that mineralocorticoid receptor (MR) antagonism with eplerenone or spironolactone has beneficial effects on the cerebral vasculature that could function to reduce an individual's risk of having a stroke [5-8]. Our understanding of the molecular mechanisms underlying the effects of aldosterone in the vasculature is limited when compared to our knowledge of its effects in the kidney. However, one thing is clear: a nephrocentric view of aldosterone as a hormone that merely regulates salt and water balance is no longer appropriate. There are clear links between aldosterone and hypertension and between hypertension and stroke risk, therefore, this review will focus primarily on the potential effects of aldosterone in the cerebral vasculature and how that might affect the outcome of cerebral ischaemia. We will also discuss potential mechanisms for aldosterone induced vascular changes and review the evidence that in some models of hypertension the MR is activated not by aldosterone but by glucocorticoids.

Vascular Structure and Stroke

Hypertension is one of the primary risk factors for stroke [9] and prior to discussing how aldosterone might affect the cerebral vasculature it is necessary to consider how hypertension in general affects the cerebral vessels and the outcome of stroke. At the level of the vasculature

there are two ways that the outcome of ischaemia could be affected, a reduction in the number of collateral vessels would reduce blood flow and therefore increase infarct size. Similarly, remodeling of the vessels in a manner that would reduce the lumen diameter would also reduce flow and increase the damage post-stroke. As the number of collateral vessels present in the brain of the SHRSP and WKY rats does not differ [10] a reduction in vessel lumen diameter seems to be the likely cause of the increased ischemic injury in the hypertensive rats. The stroke-prone spontaneously hypertensive rat (SHRSP), a model of essential hypertension and cerebrovascular disease, has been extensively studied to investigate the factors affecting the outcome of both ischemic and hemorrhagic strokes. SHRSP suffer spontaneous hemorrhagic strokes when fed a high-salt diet [11], and when cerebral ischaemia is induced experimentally by middle cerebral artery (MCA) occlusion the SHRSP have significantly more neuronal damage than normotensive Wistar Kyoto (WKY) rats [12].

Under non-ischemic conditions there is very little blood flow through the collateral vessels [13], but these vessels dilate in response to an ischemic insult to increase perfusion, effectively “bypassing” the blockage. The ability of these vessels to dilate is impaired in the SHRSP and this may contribute to the larger infarct observed in these rats [14,15]. The impaired ability to dilate in response to ischaemia may be caused by vascular remodeling which is a complex process and the subject of many excellent reviews [16,17]. Cerebral vessels from hypertensive rats undergo mostly inward eutrophic remodeling [18] which is characterized by a reduction in the vessel lumen and outer diameters without a significant change in wall thickness. Thus, there is thought to be little cell proliferation but that the existing cells rearrange themselves around a smaller lumen. This type of remodeling also occurs in patients with essential hypertension [19] and is thought to be particularly important in the cerebral vasculature because it impairs the vessel's ability to autoregulate [20] and dilate [14,15]. However, there is also a small amount of hypertrophy of the vessel walls of cerebral arterioles from SHRSP which have been shown to contain more vascular smooth muscle cells (VSMC) and elastin than those from WKY rats [21]. The hypertrophy appears to be pressure-dependent, whereas the rearrangement of the VSMC is pressure-independent [22]. It should be noted that these studies assessed the lumen diameter of cerebral arterioles and not of the MCA. This also raises an important point: not all branches of the cerebrovascular tree respond to hypertension in the same way. For instance, our lab has recently shown that dietary potassium supplementation improves the compliance of the branches of the MCA from SHRSP but has no effect on the MCA itself [23].

