Published in final edited form as:

Trends Endocrinol Metab. 2005 November ; 16(9): 401–406. doi:10.1016/j.tem.2005.09.002.

Aldosterone: good guy or bad guy in cerebrovascular disease?

Christiné S Rigsby, William E Cannady, and Anne M Dorrance

Department of Physiology, Medical College of Georgia, 1120 15th Street, Augusta, GA 30912, USA

Abstract

Stroke is a leading cause of disability in the Western world, yet the choices for therapeutic intervention are few. The complex role played by aldosterone in the pathogenesis of stroke is beginning to emerge. Chronic mineralocorticoid receptor (MR) blockade reduces the incidence of hemorrhagic strokes and the severity of damage caused by ischemic strokes. This appears to be a vascular phenomenon because MR blockade increases vessel lumen diameter, which presumably increases blood flow and perfusion of the tissue to reduce ischemic damage. However, the vascular protection afforded by MR antagonism is at odds with the results seen within the brain, where MR activation is required for neuronal survival. Both of these divergent effects have possible therapeutic implications for stroke.

Introduction

Stroke is the third leading cause of death in the Western world [1]. Strokes, at their simplest, can be categorized as hemorrhagic or ischemic, resulting from the rupture or blockage of a cerebral vessel, respectively. Ischemic strokes are more common and account for most of the disability caused by stroke. Few stroke therapies have successfully made the transition from bench to bedside [2], and those that have must be administered within such a tight time window post-stroke that only a small proportion of patients receive treatment. This situation is unlikely to change until our understanding of the factors affecting the outcome of stroke increases. Experimentally, stroke therapies are thought of as preventative therapies to protect the vasculature, or as treatments to improve neuronal survival post-stroke. Interestingly, mineralocorticoid receptors (MRs) have been identified in the brain [3] and blood vessels [4], making them a potential target for both types of intervention. Paradoxically, preventative therapy would require MR blockade and neuronal therapy would require MR activation. In this review, we present evidence of the opposing actions of MR activation in the vasculature and brain on the outcome of stroke. The molecular mechanisms of neuronal death are not discussed at length here; others have eloquently described them [5,6], and Box 1 contains a brief overview of the subject.

Vascular structure and stroke

Stroke is defined as an interruption in cerebral blood flow. Blood flow is controlled by vascular resistance, which in turn is controlled by blood viscosity, vessel length and lumen diameter. Because vessel length and blood viscosity vary little, changes in lumen diameter, caused by vasoconstriction or vascular remodeling, are the important determinant of resistance. Vascular remodeling is a complex process and the subject of many excellent reviews [7,8].

Hypertension is a risk factor for stroke [9]. Stroke-prone spontaneously hypertensive rats (SHRSP), a model of essential hypertension and cerebrovascular disease, have been used to study factors affecting the outcome of cerebral ischemia and hemorrhage. When fed a high Na^+ diet, SHRSP have more spontaneous hemorrhagic strokes than normotensive Wistar Kyoto (WKY) rats [10]. When cerebral ischemia is induced experimentally, the volume of the resultant infarct is greater in SHRSP than in WKY rats [11] (Figure 1). This might reflect differences in the morphology of the cerebral vessels between the two strains. In normotensive rats under non-ischemic conditions, little blood flows although the collateral vessels, but when an ischemic blockage occurs, these vessels dilate to increase tissue perfusion, effectively 'bypassing' the blockage. The ability of these vessels to dilate in response to ischemia is impaired in SHRSP, which might contribute to the larger infarct seen in these rats [12,13].

Cerebral vessels from hypertensive rats show primarily inward eutrophic remodeling, involving rearrangement of the vascular smooth muscle cells (VSMC) around a narrower lumen, with the end result being a vessel with a smaller external and internal diameter [14]. This type of remodeling is well characterized; it occurs in patients with essential hypertension [15] and is particularly important in the cerebral vasculature because it impairs autoregulation [16] and dilation [12,13]. Therefore, treatments that prevent or reverse remodeling should reduce ischemic damage by increasing blood flow.

Although the reduction in cerebral vessel lumen diameter is mostly caused by rearrangement of the VSMC, some hypertrophy is involved in the process. Morphological assessment of cerebral arterioles from SHRSP shows that they contain more VSMC and elastin than those from WKY rats [17]. The hypertrophy appears to be pressure dependent, whereas the rearrangement of the VSMC is pressure independent [18].

