Renin-angiotensin-aldosterone system and oxidative stress in cardiovascular insulin resistance

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Departments of 1Internal Medicine and 2Medical Pharmacology and Physiology and 3Diabetes and Cardiovascular Center of Excellence, University of Missouri School of Medicine, and 4Harry S. Truman Veterans Affairs Medical Center, Columbia, Missouri

Cooper SA, Whaley-Connell A, Habibi J, Wei Y, Lastra G, Manrique C, Stas S, Sowers JR. Renin-angiotensin-aldosterone system and oxidative stress in cardiovascular insulin resistance. Am J Physiol Heart Circ Physiol 293: H2009–H2023, 2007. First published June 22, 2007; doi:10.1152/ajpheart.00522.2007.—Hypertension commonly occurs in conjunction with insulin resistance and other components of the cardiometabolic syndrome. Insulin resistance plays a significant role in the relationship between hypertension, Type 2 diabetes mellitus, chronic kidney disease, and cardiovascular disease. There is accumulating evidence that insulin resistance occurs in cardiovascular and renal tissue as well as in classical metabolic tissues (i.e., skeletal muscle, liver, and adipose tissue). Activation of the renin-angiotensin-aldosterone system and subsequent elevations in angiotensin II and aldosterone, as seen in cardiometabolic syndrome, contribute to altered insulin/IGF-1 signaling pathways and reactive oxygen species formation to induce endothelial dysfunction and cardiovascular disease. This review examines currently understood mechanisms underlying the development of resistance to the metabolic actions of insulin in cardiovascular as well as skeletal muscle tissue.
Inappropriate activation of these phosphatases may contribute to insulin/IGF-1 resistance in CV tissue as well as liver, skeletal muscle, and adipose tissue (27, 202, 220).

**Vascular Actions of Insulin/IGF-1**

Vascular relaxation effects of insulin/IGF-1 are mediated, in part, by endothelial cell production of NO (186, 190, 224, 235, 236) (Fig. 1A). IR/IGF-1 receptor mediation of PI3K/PDK-1/Akt phosphorylation/activation leads to stimulation of endothelial NO synthase (eNOS). AT1R activation decreases the availability of NO via the induction of insulin resistance, diminishing eNOS mRNA stability and promoting NADPH oxidase-induced ROS production. Mineralocorticoids also activate NADPH oxidase with secondary superoxide production (O2⁻) and consequent generation of peroxynitrite (ONOO⁻). Akt, PKB; GRE, glucocorticoid response element; Gαi, Goi subunit; IRS, insulin receptor substrate; NOX2, catalytic subunit of NADPH oxidase; p22, p47, p40, and p67, subunits of NADPH oxidase; PH, pleckstrin homology domain; PI3-K, phosphatidylinositol 3-kinase; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; ROK, Rho kinase; SOD, superoxide dismutase. Akt, PKB; GRE, glucocorticoid response element; Gαi, Goi subunit; IRS, insulin receptor substrate; NOX2, catalytic subunit of NADPH oxidase; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; ROK, Rho kinase; SOD, superoxide dismutase. Fig. 1. A: vascular effects of insulin (INS)/IGF-1 and counteregulatory effects of angiotensin II (ANG II) type 1 receptor (AT1-R) and mineralocorticoid receptor (MR) activation in endothelial cells. Insulin actions on the blood vessel are partially mediated by increased production of nitric oxide (NO) through phosphorylation and secondary activation of endothelial NO synthase (eNOS). AT1R activation decreases the availability of NO via the induction of insulin resistance, diminishing eNOS mRNA stability and promoting NADPH oxidase-induced ROS production. Mineralocorticoids also activate NADPH oxidase with secondary superoxide production (O2⁻) and consequent generation of peroxynitrite (ONOO⁻). Akt, PKB; GRE, glucocorticoid response element; Gαi, Goi subunit; IRS, insulin receptor substrate; NOX2, catalytic subunit of NADPH oxidase; p22, p47, p40, and p67, subunits of NADPH oxidase; PH, pleckstrin homology domain; PI3-K, phosphatidylinositol 3-kinase; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; ROK, Rho kinase; SOD, superoxide dismutase. Akt, PKB; GRE, glucocorticoid response element; Gαi, Goi subunit; IRS, insulin receptor substrate; NOX2, catalytic subunit of NADPH oxidase; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; ROK, Rho kinase; SOD, superoxide dismutase. Akt, PKB; GRE, glucocorticoid response element; Gαi, Goi subunit; IRS, insulin receptor substrate; NOX2, catalytic subunit of NADPH oxidase; p22, p47, p40, and p67, subunits of NADPH oxidase; PH, pleckstrin homology domain; PI3-K, phosphatidylinositol 3-kinase; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; ROK, Rho kinase; SOD, superoxide dismutase.
(i.e., ANG II)-induced increases in cytosolic calcium \([\text{Ca}^{2+}]\) and myosin light chain (MLC) kinase activity (13, 174, 191). By enhancing MLC phosphatase activity, insulin and IGF-1 reduce MLC kinase activity and thus \([\text{Ca}^{2+}]\)-sensitive contraction (13, 125, 174, 191, 199) (Fig. 1B).