Mineralocorticoids and Stroke

Various studies have suggested that elevated plasma aldosterone or MR activation increases both the risk for and severity of cerebrovascular events. Studies of patients with primary aldosteronism (PA) performed by Conn in the 1960s were some of the first to suggest a link between hypertension, stroke and elevated plasma aldosterone levels [24]. Like PA patients, patients with increased plasma aldosterone caused by glucocorticoid-remediable hypertension have an increased frequency of stroke and hypertension [25,26]. Interestingly, when PA patients and patients with essential hypertension are compared, the PA patients suffer from more strokes despite having slightly lower blood pressure than patients with essential hypertension [27]. This suggests that elevated plasma aldosterone may increase an individual's risk of having a stroke in a blood pressure-independent manner. The link between aldosterone and stroke has been further strengthened by studies using inhibitors of the renin-angiotensin-aldosterone system (RAAS). RAAS inhibition confers a greater protection from stroke than can be accounted for by the reduction in blood pressure alone [28,29], presenting the possibility that reduced aldosterone production in these patients is responsible for at least part of the beneficial effects. Importantly, it is now accepted that aldosterone is involved in 10-15% of the cases of hypertension previously classified as essential hypertension [30]. If a causative

link between aldosterone and stroke exists then this finding significantly increases the percentage of the population at risk for a cerebrovascular event.

The link between mineralocorticoids and stroke has been established experimentally. Administration of MR antagonists (spironolactone or eplerenone) to SHRSP fed a high-salt or stroke-prone diet prevents spontaneous hemorrhagic strokes without lowering blood pressure [8]. Administration of captopril, an angiotensin converting enzyme (ACE) inhibitor, to salt-loaded SHRSP has a similar effect. Interestingly, the beneficial effects of captopril are lost when a mineralocorticoid is administered with the ACE inhibitor [31,32], suggesting that some of the benefits of ACE inhibition result from a reduction in angiotensin II (AngII) stimulated aldosterone release. Spironolactone treatment has extremely dramatic effects on the outcome of acute ischemic strokes, in fact it reduces the damage caused by cerebral ischaemia by 50% in SHRSP [5]. In this study spironolactone had no effect on the systolic blood pressure suggesting again that aldosterone has deleterious actions on the cerebral vasculature that occur independently of its ability to increase blood pressure. The possibility that the effects of MR activation on the cerebral vasculature are blood pressure-independent is further corroborated by studies showing that treatment of normotensive WKY rats with spironolactone also reduces the ischemic cerebral infarct size [33].

Mineralocorticoids and Vessel Structure

As mentioned above, the structure of the cerebral vasculature is a major determinant of the outcome of cerebral ischaemia. The effect of spironolactone on MCA structure has been studied in SHRSP. Studies in which SHRSP were treated with spironolactone from six to twelve weeks of age showed that MR antagonism significantly increases the MCA lumen and outer diameters but had no effect on vessel wall area. This is consistent with a prevention of the eutrophic remodeling during the development of hypertension [6]. Interestingly, an increase in myogenic tone with spironolactone treatment was also observed in these rats; this may represent an improvement of the ability of these MCAs to autoregulate, which may in turn be the mechanism for the protective effects of spironolactone against hemorrhagic strokes. While these studies suggest spironolactone can prevent the hypertension driven vessel remodeling, the reversal of remodeling is more important clinically. Therefore, the studies described above were repeated in adult SHRSP. SHRSP were treated with spironolactone from twelve to eighteen weeks of age. Importantly, at twelve weeks of age the MCA remodeling in the SHRSP is marked and maintained as there is no difference between the structure of the MCAs from the twelve and eighteen week old rats. Spironolactone treatment also increased the lumen and outer diameters of the MCAs from the adult rats [34]. What is of interest is that in the young rats (age 6-12 weeks) the prevention of the remodeling was complete, i.e. the vessels from the spironolactone treated SHRSP behaved for all intents and purposes like a vessel from a WKY rat. However, in the older rats (age 12-18 weeks) the lumen diameter of the spironolactone treated SHRSP was between that of the control SHRSP and the WKY rats; the lumen is significantly larger than the former and smaller than the latter. What prevents these older rats from “completely” remodeling in response to spironolactone treatment is unclear; it could be an effect of the duration of the hypertension or it is possible that in the older rats the remodeling process is “set”, where as in the young rats the process is still active and therefore more pliable.

