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REVIEW

Aldosterone affects blood flow and vascular tone regulated by endothelium-derived NO: therapeutic implications

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Aldosterone, in doses inappropriate to the salt status, plays an important role in the development of cardiovascular injury, including endothelial dysfunction, independent of its hypertensive effects. Acute non-genomic effects of aldosterone acting on mineralocorticoid receptors are inconsistent in healthy humans: vasoconstriction or forearm blood flow decrease via endothelial dysfunction, vasodilatation mediated by increased NO actions, or no effects. However, in studies with experimental animals, aldosterone mostly enhances vasodilatation mediated by endothelium-derived NO. Chronic exposure to aldosterone, which induces genomic responses, results in impairments of endothelial function through decreased NO synthesis and action in healthy individuals, experimental animals and isolated endothelial cells. Chronic aldosterone reduces NO release from isolated human endothelial cells only when extracellular sodium is raised. Oxidative stress is involved in the impairment of endothelial function by promoting NO degradation. Aldosterone liberates endothelin-1 (ET-1) from endothelial cells, which elicits ET_A receptor–mediated vasoconstriction by inhibiting endothelial NO synthesis and action and through its own direct vasoconstrictor action. Ca^{2+} flux through T-type Ca^{2+} channels activates aldosterone synthesis and thus enhances unwanted effects of aldosterone on the endothelium. Mineralocorticoid receptor inhibitors, ET_A receptor antagonists and T-type Ca^{2}+ channel blockers appear to diminish the pathophysiological participation of aldosterone in cardiovascular disease and exert beneficial actions on bioavailability of endothelium-derived NO, particularly in resistant hypertension and aldosteronism.

Abbreviations

ACE, angiotensin-converting enzyme; BH_4, tetrahydrobiopterin; eNOS, endothelial NOS; ET, endothelin; G6PD, glucose-6-phosphate dehydrogenase; L-NMMA, N^G-monomethyl-L-arginine; MR, mineralocorticoid receptor; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; SHRSP, stroke-prone spontaneously-hypertensive rat; SNP, sodium nitroprusside

Introduction

The mineralocorticoid aldosterone is synthesized from cholesterol in the zona glomerulosa of the adrenal gland and extra-adrenal tissues. In blood vessels, it is produced in both endothelial and smooth muscle cells (Schiffrin, 2006; Skøtt *et al.*, 2006). The main role of aldosterone is to maintain body sodium homeostasis. In normal physiological situations, aldosterone is regulated inversely with salt (NaCl) status. If aldosterone becomes inappropriately high for the salt status, elevated plasma aldosterone results in endothelial dysfunction, vasculopathy, vascular and ventricular remodelling and renal injury. Most of these effects are mediated via mineralocorticoid receptors (MR) (Brown, 2005; receptor nomenclature follows Alexander *et al.*, 2011). Substantial evidence has emerged to show that aldosterone plays an independent role in the development of cardiovascular tissue damage. The Randomized Aldactone Evaluation Study (RALES) has demonstrated that inhibition of MR leads to a 30% reduction in mortality rates in patients with heart failure after myocardial

infarction (Pitt *et al.*, 1999). Aldosterone is associated with increased risk for end organ-damage and cardiovascular events. MR antagonism is a potential prevention therapy for patients at risk of ischaemic stroke (Osmond *et al.*, 2008).

Risk factors for endothelial dysfunction include cardiovascular and metabolic diseases, such as hypertension (Busse and Flemming, 1999), diabetes mellitus/insulin resistance (Toda *et al.*, 2010), hyperhomocysteinaemia (Austin *et al.*, 2004), hyperlipidaemia (Francois and Kojda, 2004) and Alzheimer's disease (Toda and Okamura, 2012); poor life-style choices, such as smoking (Toda and Toda, 2010), chronic alcohol intake (Toda and Ayajiki, 2010), high salt intake (Toda and Arakawa, 2011), being sedentary (Di Francescomarino *et al.*, 2009) and mental stress (Toda and Nakanishi-Toda, 2011); and chronological age (Toda, 2012). In addition to high salt intake, aldosterone should also be included as a risk factor.

The endothelium exerts important effects on vascular tone through the release of vasodilator (endothelium-derived relaxing factor; Furchgott and Zawadzki, 1980) and vasoconstrictor molecules. The former includes NO (Palmer *et al.*, 1987; Furchgott, 1988), prostacyclin and endothelium-derived hyperpolarizing factor and the latter includes endothelin (ET; Yanagisawa *et al.*, 1988) and vasoconstrictor prostanoids. NO liberated from endothelial cells in response to chemical or physical stimuli exerts vasodilatation, decreased vascular resistance, increased blood flow, lowering systemic blood pressure and thrombosis prevention (Moncada *et al.*, 1991). ET-1, also liberated from the endothelium, acts as a counteracting molecule against NO via interfering with NO synthesis and its vasoconstrictor property (Bourque *et al.*, 2011).

Aldosterone exerts actions in the vascular endothelium through acute, non-genomic and chronic, genomic effects that modulate vascular resistance and blood flow. Aldosterone-induced vasculopathy is characterized by a reduction of endothelial NO synthesis and bioavailability and by increased generation of superoxide radicals that degrade endogenous NO. The present article describes how endothelial function is altered by acutely administered aldosterone and in addition compares it with the effect of chronic exposure to aldosterone in humans, experimental animals and isolated endothelial cells. We will discuss the mechanisms of its unwanted actions and the interactions between aldosterone and ET-1, Ca^{2+} flux through T-type Ca^{2+} channels and sodium, with reference to the bioavailability of endothelial NO. Therapeutic efficacies of MR inhibitors, ET_A receptor antagonists, and T-type Ca^{2+} channel blockers through beneficial actions of NO on blood flow against inappropriately elevated plasma aldosterone concentrations or aldosteronism and resistant hypertension are also summarized.

Synthesis and actions of endothelial nitric oxide (eNOS) and endothelin

NO is produced when L-arginine is transformed to L-citrulline via catalysis by NOS in the presence of oxygen and cofactors including reduced NADPH, tetrahydrobiopterin (BH_4), haem, FAD, FMN and calmodulin. Ca^{2+} is required for the activation of eNOS that is constitutively expressed mainly in endothelial cells (Förstermann *et al.*, 1991). eNOS binds to caveolin-1 in the caveolae, microdomains of the plasma membrane, which keeps eNOS in an inactive state. In response to increased cytosolic Ca^{2+}, calmodulin displaces eNOS from caveolin-1, making it active for NO synthesis (Figure 1). The transmembrane influx of Ca^{2+} and its mobilization from intracellular storage sites are caused via stimulation of receptors located on the endothelial cell membrane by ACh, bradykinin and substance P or via mechanical stimuli such as shear stress. On the other hand, shear stress, bradykinin or insulin induce the phosphorylation of $Ser^{1177/1179}$ of eNOS through PI_3K and the downstream Akt, serine/threonine protein kinase (PKB), resulting in enhanced NO formation (Dimmeler *et al.*, 1999). Endothelial NO causes vasodilatation, decreases vascular resistance, increases regional blood flow and lowers blood pressure. It also inhibits platelet aggregation and adhesion, reduces leukocyte adhesion and migration and inhibits smooth muscle proliferation, thus, leading to prevention of atherosclerosis. These actions of NO are mediated by cyclic GMP synthesized by soluble guanylyl cyclase from GTP. Non-invasive techniques to assess the endothelium-dependent vasodilatation include forearm blood flow measurement by strain gauge plethysmography using the venous occlusion technique and flow-mediated vasodilatation by high-resolution ultrasonography.