**ANG II Actions on the Vasculature**

There is accumulating evidence that ANG II, in addition to its vasoconstriction effects, attenuates the CV and skeletal muscle metabolic actions of insulin and IGF-1 (115, 186, 190). The mechanisms involved in these inhibitory effects of ANG II include the generation of ROS and the activation of small-molecular-weight proteins such as RhoA and Rac1 (9, 66, 189, 190) (Figs. 1 and 2). Indeed, there is increasing evidence indicating that ANG II contributes to insulin resistance and other components of CMS such as hypertension, dyslipidemia, central fat deposition, hepatic steatosis, CKD, and proteinuria (69, 77, 186, 190, 194, 229, 230).

ANG II exerts inflammatory effects and promotes vascular growth/remodeling, apoptosis, and fibrosis. There is mounting evidence that increased generation of ROS partially mediates these effects (66, 189). These markedly reactive ROS molecules oxidize lipids, protein, and DNA as well as cause cellular injury and enhance vasoconstriction, in part by converting NO to peroxynitrite (ONOO\(^-\)), itself a potent ROS. ROS activate transcription factors such as TNF-\(\alpha\), monocyte chemoattractant protein (MCP)-1, IL-6, and C-reactive protein (CRP). TNF-\(\alpha\), in turn, impedes insulin- and IGF-1-mediated eNOS activation as well as the antiapoptotic actions of insulin and IGF-1 (128, 186, 190).

**Animal Model to Investigate the Role of ANG II in Mediating Insulin/IGF-1 Resistance**

Our laboratory has utilized the transgenic TG(mRen2)27 rat, which harbors the mouse renin gene and displays an activated tissue renin-angiotensin-aldosterone system (RAAS) with increased ANG II levels and increased plasma mineralocorticoids, to evaluate the role of increased tissue ANG II and mineralocorticoids in mediating CVD as well as skeletal muscle insulin resistance (Fig. 3) (18, 229, 231). Indeed, this rodent model develops proteinuria (77, 231) as well as insulin resistance (18), fatty liver steatosis, and hypertension (18, 77, 229, 231), making it a relevant model of CMS.

A recent study (230) from our laboratory has observed that the vasculature from young Ren2 rats exhibits increased NADPH oxidase activity, ROS levels (Fig. 4), lipid peroxidation, inflammation (increased expression of TNF-\(\alpha\) and CRP), and indexes of apoptosis compared with Sprague-Dawley rats. Furthermore, in the vasculature, there was a marked reduction in insulin stimulation of Akt signaling eNOS Ser\(^{1177}\) phosphorylation/activation. These abnormalities were markedly improved by in vivo treatment with an AT\(_1\)R blocker or the superoxide dismutase (SOD)/catalase mimetic tempol. Available data have suggested that vascular RAAS activation and insulin/IGF-1 resistance perpetuate each other and concomitantly aggravate the deleterious effects of ANG II on the vasculature.
dantly contribute to endothelial dysfunction, vascular inflammation/remodeling, and hypertension (230). Similar observations have also been made in the left ventricle of hearts taken from young insulin-resistant Ren2 rats (192, 231; Cooper SA, Whaley-Connell A, Habibi J, Stump CS, Link CD, Hayden MR, Ferrario C, Sowers JR, unpublished observations).