Studies using ACE inhibitors and angiotensin receptor blockers also support a role for the RAAS in hypertensive remodeling of the cerebral vasculature. Both ACE inhibition and angiotensin receptor blockade increase cerebral arteriolar lumen diameter [35,¹⁶,32]. Whether this is an aldosterone mediated effect or strictly mediated by AngII has not been investigated, but it seems a likely possibility in light of the studies of hemorrhagic stroke with ACE inhibition which show that aldosterone administration rescues the SHRSP phenotype [31,32]. Interestingly, there appears to be some crosstalk between the MR and the AT1 receptor, which

is elevated in the cerebral microvessels from hypertensive rats [35]. Jaffe and Mendelsohn [36] have shown that AngII regulates gene transcription in cultured VSMC. Surprisingly, the effects of AngII could be inhibited by spironolactone, suggesting that the MR is involved in the AngII-dependent modulation of gene transcription and that AngII may transactivate the MR. Aldosterone synthase, was not present in the VSMC, ruling out the possibility that the actions of AngII were via local aldosterone production. If AngII transactivates the MR in the cerebral vasculature this could potentially enhance the actions of aldosterone and increase the risk of stroke.

One caveat that must be included in the discussion of these studies is that they all took place in genetically hypertensive rats. This makes it difficult to distinguish the specific effects of MR activation versus blood pressure dependent effects. One other concern is that spironolactone has several effects other than MR antagonism; of particular importance here is its antagonistic effects on the testosterone receptor. Short term testosterone administration increases the lesion size after ischaemia-reperfusion injury in rats [37], thus it is possible that testosterone receptor antagonism could reduce infarct size. However, it should be noted that these studies were of transient focal ischaemia, the pathogenesis of which is different from that of permanent ischaemia. Additionally, there are also studies showing that unlike spironolactone, testosterone has no effect on vessel structure [38]. This, taken with the knowledge that eplerenone, a more specific MR antagonist with no anti-testosterone actions, is as effective as spironolactone at reducing the number of hemorrhagic strokes in salt-loaded SHRSP [39] suggests that the effects of spironolactone on the vasculature are MR and not testosterone receptor dependent.

To further confirm a role for MR activation in cerebral vessel remodeling we have performed studies to directly activate the MR with deoxycorticosterone acetate (DOCA) [40]. In these studies rats were treated with DOCA but did not undergo uninephrectomy or receive salt water. This treatment causes a modest increase in systolic blood pressure (157 ± 5.9 vs 124 ± 3.1 mmHg, DOCA vs control) and an approximately 40% increase in the amount of damage caused by cerebral ischaemia. The effects on the MCA were also dramatic; DOCA treatment reduced the lumen and outer diameters. Surprisingly, DOCA treatment also caused a significant increase in vessel wall thickness, suggesting that direct MR activation perhaps has an effect on VSMC hypertrophy. These results challenge the accepted dogma that there is little proliferative remodeling in the cerebral vasculature, but the question of the blood pressure dependency of this effect remains.

A recent study from Watts *et al* [41] may shed some light on this issue. In this study aorta and vena cava from DOCA-salt hypertensive rats were compared. As expected the aorta showed marked remodeling whereas none could be detected in the vena cava. Both vessel types were exposed to similar levels of circulating mineralocorticoids but only the aorta was exposed to the increase in pressure, suggesting that an elevation in blood pressure may be required for MR dependent remodeling to occur. However, it should be noted that remodeling is extremely difficult to assess in veins because they have so little smooth muscle mass and such a large amount of collagen. It is also possible that vena cava and aorta have differential expression of the MR, but to the best of our knowledge there have been no studies comparing the expression of the MR between arteries and veins. It should also be noted that these studies were conducted in conduit vessels and that the response in resistance vessels may prove to be quite different.