Box 1

How neurons die during stroke

Neurons within the ischemic core die primarily from necrosis. Within minutes after the loss of blood flow to the brain, the amount of oxygen and glucose available to the cells falls and this causes acidification of the cell as the byproducts of metabolism increase. This reduction in pH causes the electron transport chain in the mitochondria to fail so that ATP levels fall. The lack of ATP causes a reduction in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, and an increase in intracellular Na^+ causes the cell membrane to depolarize and Ca^{2+} flow into the cell to increase. Eventually, the membrane depolarizes enough to reach its electrical threshold and the neurons exhibit ischemic discharges; they fire repetitively, releasing neurotransmitters, particularly glutamate, into the surrounding area.

The high intracellular Ca^{2+} concentration also increases the activity of calpain protease; this affects the structural integrity of the cell and damages the cell membrane by increasing phospholipase activity. Ca^{2+} also increases the synthesis and activity of nitric oxide synthase, causing the formation of nitric oxide and the free radical peroxynitrate. Eventually the mitochondria, which try to sequester the excess Ca^{2+} succumb to Ca^{2+} toxicity and release cytochrome C into the cytoplasm. The cells eventually become so damaged that they are no longer viable.

The ischemic penumbra surrounds the ischemic core and here blood flow is reduced to a lesser extent than in the core; thus, the changes to the cells are less catastrophic. Glutamate levels increase within the penumbra as a result of the ischemic depolarization and release from damaged neurons. Glutamate activates the NMDA (*N*-methyl-D-aspartate) and AMPA receptors to increase intracellular Na^+ and Ca^{2+} . Glutamate also activates the metabotropic receptor, resulting in an increase in cAMP levels, which alters protein kinase activity and increases proteolysis and lipolysis. Free radicals also contribute to the damage in the

penumbra; free radicals can emanate from the core or can be produced within the penumbra, particularly during reperfusion, to cause damage to the DNA and cell membrane. The cells within the penumbra primarily die by apoptosis and this process, unlike necrosis, requires energy and protein synthesis. Apoptosis might be initiated by the increases in cytochrome C and calpain activity. For more details of the mechanisms of ischemia-induced cell death see [2,5,6].

Mineralocorticoids and stroke

The link between aldosterone and hypertension has been evident since studies by Conn *et al.* showed that hypertension was prevalent in a cohort of patients with primary aldosteronism (PA) [19]. Similarly, patients with glucocorticoid-remediable hypertension, and therefore raised plasma aldosterone, have an increased frequency of stroke and hypertension [20,21]. Comparisons of patients with PA and essential hypertension show that PA patients suffer from more strokes, despite having lower blood pressure than patients with essential hypertension [22]. This suggests that the increased stroke risk associated with raised plasma aldosterone is blood pressure independent. This is supported by studies showing that inhibition of the renin-angiotensin system reduces the risk of stroke with only a small reduction in blood pressure [23,24]. Importantly, it is now apparent that aldosterone plays a role in 10–15% of cases of hypertension previously classified as essential hypertension [25]. If a causative link between aldosterone and stroke exists, then this finding significantly increases the proportion of the population at risk for a cerebrovascular event.

In the laboratory, the link between mineralocorticoids and hemorrhagic stroke is well established. Administration of MR antagonists (spironolactone or eplerenone) to SHRSP fed a high salt diet does not reduce blood pressure, but prevents hemorrhagic strokes [26]. Administration of the angiotensin-converting enzyme (ACE) inhibitor, captopril, to salt-loaded SHRSP had a similar effect. Co-administration of mineralocorticoid negates the beneficial effect of captopril [27,28], suggesting that some of the benefits of ACE inhibition result from a reduction in angiotensin II-stimulated aldosterone release. The effect of MR blockade on ischemic stroke in SHRSP has been studied using a model of permanent focal ischemia. Spironolactone treatment reduced the ischemic infarct in SHRSP by ~50% [11] (Figure 1). Similar to the studies by Rocha and Stier [26], spironolactone did not lower blood pressure, suggesting that aldosterone plays an important role in the predisposition to vascular injury that is not linked to hypertension. Many mechanisms could be responsible for this effect, but for the purpose of this review we focus on the possibility that the beneficial effects of MR blockade on stroke risk and outcome are the result of changes in cerebral vessel structure.