Insulin and ANG II in the Heart

Insulin regulates metabolism in CV tissue by modulating glucose uptake and utilization, glycogen synthesis, lipid metabolism, proliferation, contractility, remodeling, and apoptosis in cardiomyocytes (Fig. 2). Insulin and IGF-1 exert a number of metabolic and functional effects on the heart (2, 22, 62, 64, 70, 84, 98, 105, 117, 137, 146, 153, 163–165, 179, 180, 192, 198, 222; Cooper SA et al., unpublished observations) (Fig. 2).

Both peptides regulate glucose uptake, glycogen and protein synthesis, growth, and lipid metabolism (2, 98, 137). As in skeletal muscle, glucose uptake in cardiomyocytes involves mobilization of insulin-responsive GLUT4 via a PI3K/Akt signaling pathway (2, 98) (Fig. 2). Furthermore, in cardiomyocytes, insulin stimulation of the PI3K/Akt pathway results in the phosphorylation and nuclear exclusion of the forkhead transcription factor FOXO-1, which further modulates glucose and lipid metabolism (125, 137).

Insulin and IGF-1 normally enhance cardiac contractility (22, 153, 163–165, 186, 190) via signaling through the PI3K/Akt pathway. This signaling is associated with enhanced Ca$^{2+}$ influx via the activation of L-type Ca$^{2+}$ channels and reversed Na$^{+}$/Ca$^{2+}$ exchange (117, 222). Insulin and IGF-1 also enhance cardiomyocyte myofilament Ca$^{2+}$ sensitivity (42).

Increases in myocardial NO production through the PI3K/Akt/eNOS pathway also appear to contribute to the inotropic effects of these peptides (62, 164).

Insulin- and IGF-1-induced increases in myocardial contractility result in increased oxygen consumption (198). Cardiac oxygen demand is a potent determinant of myocardial blood flow (MBF), and insulin and IGF-1 enhance MBF and promote capillary recruitment in the heart (84, 198). Hyperinsulinemia...
increases MBF, particularly in areas of the myocardium associated with high rates of glucose uptake (64, 105, 146, 179, 180) (Fig. 5). These observations suggest coupling between the metabolic and coronary vascular actions of insulin and IGF-1 in the heart, with increases in capillary recruitment and MBF-enhancing insulin-stimulated increases in the delivery of insulin and glucose. These actions of insulin and IGF-1, as well as their direct effects on cardiomyocytes, also enhance glucose transport (2, 98, 137).

Insulin and IGF-1 also regulate developmental and physiological growth and remodeling of the heart (41, 78, 94, 102, 142, 163, 223, 234) (Fig. 2). The peptides accomplish these effects by signaling through the PI3K/Akt pathway (78, 102, 223). Downstream from Akt, activation of the mammalian target of rapamycin promotes cardiac growth, whereas suppression of glycogen synthase kinase-3 (GSK-3β), as well as FOXO phosphorylation, also modulates cardiomyocyte growth (78, 223). Signaling through the Akt pathway also exerts antiapoptotic effects on the myocardium (163), by negatively regulating apoptotic factors and positively regulating factors that induce survival genes.