Studies of the effects of aldosterone on vessel structure in humans are few. Recently an elegant study compared the resistance vessels of patients with hypertension to patients with PA. Interestingly, the pattern of remodeling in arteries from gluteal biopsies appears to be different between the two groups despite similar blood pressure. Both the hypertensive and PA patients had increased media thickness and media/lumen ratio when compared to normotensive

controls. However, only the PA patients had a significant reduction in lumen diameter probably driven by an enhanced increase in the media thickness compared to the hypertensive patients [42]. This suggests that, as indicated above, direct MR activation does stimulate a small amount of vascular hypertrophy.

The possibility that MR activation results in VSMC proliferation has been further corroborated by studies showing that aldosterone increases the levels of murine double minute type 2 (MDM2) protein in VSMC [43]. The MDM2 protein has previously been shown to be involved in cell cycle regulation [44]. Interestingly, MDM2 levels were not only found to be increased in VSMC treated with aldosterone, but also in resistance vessels from patients with PA (Figure 1) [43].

It should also be pointed out that not all of the effects of aldosterone or spironolactone on the vasculature occur at the level of the smooth muscle. Recent studies have suggested that spironolactone has beneficial effects on endothelial cells. Several studies have shown that spironolactone treatment improves endothelium dependent vasodilatation in patients with congestive heart failure [45,46]. Studies using human umbilical vein endothelial cells (HUVEC) suggest that spironolactone prevents endothelial cell apoptosis induced by serum deprivation. Interestingly however, this protective effect of spironolactone appears not to be mediated by its ability to antagonize the MR but may instead be a function of its ability to activate the progesterone receptor [47]. Other studies using HUVECs suggest that aldosterone can increase reactive oxygen species (ROS) generation from endothelial cells and increase the expression of the p47^{phox} subunit of the NADPH oxidase enzyme. The function of endothelial nitric oxide synthase (eNOS) is also reduced by aldosterone this is caused by a reduction in the availability of the eNOS co-factor 5,6,7,8-tetrahydrobiopterin, activation of protein phosphatase 2A [48] and a reduction in eNOS phosphorylation [49]. While these studies may explain why spironolactone therapy increases endothelium dependent vasodilation in heart failure patients and in patients with PA [45,46], a note of caution is warranted: the concentration of aldosterone used in some of these studies was 100 fold higher than what would be considered to be physiological levels.

Potential Mechanisms for the Effects of MR Activation

As mentioned previously, our understanding of the molecular mechanisms underlying the effects of aldosterone in the vasculature are limited. Therefore, we will limit our discussion to pathways which evidence suggests are important in cerebral vessel remodeling (Figure 1).

Enhanced Epidermal Growth Factor (EGF) Receptor Expression

Studies of MCA structure after DOCA administration suggest there may be VSMC proliferation [40] and aldosterone has been shown to stimulate VSMC proliferation in culture [50]. EGF and its receptor (EGFR) are possibly the best candidates for a signaling mechanism for this and several studies have linked this pathway and aldosterone. EGF is a VSMC mitogen that has previously been implicated in the hypertensive remodeling process [51-53]. That said, when one considers the effect of spironolactone on the MCA of SHRSP [6] it is difficult to envision a role for cell proliferation in the remodeling process as spironolactone has no effect on the amount of vessel wall material. However, it should be noted that EGFR activation is not only involved in proliferation but also in cell migration [54], as is required for the inward eutrophic remodeling process. Also, in our initial studies of the effects of spironolactone on the outcome of cerebral ischaemia we observed an increase in the mRNA for EGF and EGFR in vessels from SHRSP compared to WKY rats. Importantly, spironolactone treatment reduced the expression of both the receptor and ligand [5]. As discussed earlier, spironolactone prevents the hypertension induced remodeling process [6] and the possibility that EGF is involved in this process cannot be ruled out.