The synthesis of NO by eNOS is inhibited by L-arginine analogues, including N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methylester (L-NAME). Nitro compounds, such as nitroglycerin and sodium nitroprusside (SNP), are capable of liberating NO. 1*H*[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (Garthwaite *et al.*, 1995) decreases the synthesis of cyclic GMP by inhibiting guanylyl cyclase activity. Deficiency of the NOS cofactor, BH_4 makes uncouples NOS, which results in superoxide anions being produced instead of NO. Superoxide anions are also generated by NAD(P)H oxidase (Figure 1) and xanthine oxidase. Superoxide dismutase, catalase and dimethyl sulfoxide scavenge free radicals. NO reacts with superoxide anions, generating highly toxic compounds such as peroxynitrite.

Vascular endothelial cells generate not only vasodilator mediators, such as NO, prostacyclin (Moncada and Vane, 1981) and endothelium-derived hyperpolarizing factors but also vasoconstrictor prostanoids (Félétou *et al.*, 2010) and ET-1, a 21-amino-acid polypeptide synthesized through the process shown in Figure 2. The potent vasoconstrictor property of ET-1 is mediated via activation of ET_A receptors located on smooth muscle cell membranes. ET-1 has been implicated in the pathogenesis of many diseases of the cardiovascular-system (Rodríguez-Pascual *et al.*, 2011). The main outcome of ET-1 binding to ET_B receptors in the endothelium is an increased production of NO and prostacyclin whereas ET-1, by binding to ET_A receptors interferes with NO synthesis and acts to counter NO-induced vasodilatation (Bourque *et al.*, 2011).

Studies on humans

Studies in vivo

Aldosterone exerts actions in the vascular endothelium through non-genomic effects, which are acute (occurring in

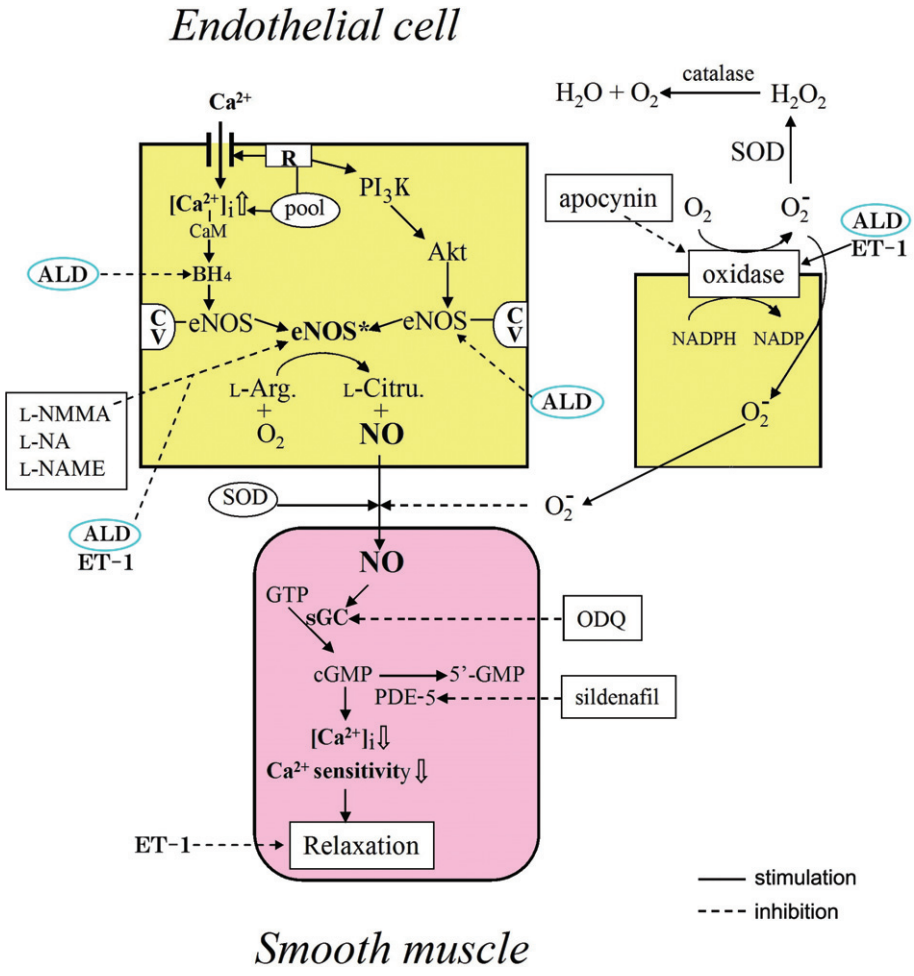

Figure 1

Schematic presentation of information pathways via NO liberated from endothelial cells to vascular smooth muscle cells. Superoxide generation via NAD(P)H oxidase is also included in the right part of the figure. Possible sites of action of aldosterone (ALD) and ET on eNOS and superoxide generation are shown in the figure. R in the square on the endothelial membrane, drug receptor or mechanoreceptor; pool, Ca^{2+} storage site; CV, caveolin-1; CaM, calmodulin; Akt, serine/threonine protein kinase Akt; eNOS*, activated eNOS; L-Arg., L-arginine; L-Citru., L-citrulline; O_2^-, superoxide anion; L-NA, N^G-nitro-L-arginine; SOD, superoxide dismutase; sGC, soluble guanylyl cyclase; cGMP, cyclic GMP; ODQ, 1H[1,2,4] oxadiazolo[4,3-a]quinoxalin-o1-one.

minutes) and do not involve transcriptional mechanisms, or through genomic effects, which are chronic (occurring in hours) and involve transcriptional mechanisms.

Acute effects of aldosterone. In healthy male volunteers, forearm blood flow measured using venous occlusion plethysmography was reduced by brachial arterial infusion of aldosterone at 2.5 pmol·min^{-1} within 4 min, reaching its nadir at 12 min, suggesting that impairment of endothelial function contributes to the acute vasoconstrictor effect of aldosterone (Romagni *et al.*, 2003). On the other hand, local intra-arterial infusion of aldosterone (10–100 ng·min^{-1}) had no acute effect on forearm resistance vessels in healthy males (Gunaruwan *et al.*, 2002). No change in forearm blood flow was seen with aldosterone alone (3.3–55 pmol·min^{-1} per 1000 mL forearm volume for 15 min) and acute treatment with aldosterone increased vasodilation in response to SNP or ACh (Nietlispach *et al.*, 2007). Aldosterone appears to acutely enhance vasodilatation to exogenous NO. Aldosterone infusion in healthy male volunteers increased forearm blood flow. In the presence of aldosterone, L-NMMA induced a greater vasoconstriction and phenylephrine induced an exaggerated vasoconstriction as compared with placebo, suggesting that aldosterone acts through non-genomic effects on the endothelium by increasing NO release and on the vascular smooth muscle cells by promoting vasoconstriction (Schmidt *et al.*, 2003). In healthy males, i.v. aldosterone (500 μg) did not change renal plasma flow. L-NMMA alone decreased renal plasma flow and combined treatment with aldosterone and L-NMMA resulted in an additional decrease in plasma flow (Schmidt *et al.*, 2006). This inconsistency in the acute effects of aldosterone effects may be due to the variety of doses of aldosterone used (Table 1). On the basis of findings obtained so far with healthy male volunteers, Schmitt *et al.* (2004) proposed that vasoconstriction was induced at low plasma levels of aldosterone, that there was a neutral effect at mod-

Table 1

Acute (non-genomic) effects of aldosterone (ALD) on endothelial function in humans and experimental animals