Indeed, one apoptotic signaling system modulated by the Akt pathway involves the phosphorylation and nuclear exclusion of the FOXO subgroup of the forkhead family of transcription factors. Insulin and IGF-1 also promote survival by direct phosphorylation/inactivation of Bad, a member of the Bcl-2 family, which promotes apoptosis by binding to and antagonizing the action of prosurvival members of the family such as Bcl-2 and Bcl-XL. Insulin/IGF-1 activation of Akt may also interfere with stress-activated protein kinases such as JNK, p38, and MAPK pathways critically involved in the induction of apoptosis following exposure of cardiomyocytes to physical stress stimuli (94, 234). Finally, Akt activation increases the expression of c-FLIP, a caspase-8 homologene that inhibits TNF receptor family-induced apoptosis (142). In conditions of insulin resistance/hyperinsulinemia, pathological cardiomyocyte hypertrophy is promoted by interactions of insulin/IGF-1 with growth factors such as ANG II, catecholamines, endothelin, and mineralocorticoids to stimulate signaling pathways involving MAPK, p38, MAPK JAK/STAT, and the small-molecular-weight G proteins Rho and Ras (163, 180, 186, 188, 190).

**Insulin Resistance and CVD: Role of RAAS and Other Factors**

There is accumulating evidence that hypertension predisposes an individual to diabetes independently of other CVD risk factors, such as obesity (186, 187, 190). Clinical evidence supports a link between insulin resistance/hyperinsulinemia and hypertension, including positive associations between blood pressure and fasting insulin levels in patients with essential hypertension (42, 118, 162, 186, 190). Mechanisms to explain this linkage include cellular abnormalities in insulin signaling (186, 190), cellular cation alterations, enhanced sympathetic nervous system activity (162), and enhanced RAAS activity (186, 190) as well as inflammation and oxidative stress (186, 190). Importantly, resistance to the metabolic and pro-
liferative actions of insulin appears to be differential. Indeed, a seminal feature of insulin resistance is impairment in PI3K/Akt signaling metabolic pathways, whereas other insulin signaling growth pathways, including RAS/MAPK/JAK/STAT signaling, are not inhibited (36, 89, 150, 186, 190). In the vasculature, this leads to diminished endothelium-mediated vasodilation and increased growth remodeling and atherosclerosis (18, 89, 50).

In addition, proinflammatory effects of chronically elevated levels of glucose and fatty acids contribute to endothelial dysfunction, chronic low-grade inflammation, and insulin resistance. For example, exposure of the vasculature and myocardium to elevated levels of free fatty acids leads to impaired insulin signaling (47, 226), enhancement of vascular RAAS (227), and oxidative stress (83) as well as impaired insulin-stimulated eNOS activity and NO production (47). Chronic hyperglycemia also increases oxidative stress in the vasculature (166). Increased ROS induced by hyperglycemia and dyslipidemia further impair insulin signaling, decrease NO bioavailability, reduce cellular tetrahydrobiopterin levels, and promote the generation of superoxide by eNOS.

Role of RAAS in Vascular Insulin Resistance

As noted previously, physiological concentrations of insulin increase vasodilatation through NO release and exert antioxidant and anti-inflammatory effects via signaling through the PI3K/Akt metabolic pathway (186, 190). ANG II and mineralocorticoids, in contrast, cause vasoconstriction and enhance the expression of proinflammatory cytokines, adhesion molecules, growth, and inflammatory pathways (80, 85, 86, 135, 153, 192, 207, 208). Furthermore, ANG II and aldosterone interfere with many of the metabolic signaling actions of insulin and IGF-1 in the CV system (9, 80, 85, 86, 102, 135, 153, 186, 188–190, 207, 208, 230).

ANG II, acting through the AT1R, increases the generation of ROS in the vasculature, primarily through activation of the membrane-bound NADPH oxidase enzyme complex (Fig. 1, A and B) (8, 16, 31, 57, 63, 101, 112, 141, 155, 159, 189, 201, 206, 213). Infusion of ANG II impairs endothelium-dependent vasorelaxation (31), and this impairment is corrected by administration of SOD (105), indicating the critical role of ROS in ANG II-mediated endothelial dysfunction (189). ANG II-stimulated ROS inhibit insulin/IGF-1 signaling through the PI3K/Akt signaling pathway to activate eNOS (13, 125, 199, 224, 235, 236). Furthermore, ROS generated by ANG II inactivate NO (20, 120, 152, 203), and the resultant decrease in bioavailable NO, in turn, upregulates the AT1R on vascular cells (81). This creates a cycle of impaired endothelium-derived vasodilation and increased ANG II-mediated vasoconstriction. ANG II also stimulates RhoA/Rho kinase activation, which decreases eNOS expression, in part, by decreasing eNOS mRNA stability (122, 200) (Fig. 1A). ANG II, acting via its AT1R, increases VSMC contraction by increasing intracellular \([\text{Ca}^{2+}]\) and \(\text{Ca}^{2+}\)-MLC sensitization (129, 233) (Fig. 1B).