Some of the first studies suggesting a link between aldosterone and EGF signaling pathway were studies of vascular reactivity. Florian and Watts showed that EGF causes a marked contraction of aorta from DOCA-salt hypertensive compared to control rats [55]. Later studies showed that this was not just an effect of elevated blood pressure [56]. These studies utilized Wistar and Wistar Furth rats, the former become hypertensive with uninephrectomy and administration of DOCA and salt, while the Wistar Furth rats remain normotensive [57]. Vessels from both Wistar and Wistar Furth rats treated with DOCA responded to EGF by contracting more than their respective controls [56], this contraction appears to be dependent on activation of the PI3 kinase and extracellular regulated kinase (ERK1/2) pathway [58]. These studies also add weight to the argument that the effects of aldosterone on the vasculature occur independently of its effects on blood pressure. Additional studies carried out to assess the effects of DOCA-salt administration on the expression of mRNA and protein for the EGFR showed that the mRNA levels were increased but that the protein itself was not elevated [59]. Proof that aldosterone increases the expression of EGFR protein in the vasculature remained elusive until recently. Grossmann *et al* [60] showed that aldosterone increases EGFR protein expression in aorta from adrenalectomized rats and in cultured human aortic VSMC. These studies identified the area of the EGFR promoter with which the MR interacts. Importantly, the glucocorticoid receptor does not interact with this area, suggesting that the authors may have identified a novel mineralocorticoid responsive element. The increase in expression of EGFR was confirmed to be due to increased EGFR production, as EGFR degradation was unaffected by aldosterone. The newly produced EGFR was also shown to be functional; EGF induced ERK1/2 phosphorylation was also enhanced with aldosterone treatment.

Rapid EGFR and Tyrosine Kinase Activation

The rapid non-genomic effects of aldosterone occur within minutes and are insensitive to protein synthesis inhibition or to the actions of classical MR antagonists [61]. This suggests that aldosterone activates the EGFR and other tyrosine kinase pathways. The initial studies documenting the effects of aldosterone on EGFR were carried out in Chinese hamster ovary cells [62] and in renal epithelial cells [63]. In VSMC, aldosterone and AngII act synergistically to increase EGFR activation. Low doses of aldosterone or AngII alone have no effect on proliferation, but when the two are combined they increase proliferation via an EGFR dependent mechanism [64]. In these studies the increase in EGFR phosphorylation also resulted in an increase in the activation of ERK1/2. In other studies the AngII induced phosphorylation of ERK1/2 and c-Jun N-terminal kinase (JNK) has been shown to be enhanced by the presence of aldosterone. Interestingly, spironolactone prevented not only the response to AngII and aldosterone together, but also the increase in ROS production and EGFR activation caused by AngII alone [65]. These studies suggest that AngII might stimulate the release of aldosterone from VSMC and that this locally produced aldosterone is responsible for the effects that can be inhibited by spironolactone. The notion that the cardiovascular system can produce aldosterone has become increasingly controversial. Studies have suggested that VSMC can produce significant amounts of aldosterone and that the enzymes responsible for aldosterone production are present in these cells [66,¹⁷,67,68]. However, more recent studies using state-of-the-art technology have failed to show evidence of aldosterone production at least in the heart [69]; as yet the presence of aldosterone production in VSMC has not been confirmed or refuted.

The non-genomic actions of aldosterone that could stimulate cell proliferation are not limited to the EGFR. Recently, Big MAP kinase 1 (BMPK1), a MAP kinase involved in cell proliferation, differentiation and survival, has been shown to be rapidly activated by aldosterone in VSMC. This activation resulted in VSMC proliferation that could be inhibited by eplerenone [50]. Aldosterone also activates p38 MAP kinase and NADPH oxidase; this effect appears to be dependent on c-Src activation as it can be inhibited by PP-2, the Src-kinase

inhibitor [70]. In these studies an increase [^3H] proline incorporation, a marker of collagen synthesis, was observed in response to aldosterone, and this will be discussed further later. These effects of aldosterone were enhanced in cells from hypertensive rats [71].