Author and year	Species and condition	Duration and dose of ALD	Endothelial function or FBF change	Mechanisms
Human				
Gunaruwan *et al.* (2002)	healthy male	ib-inf, 10 min, 10–100 ng·min^{-1}	no change in FBF	
Romagni *et al.* (2003)	healthy male	ib-inf, 12 min, 2.5 pmol·min^{-1}	decrease (FBF ↓)	
Schmidt *et al.* (2003)	healthy male	ib-inf, 8 min, 500 ng·min^{-1}	increase (FBF ↑)	NO release ↑
Gunaruwan *et al.* (2005)	patient with CHF	ib-inf, 10 min, 10 & 50 ng·min^{-1}	decrease (FBF ↓)	
Schmidt *et al.* (2006)	healthy male	intravenous, 30 min, 500 μg	renal VR ↑ with L-NMMA	Vasoconst: ALD + L-NMMA > L-NMMA
Nietlispach *et al.* (2007)	healthy male	ib-inf, 15 min, 55 pmol·L·min^{-1}	FBF ↑ by ACh and SNP	NO action ↑
Experimental animal				
Liu *et al.* (2003)	rat aorta	2 min, 0.01 nmol·L^{-1}	increase	eNOS activity ↑(PI3K-dependent)
Uhrenholt *et al.* (2003)	rabbit renal arteriole	20 min, 0.1–10 pmol·L^{-1}	increase	NO action ↑ (PI3K, PKB, cGMP-dependent)
Michea *et al.* (2005)	rat mesenteric arteriole	10 min, 10 nmol·L^{-1}	decrease	contraction ↑ (PI3K ↓, PKB ↓, PKC ↑)
Mutoh *et al.* (2008)	bovine aortic EC	10 min, 10–100 nmol·L^{-1}	increase	NO production ↑ (PI3K-dependent)
Heylen *et al.* (2009)	rat arteriole	5 min, 1 pM–100 nmol·L^{-1}	increase	NO action ↑

FBF, forearm blood flow measured using venous occlusion plethysmography; ib-inf, intrabrachial infusion; CHF, chronic heart failure; renal VR, renal vascular resistance; Vasoconst, vasoconstriction.

erate levels and that vasodilatation was induced at high levels. Physiological plasma levels of aldosterone are approximately 100 pmol·L^{-1} (Arima, 2006). To conclusively establish the reason for the discrepant results, studies using various aldosterone doses and administration routes, if ethically permitted, are required.

Because the non-genomic effects of aldosterone in disease states associated with endothelial dysfunction may differ from those in healthy individuals, Gunaruwan *et al.* (2005) performed studies in patients with chronic heart failure, who showed constriction of forearm resistance vasculature, but not capacitance vasculature, in response to locally infused aldosterone. These authors previously (Gunaruwan *et al.*, 2002) noted that there was no acute effect of aldosterone on forearm vascular resistance in healthy volunteers. Therefore, they proposed that in disease states, such as chronic heart failure, beneficial effects of endothelial NO, opposing aldosterone-induced vasoconstriction, are impaired because of endothelial dysfunction.

Effects of chronic aldosterone. Infusions of aldosterone (12 pmol·min^{-1} kg^{-1} for 4 h; i.v.) in healthy males attenuated endothelium-dependent forearm vasodilatation induced by ACh, compared with either oral prednisolone or placebo. Endothelium-independent vasodilatation induced by SNP and vasoconstriction in response to angiotensin II and norepinephrine were not affected by either aldosterone or prednisolone. Blood pressure and baseline blood flow did not differ between any of the study phases (Farquharson and Struthers, 2002). The persistent i.v. administration of aldosterone appears to impair endothelial vasodilator function when the vessels are stimulated but not under basal conditions. Spironolactone improves endothelial dysfunction and increases NO bioactivity in patients with heart failure (Farquharson and Struthers, 2000). Brachial artery flow–mediated, endothelium-dependent vasodilatation was lower in resistant hypertensive subjects with hyperaldosteronism compared with the subjects without hyperaldosteronism. Flow-mediated vasodilatation was negatively correlated with plasma aldosterone and the ratio of plasma aldosterone to plasma renin activity but was independent of blood pressure and age. Nitroglycerin-induced vasodilatation was similar in both groups. Treatment with spironolactone increased flow-mediated vasodilatation independently of blood pressure change (Nishizaka *et al.*, 2004). Chronic aldosteronism may result in a blood pressure–independent endothelial dysfunction in subjects with resistant hypertension. Flow-mediated vasodilatation in patients with primary aldosteronism was lower than that in the control subjects. Nitroglycerin-induced vasodilatation was not affected. Flow-mediated vasodilatation was negatively correlated with plasma aldosterone concentration, aldosterone–renin ratio and systolic blood pressure. Treatments with unilateral adrenalectomy and spironolactone improved flow-mediated vasodilatation,

Table 2

Chronic (genomic) effects of aldosterone on endothelial function in humans, experimental animals and isolated endothelial cells

Author and year	Species and materials	Time of exposure to ALD Dose of ALD	Endothelial function	Mechanisms and remarks
Human				
Farquharson and Struthers, 2000	heart failure patient	increased ALD as assessed by treatment with spironolactone	decrease	endothelial NO action ↓, ANG I/ANG II conversion ↑
Farquharson and Struthers, 2002	healthy male	4 h, 12 pmol min^{-1} kg^{-1}	decrease	ACh, but not SNP, action impaired
Nishizaka et al., 2004	hyperaldosteronism	plasma ALD, patient > control	decrease (FMD ↓)	MR blocker effective
Nietlispach et al., 2007	healthy male	2 weeks, 0.8 μmol day^{-1}*	increase	ACh effect ↑, L-NMMA effect ↑
Tsuchiya et al., 2009	primary aldosteronism	plasma ALD increased	decrease (FMD ↓)	MR blocker effective
Experimental animal				
Virdis et al., 2002	rat mesenteric artery	14 days, 2 nmol h^{-1}, sc inj	decrease	ACh, but not SNP, action ↓ NADPH oxidase activity ↑
Bauersachs et al., 2002	rat (heart failure)	increased ALD assessed by treatment with SP + ACEI	decrease	ACh action ↓ NADPH oxidase expression ↑
Endemann et al., 2004	SHRSP, salt-loading	increased ALD assessed by treatment with eplerenone	decrease	ACh action ↓ MR blocker effective
Sanz-Rosa et al., 2005	SHR	increased ALD assessed by treatment with eplerenone	decrease	eNOS ↓, NADPH oxidase ↑
Kobayashi et al., 2006	Dahl rat (heart failure)	increased ALD assessed by treatment with eplerenone	decrease	eNOS ↓through Akt NADPH oxidase ↑
Thai et al., 2006	rat (heart failure)	increased ALD assessed by treatment with spironolactone	decrease	ACh action ↓ eNOS ↓,
Leopold et al., 2007	mouse	14 days, 0.14 μmol kg^{-1} day^{-1}	decrease	vascular G6PD ↓
Bayorh et al., 2011	Dahl rat	4 weeks, 0.6 μmol pellet	decrease	Plasma NO ↓, NADPH oxidase 4 ↑
Isolated cell				
Nagata et al., 2006	HUVEC	6 h, 100 nmol L^{-1}	NO ↓	eNOS ↓ by BH4 ↓ & PP 2A ↑
Hashikabe et al., 2006	HUVEC	16 h, 1 μmol L^{-1}	NO ↓	Nox4 mRNA ↑
Oberleithner et al., 2007	bovine EC	3 days, 0.45 nmol L^{-1}	NO ↓	endothelial NO ↓on ambient sodium ↑
Leopold et al., 2007	bovine EC	24 h, 1–100 nmol L^{-1}	NO ↓	oxidative stress ↑, G6PD ↓
Iwashima et al., 2008	rat aortic EC	6–24 h, 10 nmol L^{-1} μmol L^{-1}	NO ↓	NADPH oxidase ↑, Rac 1 ↑

*Fludrocortisone; ALD, aldosterone; ANG, angiotensin; SNP, sodium nitroprusside; FMD, flow-mediated vasodilatation; sc inj, subcutaneous injection; SP + ACEI, spironolactone + angiotensin-converting enzyme inhibitor; SHR, spontaneously-hypertensive rat; SHRSP, stroke-prone SHR; Dahl rat, Dahl salt-sensitive rat; EC, endothelial cell; ET_A, ET_A receptor; G6PD, glucose-6-phosphate dehydrogenase; PP 2A, protein phosphatase 2A.

plasma aldosterone concentration and systolic blood pressure, suggesting that aldosterone excess contributes to attenuated effects of endothelial NO (Tsuchiya *et al.*, 2009). Sustained aldosterone excess appears to contribute to the development of endothelial dysfunction (Table 2). Hypertensive patients with a high aldosterone/renin ratio had impaired vasodilator responses to methacholine, but not those to SNP, in forearm resistance vessels. Low-renin status was associated with impaired responses to methacholine and SNP in hypertensive patients (Duffy *et al.*, 2005).