Both processes are mediated, in part, by ANG II-stimulated generation of ROS in endothelial cells and VSMCs (189, 205, 233). ANG II also increases \(\text{Ca}^{2+}\)-MLC sensitization by stimulating Rho kinase activity in VSMCs, whereas insulin and IGF-1 induce relaxation by increasing endothelial cell production of NO and by reducing \(\text{Ca}^{2+}\)-MLC sensitization (175). ANG II decreases the ability of insulin and IGF-1 to decrease \(\text{Ca}^{2+}\)-MLC sensitization by activating Rho kinase, which phosphorylates myosin binding protein and thereby inhibits the ability of these peptides to dephosphorylate \(\text{Ca}^{2+}\)-MLC, which leads to increased \(\text{Ca}^{2+}\)-MLC phosphorylation (186, 190) (Fig. 1B). This concept is borne out by the observation that increases in Rho kinases and a decrease in myosin binding protein activity occurs in ANG II-mediated (30) and insulin-resistant (176) hypertensive rodents.

Increased ROS also activate multiple redox signaling pathways including NF-κB (127). NF-κB, in turn, enhances other ANG II-mediated inflammatory responses by upregulating other inflammatory molecules such as TNF-α, MCP-1, and CRP (72, 124). TNF-α activates several serine kinases including JNK, Iκκ-β, and IL-1β receptor-associated kinase (91), which directly or indirectly increase serine phosphorylation of IRS-1/2, leading to decreased PI3K/Akt signaling responses and subsequent impaired insulin/IGF-1 stimulation of eNOS, production of NO, and vasodilatation (6, 51, 95, 96). TNF-α increases the expression of other inflammatory substance including IL-6 and CRP. CRP, in turn, appears to attenuate insulin-stimulated NO production in endothelial cells by increasing phosphorylation of IRS-1 at Ser307 and indirectly by enhancing Rho kinase and JNK signaling (6, 217). CRP also upregulates VSMC AT1Rs (225) and increases the expression of VCAM, ICAM, E-selectin, and MCP-1 in endothelial cells (144), thus counterbalancing the antiatherosclerotic and vasodilatory effects of insulin/IGF-1-stimulated NO production.

In addition to stimulating membrane NADPH oxidase in vascular cells, ANG II, in conjunction with other cellular stresses, may increase endoplasmic reticulum stress (139) and mitochondrial oxidative stress (186). In addition to the impact of these inflammatory changes with regard to vasomotion and atherosclerosis, alterations in microvascular blood flow may impact cardiac, adipose tissue, skeletal muscle, and liver blood flow. In adipose tissue and the liver, microvascular inflammation may affect contributing to adipose tissue inflammation (increased macrophages) (35) and nonalcoholic fatty liver disease (1), conditions frequently associated with alterations in the RAAS and insulin resistance (183). Another maladaptive effect of increased oxidative stress is enhanced DNA strand breaks (88) and depletion of cellular NAD⁺ (88, 140). Reductions in NAD⁺ concentrations further result in the depletion of cellular ATP levels and, hence, cellular energy levels (88, 140). Increased oxidative stress can also be accentuated by Cu/Zn-SOD deficiency in response to ANG II (43, 45). Indeed, deficiency in Cu/Zn-SOD, the most abundant of the three SOD isoforms, is associated with increases in ROS and vascular dysfunction (44).