Care should be taken in the interpretation of these results; all of these studies of the rapid effects of aldosterone have been performed in cell culture where the effects of flow and pressure are lost. How these rapid responses to aldosterone will affect homeostasis is not clear. The question that remains is how physiologically relevant is an event that has only been shown to occur in cultured cells, which are of a proliferative and non-contractile phenotype?

While most effects of aldosterone on tyrosine kinase activation have been described in VSMC there is some evidence that aldosterone activates tyrosine kinases in endothelial cells. Incubation of endothelial cells with aldosterone caused an increase in the mRNA for ACE; this was inhibited by genistein, a broad-spectrum tyrosine kinase inhibitor, and by JAK2, src and EGFR inhibitors. The phosphorylation of JAK2 was measured, and interestingly, it does not appear to follow the classical non-genomic timeline. The maximum activation of JAK2 appears to occur after about 2 hours incubation with aldosterone. It is also worth noting that the concentration of aldosterone used in these studies was extremely high (10^{-6}M) [72].

The location of the receptor responsible for the rapid actions of aldosterone remains, in essence, a mystery. It has been proposed that it is a membrane bound receptor that co-localizes with caveolin-1 [73]. Conversely, others have suggested that an intracellular receptor is responsible for the rapid effects of aldosterone [74,65] and that the MR does not colocalize with $\alpha 1$ -integrin which is a membrane marker [46]. However, care should be taken in the interpretation of this result as these studies used a commercially available MR antibody directed to the classical MR, it is possible that the MR that causes the rapid non-genomic responses does not react with this antibody. Because so many of the rapid actions of aldosterone occur via membrane associated proteins it seems more likely that the receptor responsible for this effect is membrane bound itself.

Collagen Deposition

Studies of the MCA show that when the MR is inhibited in hypertensive rats the vessels become less stiff or more compliant [6] and that the opposite is true when the MR is activated by DOCA [40]. The suggestion that MR activation increases fibrosis and collagen deposition is not new and has been eloquently reviewed by other groups [75,76]. For instance, congestive heart failure increases aldosterone levels, which in turn appears to increase carotid artery stiffness and collagen deposition, that can be reduced by spironolactone [77]. What is rather surprising is the apparent lack of understanding of how aldosterone modulates collagen deposition in the vasculature. In theory, collagen levels can increase in two ways, by an increase in collagen synthesis or by a reduction in collagen metabolism.

Some of the cell culture studies already discussed with reference to tyrosine kinase activation also reported an increase in [^3H] proline incorporation into cells treated with aldosterone and that this response is enhanced in cells from hypertensive rats [71,70]. Although actual collagen release was not measured, these studies suggest an increase in collagen deposition in response to aldosterone that is exacerbated by hypertension. One well-designed study carried out using human aortic VSMC suggests that aldosterone alone is not sufficient to increase collagen synthesis, but that aldosterone can potentiate the hydrogen peroxide induced collagen accumulation. Interestingly, this effect of aldosterone was prevented by GM6001, a compound that inhibits the shedding of membrane bound EGFR ligands and therefore reduces EGFR activation. An increase in EGFR activation by aldosterone in the presence of hydrogen peroxide was confirmed as was the ability of EGFR blockade to prevent the increase in collagen accumulation [78]. Therefore, these studies suggest that the interaction between EGFR and

aldosterone may not only drive the remodeling process, but also the vessel stiffness that is observed with hypertension.