Mineralocorticoids and vessel structure

Many factors influence vascular structure including hormones, reactive oxygen species (ROS) and inflammatory mediators [29]. There is clear evidence that aldosterone has deleterious effects on the cardiovascular system. The effects on the heart, where aldosterone causes cardiac hypertrophy and fibrosis, are best characterized [30]. The effects of aldosterone on the vasculature, the cerebral vasculature in particular, are less well characterized.

Studies of the systemic vasculature suggest that MR activation affects vessel structure. The media to lumen ratio in coronary and mesenteric arteries is increased in rats made hypertensive by the administration of aldosterone or deoxycorticosterone (DOC) and salt [31,32]. Aortic hypertrophy has also been seen in DOC-salt hypertensive rats [33,34]. These effects can be inhibited by endothelin antagonism, ACE inhibition and neutral endopeptidase and proteasome inhibition [31-34]. It is important to remember that although these rats have high circulating

mineralocorticoids, they also have malignant hypertension, making it difficult to separate the effects of MR activation and blood pressure *per se*.

Studies using MR antagonists provide a better reflection of the actions of aldosterone without the confounding effects of large changes in blood pressure. Benetos *et al.* [35] found that chronic spironolactone treatment of spontaneously hypertensive rats (SHR) prevented aortic fibrosis by preventing collagen accumulation. In angiotensin II hypertensive rats, MR blockade inhibits the anticipated increase in mesenteric artery media to lumen ratio [36], again supporting the theory that some effects of angiotensin II on the vasculature are mediated by aldosterone. In SHRSP, eplerenone reduces the salt-dependent changes in vascular structure. Eplerenone did not reduce blood pressure in the control SHRSP, but prevented the increase in pressure seen with salt loading. Salt loading also caused an increase in the media to lumen ratio of mesenteric arteries and an impairment of vasodilation. These effects were inhibited by eplerenone [37].

Studies of the effects of aldosterone on the cerebral vasculature are less common. However, ACE inhibition and angiotensin receptor blockade have been found to increase cerebral vessel lumen diameter [8]. Recently, levels of the angiotensin type 1 (AT1) receptor were shown to be increased in the cerebral vasculature of SHR, and AT1 receptor blockade normalized vascular structure [38]. The increase in AT1 receptor levels is of particular interest in light of a recent study by Jaffe and Mendelsohn [39] showing that angiotensin II regulates gene transcription in cultured VSMC. Surprisingly, the effects of angiotensin II could be inhibited, not only by AT1 receptor blockade, but also by spironolactone, suggesting that the MR is involved in the angiotensin II-dependent modulation of gene transcription. Aldosterone synthase was not present in the VSMC, ruling out the possibility that the actions of angiotensin II were via local aldosterone production. If angiotensin II *trans*-activates the MR in the cerebral vasculature, this could potentially enhance the actions of aldosterone and increase the risk of stroke.

The effect of spironolactone on cerebral vessel structure has been studied in SHRSP. The external and internal diameter of the middle cerebral artery (MCA) was significantly increased by chronic spironolactone treatment. The MCAs from spironolactone-treated SHRSP did not differ structurally from the MCAs of WKY rats, despite there being no change in blood pressure with spironolactone treatment [40]. These results suggest that spironolactone prevents or reverses euthrophic remodeling in SHRSP.

The mechanism responsible for the changes in vessel structure remains elusive. Studies suggest that increased collagen deposition plays a role in the deleterious effects of aldosterone on the cardiovascular system [30,35]. Epidermal growth factor (EGF), a key VSMC mitogen, has also been implicated in the actions of aldosterone. Interestingly, both classic genomic [11] and rapid non-genomic effects [41] appear to be involved in the increased activity of the EGF signaling pathway caused by aldosterone. However, the current consensus is that most of the structural changes in the cerebral vasculature are caused by rearrangement of the VSMC and not increased cell proliferation.