In contrast to the above-mentioned findings, Nietlispach *et al.* (2007) provided evidence that oral fludrocortisone (0.3 mg·day^{-1} for 2 weeks) enhanced basal NO actions, evaluated by increased responses to L-NMMA, and also forearm vasodilatation in response to ACh in 10 healthy subjects. Fludrocortisone at oral doses of 0.05–0.2 mg·day^{-1} is used in mineralocorticoid replacement therapy with no appreciable glucocorticoid effect (Brunton, 2011).

Studies on isolated cells

Treatment of HUVECs with 10 nmol·L^{-1} aldosterone for 72 h increased the size, stiffness, apical membrane tension and protein leakage (Oberleithner, 2005). The increase in cell rigidity could trigger endothelial dysfunction observed in

hyperaldosteronism. In response to treatment with aldosterone (1 nmol·L^{-1}), HUVECs swelled, cell water decreased and intracellular organic matter increased. These changes were paralleled by a rise in cell pressure. The endothelial monolayer after blood perfusion disclosed large gaps between cells treated with aldosterone. Spironolactone and eplerenone prevented the actions of aldosterone (Oberleithner *et al.*, 2006a). These results explain why high blood pressure combined with high plasma aldosterone concentration may damage the vascular endothelium.

High extracellular sodium concentration in conjunction with aldosterone stiffens cultured human endothelial cells and reduces NO release from the endothelium via down-regulation of NO formation, but in the absence of aldosterone, the cells are insensitive to changes in ambient sodium (Oberleithner *et al.*, 2007). Endothelial cells express epithelial sodium channels (Golestaneh *et al.*, 2001; Oberleithner *et al.*, 2006b) and blockade of these channels with amiloride attenuated the increased stiffness induced by exposure to high sodium levels plus aldosterone (Oberleithner *et al.*, 2007). Aldosterone mediates genomic and non-genomic pathways in the endothelium. Both ways of action seem to affect the epithelial sodium channel (Kusche-Vihrog *et al.*, 2010). Non-genomic actions of aldosterone include a stiffening of the endothelium (Oberleithner *et al.*, 2006a,b), which is accompanied by a reduced release of NO (Oberleithner *et al.*, 2007). As the effect is prevented by amiloride, it is likely that aldosterone activates the insertion of epithelial sodium channels into the plasma membrane of endothelial cells, leading to a decrease in NO release from the endothelium and vascular smooth muscle contraction (Kusche-Vihrog *et al.*, 2010).

The enzyme NAD(P)H oxidase (Nox) is a multi-subunit enzyme. p47phox, a cytoplasmic subunit of Nox, is essential for activation of Nox and subsequent reactive oxygen species (ROS) production through this enzyme. ROS increased in aldosterone-treated HUVECs but was abolished by pretreatment with eplerenone. Aldosterone increased transcripts of NAD(P)H oxidase p47phox, but reduced the level of phosphorylation of eNOS at Ser^{1177} induced by vascular endothelial growth factor. Either eplerenone or okadaic acid restored eNOS phosphorylation. The decrease in NO output caused by aldosterone was reversed by BH_4, GTP cyclohydrolase-1 (a rate-limiting enzyme of BH_4 synthesis) overexpression, or p47phox knockdown (Nagata *et al.*, 2006). Aldosterone inhibits eNOS function possibly through a bimodal mechanism comprising BH_4 deficiency and protein phosphatase 2A activation. Michell *et al.* (2001) have shown that protein phosphatase 2A is responsible for dephosphorylation of Ser^{1177} in eNOS. Incubation of HUVEC with aldosterone diminished eNOS phosphorylation by vascular endothelial growth factor and decreased NO production in response to vascular endothelial growth factor. Aldosterone also increased Nox4 mRNA expression in HUVECs, suggesting that aldosterone directly caused the dysregulation of endothelial cell function (Hashikabe *et al.*, 2006) (Table 2).

No difference was found in the senescence rate between endothelial progenitor cells from patients with primary aldosteronism and normotensive controls. Incubation of endothelial progenitor cells with aldosterone (10–100 nmol·L^{-1}) did not modify their senescence rate and cell cycle distribution, suggesting that aldosterone exerts no effect on endothelial progenitor cell growth characteristics (Verhovez *et al.*, 2008).

In summary, aldosterone stiffens human endothelial cells and appears to activate the insertion of epithelial sodium channels into endothelial cells, leading to endothelial dysfunction. ROS and dephosphorylation of eNOS at Ser^{1177} are also involved in aldosterone-induced impairment of eNOS-derived NO actions via increased degradation and decreased synthesis of NO (Figure 1). Aldosterone does not seem to influence human endothelial progenitor cell growth and senescence.

Studies on experimental animals

Acute effects of aldosterone

Aldosterone (1–1000 pmol·L^{-1}, 2 min) attenuated phenylephrine-induced contractions of endothelium-intact rat aortic ring segments, which was sensitive to spironolactone, whereas aldosterone enhanced the amine-induced contraction in endothelium-denuded segments. In endothelial cells, short-term aldosterone exposure caused a Ser^{1177}-dependent increase in NOS activity (Liu *et al.*, 2003). Beneficial effects of aldosterone (0.1–10 pmol·L^{-1}) by stimulating the production of NO from the endothelium were seen within minutes and are not inhibited by blockers of gene transcription. The effect was mediated by the MR and involved heat shock protein 90, PI3K, PKB and eNOS in rodents (Uhrenholt *et al.*, 2003; 2004). In bovine aortic endothelial cells, aldosterone enhanced ATP-induced NO production and increased phosphorylation of eNOS at Ser^{1179}. These effects were blocked by eplerenone and the PI3K inhibitor LY294002, Aldosterone enhanced relaxation induced by ACh but not by SNP in rat isolated aortas (Mutoh *et al.*, 2008). Aldosterone enhances endothelial NO production via activation of MR, possibly involving eNOS phosphorylation. In rat isolated mesenteric and cerebral arterioles, extraluminal administration of aldosterone (0.01–100 nmol·L^{-1}) elicited a transient vasodilatation within 5 min. Intraluminally administered aldosterone induced a greater and sustained dilatation. The vasodilator responses were inhibited by spironolactone or L-NAME and were abolished by endothelial denudation, suggesting that acute elevation of aldosterone evoked an endothelium-dependent, NO-mediated vasodilatation (Heylen *et al.*, 2009) (Table 1). Pojoga *et al.* (2010) provided evidence from studies on endothelial caveolin-1-deficient mice that MR activation plays a beneficial role in regulating eNOS expression/activity and consequently the vascular function during a high-salt diet.