Studies of cardiac tissue have shed some light on the molecular mechanisms behind the increase in collagen deposition in the presence of aldosterone. Procollagens are processed into mature fibrillar collagen by procollagen C-proteinases. Procollagen C-proteinase enhancer protein (PCPE-1) increases the rate of activation of the procollagen C-proteinase to allow for increased mature collagen production [79]. Collagen I and PCPE-1 expression is positively regulated in cultured cardiac myocytes by aldosterone [80]. Recently, PCPE-1 has also been shown to be increased in the hearts of rats after myocardial infarction, a situation where aldosterone levels are markedly elevated. Importantly, the increase in PCPE-1 could be inhibited by spironolactone, suggesting an MR dependent effect [81].

Connective tissue growth factor (CTGF) is activated in situations where fibrosis occurs, promotes fibroblast proliferation and intercellular matrix deposition [82] and may also be regulated by aldosterone in the aorta. These studies showed that expression of this growth factor was higher in the vasculature of SHR and that it could be lowered by a dose of eplerenone that had no effect on blood pressure [83]. CTGF does not cause cell proliferation, instead it appears to activate caspase 3 and induce apoptosis [84]; its role in the hypertensive remodeling process has not been completely investigated.

Surprisingly, not all studies report an increase in vessel collagen with aldosterone treatment. Aldosterone-salt treatment increases carotid artery stiffness, but this increase was related to an increase in fibronectin and not collagen or elastin [85]. It is important to note that the rats used in this study were younger than those used by most groups to produce aldosterone or DOCA-salt dependent hypertension models, which may have affected the remodeling process.

Studies comparing PA patients with hypertensive subjects suggest that hypertension *per se* is not the sole regulator of collagen synthesis. An assessment of collagen levels in resistance vessels from gluteal biopsies showed that essential hypertension and PA increased the total collagen content in the vessel walls and the collagen III content, in particular. However, the magnitude of the increase was significantly greater in the PA patients compared to the essential hypertensive patients [42] indicating a role for aldosterone in the increased collagen deposition.

Matrix metalloproteinases (MMPs) digest extracellular matrix components including collagen [86]. The gelatinases (MMP-2 and -9) may play a role in the vascular remodeling caused by a combination of VSMC migration and by extracellular matrix metabolism [87]. One study showed that MMP-2 activity increases in the media of the coronary vessels from DOCA-salt treated rats [88]. As is so often the case with this model of hypertension, it is impossible to assess if this is a direct effect of aldosterone or due to the malignant hypertension that develops in these rats. However, a study using cultured cardiac myocytes has shown that aldosterone increases MMP-2 and -9 activity [89]. Interestingly, the increase in activity appears to be caused by a rapid non-genomic activation of a PKC and ROS-dependent pathway that results in the activation of MEK/ERK. Surprisingly, aldosterone has been shown to have no effect on the activity of the MMPs in cardiac fibroblasts [90], suggesting that the effects of aldosterone on MMP activity and expression are cell type dependent. Interestingly, MMPs are also thought to regulate VSMC proliferation by causing the release or activation of proteins with proliferative properties such as β -catenin [91].

MR Specificity for Aldosterone

Antagonism of the MR is beneficial in several cardiovascular pathologies even when aldosterone levels were not elevated. In recent years this has raised the question of whether a molecule other than aldosterone is responsible for increased MR activation in these conditions.

Cortisol, the predominant glucocorticoid in humans, binds the MR with the same affinity as aldosterone, and circulates at much higher concentrations than aldosterone [92,93]. However, MR specificity for aldosterone is preserved by activity of the enzyme 11 β -hydroxysteroid dehydrogenase type II (11 β HSD2) (Figure 2) [94,95]. 11 β HSD2 is expressed in aldosterone target tissues [96], including blood vessels, and converts cortisol to its inactive form cortisone (corticosterone to 11-dehydrocorticosterone in rodents), which does not have a high affinity for the MR. This process serves to protect the MR from cortisol occupancy. Aldosterone, on the other hand, is unaffected by 11 β HSD2. Due to the higher levels of circulating glucocorticoids, inactivation of 11 β HSD2 allows increased cortisol occupancy of the MR, reducing its activation by aldosterone. The activity of 11 β HSD2 is impaired congenitally in a condition known as the syndrome of apparent mineralocorticoid excess (AME) or by excessive glycyrrhetic acid consumption, the active ingredient in licorice [97,⁹⁸,95]. Both conditions mimic mineralocorticoid excess as indicated by sodium retention, hypokalemia and hypertension; however, it has been reported that in patients with AME, plasma aldosterone and renin concentrations remain low while the ratio of cortisol to cortisone metabolites is elevated [99].