Effects of RAAS on Cardiac Insulin Signaling, Structure, and Function

As previously noted, insulin and IGF-1 generally exert beneficial effects on myocardial mechanical-electrical coupling and both diastolic and systolic function (22, 62, 64, 84, 105, 117, 146, 153, 163–165, 179, 180, 198, 222). These beneficial effects appear to be lessened in conditions of RAAS activation in the heart (179, 192, 231; Cooper SA et al., unpublished observations). Many of these beneficial effects of insulin and IGF-1 are mediated largely by PI3K/Akt signaling (41, 78, 94,
102, 142, 188, 223, 234), and ANG II opposes insulin/IGF-1 mediated signaling through this pathway (18, 77, 184, 192, 194, 229–231; Cooper SA et al., unpublished observations) (Fig. 2).

There are several mechanisms whereby cardiac RAAS activation inhibits the beneficial metabolic effects of insulin and IGF-1. ANG II plays a seminal role in the genesis of cardiac hypertrophy, interstitial fibrosis, and left ventricular dysfunction (34, 46, 65, 146, 172, 173, 178, 204, 216, 219) (Fig. 2). ANG II receptors have been characterized in cardiomyocytes and cardiac fibroblasts (34, 146, 172, 216, 219) as well as in the endothelial lining of coronary arteries (65, 146, 178, 216, 237). Although both AT1Rs and ANG II type 2 (AT2Rs) receptors are present on cardiac and coronary vessel tissue, most of the adverse effects of ANG II on hypertrophy, fibrosis, and left ventricular dysfunction are mediated through AT1Rs (146, 237). There are increasing experimental data suggesting that many of the detrimental effects of both ANG II and aldosterone are triggered by redox cycling of ROS, generated by a membrane NADPH oxidase-dependent pathway, as well as mitochondria-generated ROS (11, 24, 25, 58, 37, 38, 75, 123, 126, 146, 153, 156, 189, 192, 231; Cooper SA et al., unpublished observations). In cardiomyocytes, ANG II stimulates phagocytic-type NADPH oxidase, which is composed of a membrane-bound p22phox heterodimer and four regulatory subunits (p40phox, p47phox, p67phox, and Nox2) and the small-molecular-weight G protein Rac1 (37, 130, 136, 138). ANG II activation of the NADPH oxidase enzyme affects cell signaling responses and facilitates cardiac remodeling and hypertrophy (11, 24, 38, 153), as evidenced by the attenuation of these pathological effects following treatment with free radical scavengers (153, 195, 231, 237) or AT1R blockade (146, 173, 219, 231, 237).

In a recent investigation (231), it was hypothesized that chronic ANG II overexpression in the heart was associated with structural and functional abnormalities that are driven by NADPH oxidase-mediated generation of ROS. This notion was evaluated by in vivo treatment with either an AT1R blocker or a SOD/catalase mimetic in a rodent model of chronically elevated tissue levels of ANG II, the transgenic mRen2/27 rat (Ren2). Results of this investigation indicated that the hypertensive, insulin-resistant Ren2 rat manifests increased oxidative stress in concert with structural and functional changes in the heart. Membrane NADPH oxidase activity and immunostaining of NADPH oxidase subunits p22phox, Nox2, and Rac1 were significantly increased in the Ren2 rat, in conjunction with increased levels of myocardial tissue oxidative stress. Structurally, septal wall thickness, as measured by in vivo cine MRI, was significantly increased. Additionally, light microscopy revealed substantial left ventricular coronary artery perivascular fibrosis. Citrate synthase activity and transmission electron microscopy demonstrated significant increases in mitochondrial numbers in Ren2 left ventricle tissue. Systolic function was also diminished in the Ren2 rat compared with the Sprague-Dawley control. These effects were abrogated by both the AT1R blockade and SOD/catalase mimetic, highlighting the role of ANG II in the activation of NADPH oxidase and the importance of ROS in cardiac remodeling and dysfunction. While the observations are novel in the Ren2 model, previous studies (7, 109, 113) have shown that ANG II increases ROS in cultured myocardial fibroblasts and cardiomyocytes.