In contrast to the beneficial actions of aldosterone in endothelial function presented so far, there are reports in the literature indicating that aldosterone elicits vasoconstriction via decreased eNOS activity and other mechanisms involved in increased vascular resistance. Aldosterone (10 nmol·L^{-1}) elicited a rapid constriction of rat small mesenteric resistance vessels, augmented phenylephrine-induced constriction and induced a rapid increase in intracellular concentrations

of Ca^{2+}, which were abolished by treatment with eplerenone. The vasoconstrictor response to aldosterone was related to decreased PKB phosphorylation. Pharmacological inhibitions of PI3K by wortmannin induced vasoconstriction; blockade of PKC and/or inhibition of the sodium–proton exchanger activity abolished aldosterone-induced vasoconstriction (Michea *et al.*, 2005). MR-mediated, aldosterone-induced increase in vascular tone may be related to a non-genomic mechanism that involves PI3K, PKB, PKC and sodium–proton exchanger activity. Aldosterone (1–10 $nmol \cdot L^{-1}$) added to both the bathing media and lumen rapidly caused dose-dependent constriction in afferent and efferent arterioles from rabbit kidneys that was not affected by spironolactone but was abolished by treatment with the PLC inhibitor neomycin. The vasoconstrictor action of aldosterone on afferent arterioles was inhibited by both nifedipine and efonidipine, whereas that on efferent arterioles was inhibited by efonidipine but not nifedipine (Arima *et al.*, 2003). In rabbit afferent arterioles, endothelium-derived NO modulated vasoconstrictor actions of aldosterone that were mediated by the activation of both inositol 1,4,5-triphosphate and PKC pathways (Arima *et al.*, 2004). In open-chest dogs, the infusion of aldosterone into the coronary artery reduced coronary blood flow in both non-ischaemic and ischaemic hearts, effects blunted by co-administration of GF 109203X, a PKC inhibitor, but not by spironolactone, suggesting that aldosterone non-genomically induced vasoconstriction via a PKC-dependent pathway that was independent of MR (Fujita *et al.*, 2005).

Short-term (60 min) aldosterone infusion enhanced experimental venous thrombosis, increased platelet adhesion, increased NADPH oxidase and decreased NOS expression and NO plasma levels in rats. Eplerenone only partially diminished the aldosterone effects (Gromotowicz *et al.*, 2011).

Chronic effects of aldosterone

ACh-induced vasodilation of mesenteric resistance arteries was impaired under salt-loading in stroke-prone spontaneously hypertensive rats (SHRSP), and it was improved under treatment with eplerenone, suggesting that aldosterone may play a pathophysiological role in salt-sensitive hypertension (Endemann *et al.*, 2004). ACh-induced relaxation was attenuated in aortic rings from rats with experimental heart failure. Spironolactone alone, administered for 11 weeks, had no influence and the angiotensin-converting enzyme (ACE) inhibitor trandolapril improved relaxant responses, whereas treatment with both completely restored the endothelium-dependent relaxation. Aortic superoxide formation was increased in rats with a failing heart, but this was normalized by treatment with spironolactone or spironolactone plus trandolapril. eNOS expression was increased in trandolapril-treated rats (Bauersachs *et al.*, 2002). Spironolactone added to an ACE inhibitor normalizes NO-mediated vascular relaxation in rats with heart failure by beneficially modulating the balance of NO and superoxide generation. Treatment of rats with heart failure with spironolactone for 4 weeks did not affect haemodynamics, but it improved endothelium-dependent vasodilatation in response to ACh. The eNOS levels decreased in the left ventricle and aorta by heart failure were restored by spironolactone, suggesting that aldosterone may participate in endothelial dysfunction in rats with failing heart (Thai *et al.*, 2006).

In rabbits fed a high cholesterol diet, there was an increase in superoxide generation. The selective aldosterone receptor antagonist SARA normalized superoxide generation in the aorta and reduced NAD(P)H and NAD(P)H oxidase activity to basal levels. It improved aortic relaxations to ACh to near normal levels, suggesting that MR antagonism appears to improve endothelial function and reduce ROS generation in diet-induced atherosclerosis (Rajagopalan *et al.*, 2002). In mesenteric small arteries, ACh-induced relaxation was impaired in rats with either angiotensin II or aldosterone infusion. The effect of the former substance was partially improved and that of the latter was normalized by treatment with spironolactone. Aortic NAD(P)H oxidase activity was increased by angiotensin II and aldosterone, suggesting that aldosterone may mediate some of angiotensin II–induced endothelial dysfunction (Virdis *et al.*, 2002). Systolic blood pressure was raised in aldosterone-infused rats, in which serum 8-isoporostane levels were increased. These effects were blunted by co-administration of losartan and the antioxidant tempol. Losartan and tempol totally prevented vascular, cardiac and renal increases in NAD(P)H-induced superoxide production stimulated by aldosterone (Iglarz *et al.*, 2004). Treatment of spontaneously hypertensive rats with eplerenone (30 and 100 $mg \cdot kg^{-1}$ day^{-1} for 10 weeks) reduced systolic blood pressure and normalized aortic media/lumen ratio and ACh-induced aortic relaxation. In addition, it enhanced eNOS and reduced expression of the mRNA for p22phox, one of the components of NAD(P)H oxidase, in the aorta, suggesting that aldosterone participates in the functional and structural vascular alterations of spontaneously hypertensive rats through a diminution of NO synthesis and an enhancement of superoxide generation (Sanz-Rosa *et al.*, 2005). Dahl salt-sensitive rats with heart failure showed down-regulation of eNOS expression, eNOS and Akt phosphorylation and NOS activity. Treatment with eplerenone reversed these unwanted effects and impaired ventricular function, and it inhibited up-regulated expression of aldosterone synthase, NAD(P)H oxidase p22phox, p47phox, gp91phox and inducible NOS and activated nuclear factor-κB and PKC (Kobayashi *et al.*, 2006). Cardioprotective actions of aldosterone blockade may be mediated by stimulating eNOS through Akt and inhibiting inducible NOS via NF-κB after activation of oxidative stress. Aldosterone decreased endothelial glucose-6-phosphate dehydrogenase (G6PD) expression and activity in bovine aortic endothelial cells, resulting in increased oxidative stress and decreased NO levels. Infusion of aldosterone *in vivo* decreased vascular G6PD expression and impaired vascular reactivity in mice. These effects were blocked by spironolactone or vascular gene transfer of G6PD (Leopold *et al.*, 2007). Aldosterone appears to induce a G6PD-deficient phenotype to impair endothelial function. Aldosterone (10–1000 $nmol \cdot L^{-1}$) increased superoxide generation in cultured rat aortic endothelial cells, whose effect was abolished by eplerenone, the Src inhibitor PP2 and apocynin. Aldosterone activated NAD(P)H oxidase and Rac1, a member of the Rho family GTPases, the effects being abolished by eplerenone. Aldosterone-induced superoxide generation was abolished either by a small G-protein inhibitor or dominant-negative Rac1 (Iwashima *et al.*, 2008). Aldosterone appears to induce

superoxide generation via MR-mediated activation of NAD(P)H oxidase and Rac1 in endothelial cells. Miyata *et al.* (2005) provided evidence that aldosterone-induced superoxide generation is associated with NAD(P)H oxidase activation through MR-mediated membranous translocation of p47phox and p67phox, but not p22phox, Nox-1 and Nox-4, in rat mesangial cells. Aldosterone and/or high salt were associated with increased levels of Nox-4 and p22phox in the kidney and lowered plasma NO levels in Dahl rats (Bayorh *et al.*, 2011) (Table 2).

Aldosterone has detrimental effects on various peripheral vascular beds. In the cerebral vasculature, it reduced blood flow and therefore promoted cerebral ischaemia (Rigsby *et al.*, 2005). When cerebral ischaemia was induced experimentally, the volume of the resultant infarct was greater in SHRSP than in Wistar Kyoto rats. The infarct size was reduced by spironolactone treatment to an extent similar to that seen in Wistar Kyoto rats (Dorrance *et al.*, 2001). The lumen diameter of middle cerebral arteries was greater in the spironolactone-treated (6 weeks) aldosterone SHRSP than in the control SHRSP. Spironolactone had no effect on systolic blood pressure (Rigsby and Dorrance, 2004). Chronic aldosterone appears to participate in impairment of cerebral blood perfusion. There was a cerebrovascular protective effect of spironolactone in the absence of lowered blood pressure in saline-drinking SHRSP (Rocha *et al.*, 1998). High plasma aldosterone concentration is a risk factor of cognitive impairment in hypertensive patients (Yagi *et al.*, 2011), and cerebral hypoperfusion is associated with later cognitive decline (Kitagawa *et al.*, 2009). Vascular endothelial dysfunction via high aldosteronaemia may participate in cerebral hypoperfusion that is associated with cognitive decline. Reduced cerebral blood flow associated with impairment of endothelial function and NO bioavailability leads to the generation and development of Alzheimer's disease (Toda and Okamura, 2012).