Evidence suggests that although cortisol binds the MR, binding alone may not be sufficient to activate the receptor. NAD⁺ is required for the conversion of cortisol to cortisone, and therefore activity of the enzyme 11 β HSD2 is dependent on the redox status of the cell. This concept has been reviewed in detail [100], and a study by Ward *et al.* highlights the importance of increased ROS on cortisol's ability to go beyond simply occupying the MR to activating it [101]. In this study, endogenous cortisol did not activate MR in porcine coronary vessels until the redox state was altered as a result of angioplasty-induced damage. Additionally, a study by Young *et al.* revealed that inhibition of 11 β HSD2 with carbenoxolone in combination with 0.9% NaCl mimicked the deleterious cardiac effects of deoxycorticosterone (DOC) treatment, and these effects were reversed by the MR antagonist eplerenone [102]. These studies demonstrate that with increases in ROS, the redox state of the cell changes, and cortisol becomes an MR agonist, mimicking the effects of aldosterone. The mechanism by which this occurs, however, is not completely understood.

While AME is quite rare, diseases in which oxidative stress is increased, such as obesity, diabetes and hypertension, may involve an impairment in the ability of 11 β HSD2 to protect the MR from cortisol binding due to alterations in the redox status. With this in mind, it seems possible that these disease states may be examples of disproportionate MR activation by glucocorticoids rather than aldosterone. This scenario may provide an explanation for the beneficial effects of MR antagonism observed in the Randomized Aldactone Evaluation Study (RALES) and Eplerenone neuroHormonal Efficacy and SURvival Study (EPHESUS) trials in heart failure patients possessing normal plasma aldosterone levels, but likely having elevated oxidative stress [103,104]. A recent study also demonstrated beneficial effects of MR antagonism with eplerenone in a low aldosterone model of heart failure in rats [105]. Despite decreased renin activity and aldosterone concentrations Dahl salt-sensitive rats with congestive heart failure showed improvement in left ventricular hypertrophy, inflammation and oxidative stress with a non-antihypertensive dose of eplerenone. This study further underscores the notion that many cardiovascular pathologies may involve disproportionate MR activation via glucocorticoids, and patients with these conditions will likely benefit from treatment with an MR antagonist. As the incidence of diabetes and obesity increase in the population, so does the number of people suffering from oxidative stress. If the pathway described above is correct, this also dramatically increases the number of patients who will suffer from MR-dependent vascular diseases.

Conclusions

We have presented evidence that aldosterone or MR activation increases the risk of and the damage caused by a stroke via alterations in the structure of the cerebral blood vessels. It seems that the interaction between the MR and the EGFR, both at the genomic and non-genomic level, is a likely candidate for intervention in this pathway. Still several questions remain, not least of these is the blood pressure dependency of the effects of MR activation on the vasculature. Also, the physiological relevance of the rapid effects of aldosterone and the potential that multiple forms of the MR exists needs to be evaluated, as does the notion that the MR can be activated in different ways depending on the oxidative state of the cell.

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Figure 1. Proposed mechanisms by which aldosterone alters vascular structure via genomic and non-genomic effects on the vascular smooth muscle cell.

Figure 2.

Proposed mechanism for the activation of the mineralocorticoid receptor by glucocorticoids in vascular smooth muscle cells. The dotted arrow represents alternative activation of the MR by cortisol under conditions where elevations in ROS allow cortisol to activate the MR.