MR specificity of aldosterone

Aldosterone and cortisol (corticosterone in rats) bind the MR with equal affinity. It is generally accepted that 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type II (11 β -HSD2) is responsible for the specificity of the MR for aldosterone in the vasculature. This enzyme metabolizes cortisol to cortisone (11-dehydroxycorticosterone in rats), thereby removing its ability to bind and activate the MR. A recent publication challenges this by suggesting that, under normal conditions, 11 β -HSD2 cannot convert all the cortisol in the vasculature to cortisone, and that cortisol binds to but does not activate the MR. However, under conditions

where the redox state of the cell is altered, such as would occur with inhibition of 11 β -HSD2 or an elevation of ROS, the cortisol–MR complex becomes activated [42]. This scenario might explain one of the outstanding questions about the effects of MR blockade in the cardiovascular system: why does MR blockade have such profound effects in situations where circulating aldosterone is not excessively high? It is possible that the greatly increased ROS levels seen with hypertension [43] are responsible for the activation of the cortisol–MR complex, leading to ‘aldosterone’-mediated vascular damage.

Mineralocorticoids and neurons

Although the role of MR activation as a harbinger of doom in the cardiovascular system seems clear, the situation in the brain is much less so. Aldosterone levels within the brain are low because the multidrug resistance P-glycoprotein in the blood–brain barrier (BBB) pumps aldosterone back across the barrier. Although aldosterone is produced within the brain, the physiological relevance of this is unclear [44,45]. Similarly, it is unknown how much aldosterone enters the brain post-stroke, when the BBB is breached, or what effects this might have. The effects of aldosterone on neuronal tissue are further complicated by the differential synthesis of the two 11 β -HSD isoforms. 11 β -HSD2 activity is limited to the areas of the brain that control salt appetite and blood pressure: the subcommissural organ, nucleus tractus solitarius and amygdala [46]. 11 β -HSD type 1 (11 β -HSD1) activity is more ubiquitous. This enzyme is generally thought to act as a reductase that reactivates glucocorticoids by converting cortisone to cortisol, although a recent publication questions the validity of this assumption suggesting that in brain 11 β -HSD1 has both dehydrogenase and reductase activities [47]. Both MR and glucocorticoid receptors (GR) are found in the brain. MR is found primarily in the limbic areas, such as the hippocampus, whereas the GR is more widely expressed [48].

MR activation in the hippocampus

Within the brain, adrenal steroids have been implicated in the modulation of neuron excitability, memory formation and neurodegeneration [44]. Most studies have focused on the effects of MR activation on the dentate gyrus in the hippocampus, given the role it plays in learning and memory [49,50]. 11 β -HSD2 levels are low in the hippocampus [45]. Thus, it is generally accepted that at basal cortisol levels MR are activated by cortisol and GR are only activated during stress [51,52]. Interestingly, activation of MR and GR appears to have opposing effects in the hippocampus.

Granule cells in the dentate gyrus are produced throughout adult life [53], and neurogenesis and apoptosis are controlled by MR and GR, respectively [54,55]. Adrenalectomy stimulates neurogenesis, presumably by removing the inhibitory effects of GR activation on neurogenesis [55]. At the same time, adrenalectomy increases the degeneration of adult granule cells by removing the protective actions of MR activation [56]. The net neurodegeneration exceeds neurogenesis, but administration of aldosterone increases neurogenesis [57], suggesting that MR activation also increases the formation of new cells. Similarly, adrenal steroids appear to be involved in the survival of new cells in the hippocampus [58]. Immature cells do not express MR or GR; it is thought that MR activation in neighboring adult cells initiates a signaling pathway to improve the survival of nearby immature neurons [57,58].

During neuronal death, cells release large quantities of glutamate, which is toxic to the surrounding neurons. Kainic acid is used to mimic this experimentally. MR blockade causes an increase in kainic acid-induced hippocampal cell death, suggesting again that MR activation is neuroprotective [59]. The beneficial effects of MR and deleterious effects of GR activation are controlled by a delicate balance between the expression of pro- and antiapoptotic genes. The B-cell leukemia/lymphoma 2 (*Bcl2*) gene is neuroprotective [60]; MR blockade with spironolactone reduces basal *Bcl2* expression [61,62] and MR activation increases it [63].

Conversely, GR activation shifts the balance towards apoptosis by increasing the expression of the proapoptotic gene *Bax*. Similarly, the tumor suppressor protein p53 is decreased by MR and increased by GR activation. Importantly, p53 increases *Bax* and reduces *Bcl2* transcription [63].