In contrast to these detrimental effects, aldosterone or MR activation in the brain may be required for neuronal survival by inhibiting cell death via the expression of anti-apoptotic genes (Macleod *et al.*, 2003; Rigsby *et al.*, 2005). Aldosterone facilitates neuronal damage through deleterious actions on vasculature. Conversely, MR activation in the brain may inhibit cell death.

Taken together, aldosterone non-genomically impairs or enhances endothelial function in humans, whereas it increases endothelial function in most instances of experimental animals *in situ* and in isolated preparations (Table 1). This discrepancy may not be explained by different doses of aldosterone used in humans and animals, use of different animal species in the experiments and use of *in vivo* or *in vitro* preparations. Differences in ambient redox or sodium status during experiments and in involvement of NO-independent (PLC, ET, PKC and sodium–proton exchanger) or MR-independent mechanisms may participate in the different actions of aldosterone. Chronic exposure to high aldosterone milieu is harmful to endothelial functions. Aldosterone-induced endothelial dysfunction and hypoperfusion in the brain seem to participate in the genesis of cognitive decline. An imbalance between NO synthesis and actions and superoxide anion generation plays an important role in endothelial dysfunction elicited either by acute or chronic exposures to aldosterone.

Aldosterone and ET-1

The endothelins are a family of vasoconstrictor peptides, secreted by vascular endothelium, which act through two main subtypes of receptors, ET_A and ET_B. The concept of an ET-1–aldosterone axis implies a feedback loop where ET-1 affects aldosterone but where the opposite also occurs (Rossi *et al.*, 2001) (Figure 2). In a rat model of hypertension induced with a 6 week infusion of aldosterone, modest elevation of blood pressure was associated with an increase in preproET-1 mRNA in the wall of resistance arteries, suggesting that aldosterone regulates preproET-1 gene in the vascular wall (Park and Schiffrin, 2001). Adrenalectomized rats given a single dose of aldosterone showed an increase in ET-1 mRNA levels in the kidney and colon (Wong *et al.*, 2007). There are other studies indicating that the ET-1 system plays an impor-

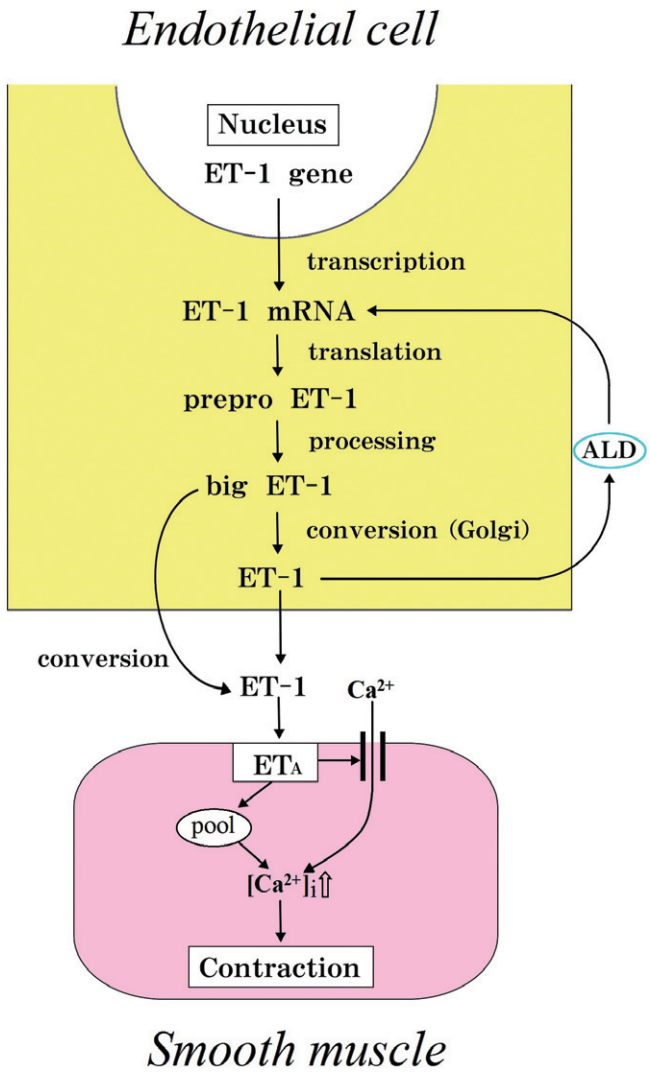

Figure 2

Information pathways via ET-1 liberated from endothelial cells to vascular smooth muscle cells. Possible site of action of ALD on ET-1 synthesis is shown in the figure. ET_A receptor; pool, Ca^{2+} storage site; $[Ca^{2+}]i$, intracellular Ca^{2+} concentration.

tant role in experimental models of hypertension mimicking hypermineralocorticoid states, including aldosterone infusion (Rossi *et al.*, 2001).

ET-1 increased the secretion of aldosterone and cortisol from human adrenocortical cells. The secretory effect of ET-1 was inhibited by the ET_A receptor antagonist BQ-123 and the ET_B receptor antagonist BQ-788 and when added together, the two antagonists suppressed the effect of ET-1, suggesting that ET-1, acting via ET_A and ET_B receptors, may exert an autocrine/paracrine regulation of the function of the human adrenal cortex (Rossi *et al.*, 1997a). In human adrenocortical carcinoma cells, ET-1 regulated the secretion of aldosterone by enhancing both aldosterone synthase transcription and raising the intracellular Ca^{2+} concentration (Rossi *et al.*, 1997b). ETs stimulate aldosterone secretion from human adrenocortical zona glomerulosa cells, acting through ET_A receptors exclusively coupled to a PLC/PKC–dependent pathway and ET_B receptors coupled to both phosphorylase C/PKC and COX-dependent cascades (Andreis *et al.*, 2002). Rossi *et al.* (2003) provided evidence that the secretagogic effect of ET-1 on aldosterone was mediated via ET_B receptors in hypertensive patients with primary aldosteronism.

In Dahl salt-sensitive rats, a high-sodium diet increased systemic blood pressure and aortic ET-1 protein content and reduced NO-mediated relaxation of aortic rings in response to ACh; ET-1 tissue levels were inversely correlated with ACh-induced relaxations. The ET_A receptor antagonist LU135252 only partially reduced blood pressure but normalized sodium-induced changes in vascular reactivity (Barton *et al.*, 1998). Vascular remodelling of small arteries occurs in aldosterone-infused rats exposed to a normal-salt diet and may be mediated by ET-1 via stimulation of ET_A receptors (Pu *et al.*, 2003). Aldosterone receptor antagonism normalizes blood pressure, prevents up-regulation of vascular ET-1 and restores NO-mediated endothelial dysfunction in liquorice-induced, 11β-hydroxysteroid dehydrogenase-2-deficient hypertension in rats (Quaschning *et al.*, 2001b). In an ovine model of persistent pulmonary hypertension of the newborn, there was a decrease in eNOS-mRNA and protein with a corresponding increase in ET-1 expression (Black *et al.*, 1998). Maeda *et al.* (2004) provided evidence that exercise-induced increase in ET-1 in rat kidney elicits a decrease in blood flow in the kidney, possibly through its vasoconstrictor action and also by attenuating endothelial NO production.