The limited endogenous antioxidant capacity, both enzymatic and nonenzymatic, of myocardial tissue renders it highly susceptible to oxidative stress induced injury (50). Thus, increased oxidative stress in the heart has been causally linked to ventricular hypertrophy and diastolic and systolic functional abnormalities as well as abnormal metabolic signaling (50, 136). Indeed, insulin-stimulated Akt phosphorylation/activation is significantly suppressed in Ren2 myocardial tissue and inversely correlated to Rac1 expression and NADPH oxidase activity (231). In the heart, Akt activation is critical for the proper regulation of proteins responsible for growth, metabolism, survival, and cardiac function (5, 32, 41, 182). Akt activity in the heart is regulated by nutritional status, insulin, pressure overload, and redox status (5, 32, 41, 182). Optimal Akt signaling, while important for physiological growth, impedes pathological cardiac hypertrophy (5, 32, 41, 182). Restored Akt activation/phosphorylation, along with abrogation of cardiac hypertrophy and dysfunction, were observed following reductions in tissue oxidative stress by treatment with either the AT1R blockade or SOD/catalase mimetic.

Investigators have evaluated the efficacy of direct renin inhibition on cardiac oxidative stress and remodeling in the Ren2 model of chronic ANG II overexpression using the novel nonpeptide renin inhibitor aliskiren (Cooper SA et al., unpublished observations). The specificity of aliskiren prevents its use in conventional rat models; however, the Ren2 rat overexpresses murine renin, which is recognized by aliskiren (148, 158, 232). Renin is the rate-limiting step in the generation of ANG II (148, 158, 232); thus, renin inhibition should reduce tissue ANG II levels as well as abrogate any direct renin effects. Previous studies (146, 231) have demonstrated the cardioprotective properties of AT1R blockade. However, AT1R blockade generates a reactive release of renin due to decreased inhibition of renal juxtaglomerular cells, which may promote myocardial injury (158, 232). Thus, the reduction of ANG II levels via direct renin inhibition is of potential therapeutic importance as it blocks the RAAS at its source (148, 158, 232).

Myocardial tissue from untreated heterozygous male Ren2 transgenic rats display significantly increased levels of ROS generated by increased NADPH oxidase activity as evidenced by increased immunostaining for the NADPH subunits p47phox and Rac1 as well as 3-nitrotyrosine. Translocation of the small GTP-binding protein Rac1 and p47phox to the cell membrane is necessary for the assembly and activation of NADPH oxidase, which has been directly implicated in ANG II-induced cardiac hypertrophy (3, 19, 50). 3-Nitrotyrosine resulting from ROS scavenging of NO produces peroxynitrite (ONOO−), which binds to protein tyrosine moities to produce stable 3-nitrotyrosine, a surrogate marker of oxidative stress (33, 70, 77, 192, 230, 231).

Direct renin inhibition in Ren2 animals significantly reduced levels of myocardial oxidative stress, as evidenced by decreased immunostaining for Rac1 and NADPH subunit p47 as well as 3-nitrotyrosine. Thus, renin blockade effectively attenuated myocardial oxidative stress, likely by downregulating NADPH oxidase. Additionally, interstitial and perivascular fibrosis were evaluated by Verhoeff-van Gieson staining, which is specific for elastin, collagen, connective tissue, and nuclei. As previously described, the Ren2 rat exhibited increases in myocardial interstitial and perivascular fibrosis,
which were abrogated by renin inhibition (Cooper SA et al., unpublished observations). The results of this study complement those of previous studies evaluating the effects of renin inhibition. In these studies (135, 148, 149, 218), renin inhibition has been shown to lower blood pressure in spontaneously hypertensive rats, double-transgenic rats, marmosets, and hypertensive humans. Renin inhibition has also been shown to significantly improve cardiac hypertrophy and diastolic and systolic dysfunction as well as reduce albuminuria and kidney inflammation/damage in the double-transgenic rat model (148). However, the beneficial effects of in vivo aliskiren administration on measures of myocardial oxidative stress, cellular remodeling, and fibrosis in the Ren2 model of tissue RAAS overactivation provide additional evidence for a critical role for the RAAS in cardiac remodeling and hypertrophy.