MR activation and cerebral ischemia

For MR activation to be important for neuronal survival post-stroke, the MR needs to be present and functional in the regions of the brain commonly damaged by ischemia, including the cerebral cortex. Recent studies suggest that this might be the case. MR protein was found to be present in cultured cortical and hippocampal neurons, and the levels of MR are increased in staurosporine-injured and aged cells undergoing spontaneous apoptosis [64], presenting the possibility that the MR is part of a cell survival mechanism. *In vivo* studies have shown that low doses of staurosporine are neuroprotective, whereas high doses cause neuronal damage [65]. It is possible that the neuroprotection seen with low doses is mediated by an increase in MR. An increase in MR synthesis was also seen in the hippocampus after transient global ischemia. Surprisingly, this only occurred in rats made hypothermic after the induction of ischemia [64]. Although hypothermia is a promising treatment for stroke [66], it is not clear whether the beneficial effects seen in other models of ischemia with hypothermia are related to an increase in MR levels. However, in this situation, the MR appears to be functionally important, because MR blockade one hour before the induction of ischemia resulted in increased cell death [64]. This contrasts with previous studies showing that chronic MR blockade reduces cerebral infarct size. Important differences exist between these studies: in the Macleod studies spironolactone treatment was acute and ischemia was transient and global, whereas in the studies by Dorrance *et al.* [11], ischemia was permanent and focal. These differences might account for the different results obtained and might be related to the differential effects of MR activation on the vasculature and the brain.

Other *in vitro* studies support the hypothesis that MR activation protects cortical neurons. Aldosterone prevents glutamate-induced cell death in cultured neurons and this was inhibited by spironolactone, suggesting that the protection occurs via MR activation (Dorrance, A.M. and Cannady, W.E. The effect of aldosterone antagonism on the outcome of cerebral ischemia. *Endocrine Societies 84th Annual Meeting*, 2002 June 19–22, San Francisco, P2-5001). These results suggest that administration of aldosterone at the onset of a stroke could be neuroprotective. This raises an important issue. Systemically administered spironolactone crosses the BBB and has central effects [67], why then is cerebral infarct size reduced by longterm administration of spironolactone (Figure 1)?

The effects of aldosterone on MR activation and neuronal survival become even more tantalizing when one considers that aldosterone is synthesized within the brain [44,45]. How neurosteroids affect neuronal function has not been elucidated, and neither has the effect of ischemia on the expression of the enzymes responsible for aldosterone synthesis within the brain. Although care should be taken not to over interpret the results obtained from *in vitro* studies, it is possible to speculate that MR expression will be increased in the ischemic penumbra as part of a cell survival mechanism.

Deleterious central actions of aldosterone

It would be misleading to present all of the actions of aldosterone or MR activation within the brain as beneficial. Activation of MR in the circumventricular organs causes a plethora of deleterious effects including increased sympathetic drive to the kidneys, heart and VSMC [45]. Similarly MR activation in the amygdala increases Na^+ intake, thereby increasing blood pressure and end organ damage. It should be noted that 11 β -HSD2 activity has not been

identified in the circumventricular areas or the amygdala, yet the actions of the MR in these regions appear to be aldosterone specific [45].

Conclusion

Here, we have presented evidence that aldosterone or MR activation could facilitate and inhibit the neuronal damage caused by stroke. Aldosterone has detrimental effects on the vasculature that reduce blood flow and therefore promote cerebral ischemia. Conversely, MR activation in the brain appears to inhibit cell death by increasing the expression of antiapoptotic genes. The therapeutic possibilities for MR antagonism or activation are intriguing and the battle between the potential protective and negative effects of receptor blockade promises to be an interesting one.

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Figure 1.

Spironolactone reduces the size of experimentally induced cerebral infarcts in SHRSP. **(a)** Representative brain slices from SHRSP±spironolactone and WKY rats. After six hours of ischemia, the brains were sliced coronally and stained with 2,3,5-triphenyltetrazolium chloride; the pink area is viable tissue and the white area is the area damaged by the cerebral infarct. **(b)** Percentage of the hemisphere that has undergone a cerebral infarct. Infarct size was quantified and expressed as a percentage of the hemisphere infarcted, the results are expressed as the mean±SEM [12]. Infarcts were greater in SHRSP ($n=8$) than either SHRSP ±spironolactone ($n=10$) or WKY ($n=8$). A $P<0.05$ was deemed significant; an asterisk (*)

indicates significant difference from control SHRSP. Reproduced, with permission, from [11].