In human vein endothelial cells, eNOS protein expression was reduced after ET-1 treatment. PKC inhibition also down-regulated eNOS protein expression, whereas the PKC agonist PMA up-regulated its expression. ET-1 exposure reduced PKC activity, suggesting that high levels of ET-1 impair endothelial NO production via an isoform-specific PKC-mediated inhibition of eNOS expression (Ramzy *et al.*, 2006). A murine model of endothelium-restricted human preproET-1 overexpression exhibited hypertrophic remodelling and oxidant excess–dependent endothelial dysfunction of resistance vessels (Amiri *et al.*, 2004). ET-1 increased H_2O_2 levels in lamb fetal pulmonary arterial smooth muscle cells in an ET_A receptor–dependent fashion. ET-1 decreased eNOS promoter activity in fetal pulmonary arterial endothelial cells in co-culture with fetal pulmonary arterial smooth muscle cells, suggesting that muscle-derived H_2O_2 contributes to ET-1–mediated down-regulation of eNOS expression (Wedgwood and Black, 2005).

Type I diabetes impaired eNOS– and neuronal NOS–dependent dilatation of rat cerebral arterioles. BQ-123, a specific antagonist of ET_A receptors, restored impaired eNOS– and neuronal NOS–dependent vasodilatation in diabetic rats. Superoxide production was higher in brain tissue from diabetic rats compared with non-diabetic rats and BQ-123 decreased the production of superoxide in diabetic rats (Arrick and Mayhan, 2010). Activation of ET_A receptors during type I diabetes mellitus appears to play an important role in impaired eNOS– and neuronal NOS–dependent dilatation of cerebral arterioles via superoxide generation. In contrast, in type II diabetic Goto–Kakizaki rats, an activated ET pathway does not appear to be involved in vascular dysfunction, including decreases in NO-mediated relaxation and NO-stimulated guanylyl cyclase activity (White *et al.*, 2003).

Taken together, ET-1 enhances aldosterone secretion. On the other hand, elevated plasma levels of aldosterone appear to participate in ET-1 release from the endothelium. ET-1–induced ET_A receptor activation would be one of the mechanisms underlying aldosterone-induced impairment of endothelial NO synthesis and bioavailability through inhibiting expression of eNOS mRNA and protein and enhancing ROS generation.

Therapeutic measures

MR inhibitors

Aldosterone blockade is required to reduce the risk of progressive target organ damage in patients with hypertension and heart failure. This may be achieved by the non-selective aldosterone blocker spironolactone or with use of the selective aldosterone receptor blocker eplerenone. This section summarizes information showing the beneficial effects of MR blockade only in reference to endothelial function.

Effects of spironolactone. In patients with chronic heart failure on diuretic/ACE inhibitor therapy, treatment with spironolactone (50 $mg \cdot day^{-1}$ for 1 month) increased the forearm blood flow response to ACh with an associated increase in vasoconstriction due to L-NMMA, suggesting that spironolactone improves endothelial dysfunction in patients with heart failure (Farquharson and Struthers, 2000). In patients with resistant hypertension, treatment with spironolactone (12.5–25 $mg \cdot day^{-1}$, 3 months) increased brachial artery flow–mediated dilatation independently of blood pressure change (Nishizaka *et al.*, 2004). Treatment of resistant hypertensive patients with spironolactone (25 $mg \cdot day^{-1}$ for over 6 months) improved endothelial function and left ventricular hypertrophy and reduced blood pressure despite the presence of aldosterone excess (Ubaid-Girioli *et al.*, 2009). In primary aldosteronism patients, flow-mediated vasodilatation, systolic blood pressure, plasma aldosterone concentration and aldosterone–renin ratio were improved after treatment with spironolactone (Tsuchiya *et al.*, 2009).

Effects of eplerenone. Eplerenone and spironolactone normalized blood pressure in rats with liquorice-induced hypertension, restored endothelium–dependent relaxation of aortic rings and blunted a decrease in vascular eNOS protein

content and nitrate tissue levels (Quaschning *et al*., 2001b). In SHRSP, ACh-induced vasodilatation was impaired under salt loading, but this was improved under eplerenone treatment. Eplerenone prevented increased heart weight and ventricular collagen deposition induced by high salt (Endemann *et al*., 2004). In two-kidney, one-clip renovascular hypertensive rats treated with eplerenone, eNOS gene expression was increased in the aorta and heart, as compared with those without the treatment (Hao *et al*., 2004). Eplerenone or candesartan partially improved endothelium-dependent relaxation, heart/body weight ratio and cardiac fibrosis and decreased systolic blood pressure in Dahl salt-sensitive rats. Combination therapy normalized blood pressure and further improved endothelial function, cardiac hypertrophy, and cardiac fibrosis (Takeda *et al*., 2007). Eplerenone increases NO bioavailability and improves endothelial dysfunction by decreasing oxidative stress (Takeda, 2009). In isolated human endothelial cells, eplerenone improves aldosterone-induced impairment of endothelial function (Nagata *et al*., 2006; Oberleithner *et al*., 2006a).

Rabbits placed on a high cholesterol diet showed an increase in superoxide generation. Eplerenone (50 $mg \cdot kg^{-1}$, twice daily for 6 weeks) normalized superoxide generation in aortas and reduced NADH and NAD(P)H oxidase activity to basal levels. Relaxations in response to ACh were improved (Rajagopalan *et al*., 2002). Targeting aldosterone by blocking its receptor has potential anti-atherosclerotic effects. Treatment with eplerenone reduced systolic arterial pressure and normalized aortic media/lumen ratio and ACh-induced aortic relaxations in spontaneously hypertensive rats; eplerenone also enhanced eNOS and reduced the expression of NAD(P)H oxidase p22phox mRNA (Sanz-Rosa *et al*., 2005). Cyclic stretch–mediated activation of NAD(P)H oxidase in rat aortic smooth muscle cells was attenuated by treatment with eplerenone (Ohmine *et al*., 2009). Aldosterone and high salt enhanced renal oxidative stress and lowered plasma NO levels in rats. Eplerenone tended to reduce kidney damage and inhibit Nox-4 expression (Bayorh *et al*., 2011).

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; therefore, these divergent effects must be kept in mind when modification of MR activity is used as a therapeutic strategy for cerebral ischaemic disease (Rigsby *et al*., 2005).

ET_A receptor antagonists

Interactions of aldosterone and ET-1 were summarized in the previous 'Aldosterone and endothelin' section. The ET system, by interacting with the renin–angiotensin–aldosterone system, may play an important role in the pathophysiology of arterial hypertension and congestive heart failure (Rossi *et al*., 2001). Aldosterone increases ET-1 synthesis. High salt intake induced an increase in plasma ET-1 levels as compared with intermediate and low-salt diet levels in salt-sensitive hypertensive patients (Ferri *et al*., 1997). Darusentan, a selective ET_A receptor antagonist, emerges as new treatment option in patients with resistant hypertension (Enseleit *et al*., 2010).

Studies on humans. In patients with atherosclerosis, BQ-123 for 1 h at a rate of 200 $mmol \cdot min^{-1}$ increased epicardial artery diameter and lowered coronary vascular resistance; the vasodilator response to ACh, corrected for the SNP response, was improved by BQ-123 in segments that constricted with ACh at baseline; cold-pressor testing–mediated vasoconstriction was reversed after BQ-123 in dysfunctional segments, suggesting that ET_A receptor activation contributes to basal human coronary arterial tone and endothelial dysfunction (Halcox *et al*., 2001). ET_A receptor antagonism may have therapeutic potential in endothelial dysfunction. In patients with coronary artery disease, substance P did not increase coronary blood flow. After ET_A receptor blockade by BQ-123 (40 $nmol \cdot min^{-1}$ of 60 min infusion), the endothelium-dependent vasodilator response to substance P was restored (Böhm *et al*., 2008). Administration of the ET_A receptor antagonist atrasentan (30 mg for 6 months) to patients with non-obstructive coronary artery disease improved changes in coronary blood flow in response to ACh (Reriani *et al*., 2010).

Studies on experimental animals. Barton *et al*. (1998) provided evidence that the ET_A receptor antagonism by LU135252 lowers vascular ET-1 content, improves endothelial function and prevents structural changes in vasculature in rats with salt-sensitive hypertension. The ET_A receptor antagonist BMS 182874 attenuated blood pressure elevation and prevented vascular remodelling or hypertrophy of the aorta and mesenteric resistance arteries in aldosterone-infused rats (Park and Schiffrin, 2001). In rats with liquorice-induced hypertension, ET_A receptor antagonism by darusentan (LU135252) improved renovascular endothelium-dependent relaxation (Quaschning *et al*., 2001a). BMS 182874 and spironolactone decreased oxidative stress, normalized the hypertrophic remodelling and decreased collagen and fibronectin deposition in the vascular wall of aldosterone-infused rats (Pu *et al*., 2003). Normalization of blood pressure through chronic ET_A receptor blockade by LU135252 prevented up-regulation of vascular ET-1 and improved endothelial dysfunction in rats with hypertension induced by inhibition of 11β-hyroxysteroid dehydrogenase, the enzyme that prevents inappropriate activation of MRs by glucocorticoids (Ruschitzka *et al*., 2001).

Ca^{2+} channel blockers

Ca^{2+} is conveyed through T-type Ca^{2+} channels to the mitochondria, where it activates aldosterone synthesis; thereafter, aldosterone stimulates T-type Ca^{2+} channel expression (Rossier *et al*., 2003; Lalevée *et al*., 2005), creating a positive feedback loop for aldosterone biosynthesis in the adrenal cells. Mibefradil, a T-type Ca^{2+} channel blocker, is highly effective in adrenal glomerulosa cells for reducing this channel activity and aldosterone biosynthesis (Rossier *et al*., 1998).

Aldosterone causes non-genomic vasoconstriction via Ca^{2+} mobilization through L- or T-type Ca^{2+} channels in afferent or efferent arterioles in the rabbit kidney, respectively, and endothelium-derived NO modulates the vasoconstrictor effects of aldosterone (Arima, 2006). These vasoconstrictor actions on the glomerular microcirculation appear to play an important role in the pathophysiology of renal diseases by elevating renal vascular resistance; therefore, blockade of Ca^{2+}

channels is expected to be a potential method for reversing untoward effects of aldosterone on blood supply. In outpatients with essential hypertension, treatment with the L-type Ca^{2+} channel blocker amlodipine (average dose of 6.2 mg·day^{-1} for more than 1 year) lowered systolic blood pressure and decreased the plasma levels of norepinephrine and active renin. Substituting efonidipine (36 mg·day^{-1} for 6 months), both a L- and T-type Ca^{2+} channel blocker, for amlodipine decreased the heart rate and plasma aldosterone levels, suggesting that Ca^{2+} influx mediated by T-type Ca^{2+} channels was involved in the release of aldosterone (Tanaka *et al.*, 2007). In outpatients with essential hypertension, the effect of efonidipine (40 mg·day^{-1}) on the suppression of plasma aldosterone levels was sustained for at least 18 months, and this long-term therapy decreased left ventricular mass index (Tsutamoto *et al.*, 2009). Efonidipine inhibits aldosterone synthesis and secretion *in vitro* (Imagawa *et al.*, 2006) and *in vivo* (Okayama *et al.*, 2006). According to Sato and Fukuda (2010), eplerenone, when given with amlodipine or the ACE inhibitor imidapril, causes a beneficial antihypertensive effect in Japanese patients with essential hypertension. Benidipine, as a T-type Ca^{2+} channel blocker, has benefits in reducing ischaemia/reperfusion-induced systemic oxidative stress through suppression of aldosterone production and increase in eNOS phosphorylation in mice (Ohtani *et al.*, 2012).

Miscellaneous

Losartan reduces the baseline plasma aldosterone levels and blocks the effect of angiotensin II to stimulate release of aldosterone in normotensive subjects under low salt conditions (10 mmol sodium day^{-1}), whereas it does not affect baseline renal plasma flow but does attenuate the renal plasma flow response to exogenous angiotensin II under high salt conditions (200 mmol sodium day^{-1}) (Gandhi *et al.*, 1996).

Renal Rac1 GTPase regulates salt sensitivity of blood pressure and kidney injury via a MR-dependent mechanism and salt-induced Rac1 and aldosterone act in concert to induce MR over-activity, suggesting that the Rac1-mediated pathway in the kidney can be an alternative therapeutic target for salt-sensitive hypertension and salt-mediated kidney injury (Shibata *et al.*, 2011).

Mice that are arrhythmic due to the deletion of Cry1 and Cry2 biological clock genes suffer from salt-sensitive hypertension and show overexpression of the 3β-hydroxyl-steroid dehydrogenase (a new type of steroid-synthetic enzyme) gene especially in aldosterone-producing cells in the adrenal cortex (Okamura *et al.*, 2011). As the 3β-hydroxyl-steroid dehydrogenase inhibitor trilostane decreases plasma aldosterone concentrations and is effective in treating hypertension in Cryl-null mice, these authors urge the development of novel 3β-hydroxyl-steroid dehydrogenase subtype-specific inhibitors for treatment of hyperaldosteronism.

Summary and conclusion

The mineralocorticoid hormone aldosterone is a critical regulator of the biochemical and biophysical properties of endothelial cells. Impaired endothelial function associated with aldosterone leads to vasoconstriction and decreased blood supply to organs and tissues. An imbalance between aldosterone and salt, associated with increased plasma aldosterone concentrations, provides a risk for resistant hypertension as well as coronary, renal and cerebral ischaemic disease. In the fast, non-genomic signalling pathway, vascular endothelial cells respond to aldosterone with an increase, a decrease, or no change in NO bioavailability in human subjects, whereas an enhancement of endothelial function is seen in experimental animal studies. Long-term exposure to aldosterone results in endothelial dysfunction and reduced NO bioavailability in humans, experimental animals and isolated endothelial cells through its genomic actions. Aldosterone in conjunction with high sodium concentrations reduces NO release from the endothelium. Aldosterone activates the insertion of epithelial sodium channels to endothelial cells leading to endothelial dysfunction. Oxidative stress through NAD(P)H oxidase and BH_4 deficiency plays an important role in impairing physiological actions of endothelial NO. Increase in plasma aldosterone levels appears to be one of the risk factors of endothelial dysfunction leading to resistant hypertension, atherosclerosis, coronary insufficiency and Alzheimer's disease. Increments in intracellular Ca^{2+} introduced through T-type Ca^{2+} channels participate in increased production of aldosterone, resulting in an enhancement of aldosterone-mediated endothelial dysfunction. ET-1 also increases aldosterone synthesis, and aldosterone stimulates the release of ETs from the endothelium. This feedback mechanism augments impaired endothelial function. MR inhibitors, T-type Ca^{2+} channel blockers and ET_A receptor antagonists are clinically evaluated to be effective in restoring endothelial function. Not only is keeping endothelial function healthy an important way to provide prophylaxis and therapeutic efficacy against serious cardiovascular and metabolic diseases but also it contributes to a long and healthy life (Toda, 2012). Novel ways to interfere with aldosterone synthesis and its mechanism of untoward action are suggested in recently reported animal studies. Constructive ideas from sophisticated animal studies, highlighting novel ways for treatment against aldosterone-induced endothelium dysfunction, and more advanced, feasible measures for prophylaxis and pharmacological therapy for individuals in health and disease are awaited.

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Conflict of interest

The authors declare no competing financial interests in relation to the work described in this report.

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