Myocardial Metabolic Signaling

Cardiac tissue is capable of remarkable metabolic flexibility. In the normal heart, ~10–40% of ATP is produced via tricarboxylic acid cycle glycolysis, whereas the remaining 60–90% is derived from β-oxidation of fatty acids. However, energy substrate preference is dynamic to fulfill the energetic requirements of one of the most metabolically active organs in the body. Substrate utilization in the myocardium is dependent on vascular perfusion, energy demand, substrate availability, and local/systemic hormonal changes (128, 215). For example, the heart preferentially shifts toward glucose rather than fatty acid or lactate metabolism under ischemic conditions (128). Similarly, a recent study (214) has shown that the hypertrophied heart is characterized by a marked shift in substrate preference from typical fatty acid to primarily glucose metabolism, which is more readily converted to ATP. However, in conditions of insulin resistance, glucose metabolism is impaired, and the heart is forced to revert to fatty acid and ketone catabolism (146), resulting in structural and other biochemical changes that ultimately lead to left ventricular hypertrophy and diastolic (impaired relaxation) and systolic dysfunction (10, 79, 111, 137, 147, 160, 212). In fact, a study (209) in humans has demonstrated that short-term depletion of serum free fatty acids in failing hearts results in impaired cardiac work as the heart typically responds to decreases in free fatty acids by increasing glucose metabolism.

In vivo evaluation of myocardial substrate preference using positron emission tomography (PET) is an area of emerging interest. Previously, PET was primarily used to evaluate myocardial viability by the preservation of glucose metabolism following infarction, ischemia, or injury using 18F-labeled deoxyglucose (18F-DG) (67, 68). However, advances in PET imaging technology now allow clinicians and investigators to evaluate myocardial metabolic flexibility by measuring myocardial fatty acid uptake, utilization, and oxidation using radiolabeled [1-11C]palmitate (14, 15, 23, 40, 92, 107, 146, 185) and myocardial efficiency using [11C]acetate (12). Numerous studies have evaluated myocardial insulin sensitivity in humans (48, 71). The application of nuclear medicine techniques to laboratory animals is another area of emerging interest. For example, our laboratory (64) has used this methodology to measure insulin-stimulated myocardial glucose uptake with 18F-FDG Cardiac Imaging

Fig. 5. Micro-positron emission tomograph (PET) and electrocardiographically gated magnetic resonance images (MRI) of SD animals with and without insulin/glucose stimulation. Rats were in a supine position, and the images are coronal views. Upon insulin/glucose stimulation, there was increased myocardial glucose uptake (noted by increased brightness) compared with the basal state. 18F-FDG, 18F-labeled deoxyglucose.
Mineralocorticoids, CVD, and Insulin Actions

Aldosterone exerts a number of maladaptive effects on the vasculature, heart, and traditional insulin-sensitive tissues, such as skeletal muscle (4, 17, 21, 26, 28, 39, 49, 52–56, 59–61, 73, 76, 82, 87, 90, 93, 97, 99, 100, 102, 108, 110, 114, 116, 121, 132–134, 142, 155, 157, 168–171, 177, 181, 194, 196, 197, 221, 228, 238) (Figs. 1–3). These maladaptive effects on the vasculature are mediated by both genomic and nongenomic actions of this hormone (53, 119, 194). Effects on the vasculature include the enhancement of tyrosine phosphorylation and inositol phosphate activation, increased Na\(^+\)/H\(^+\) exchange, and alkalization of VSMCs (4, 49, 76, 114, 228). Indeed, mineralocorticoid receptors (MRs) have been identified in the vasculature (61). As in other tissues, many of the adverse effects of mineralocorticoids on the vasculature appear result from increased oxidative stress (177).

An aldosterone infusion into rats results in impaired endothelium-dependent relaxation, and this is associated with increased oxidative stress in the vessel (155). Chronic treatment with aldosterone caused impaired endothelium-dependent vasodilation in these rats (17). Furthermore, treatment with the MR blocker spironolactone has been shown to improve endothelium-dependent vasorelaxation in rodents (221) and humans (54). It has been reported that aldosterone induces endothelial cell swelling, with concomitant increases in protein leakage through intracellular gaps that may result from increased apical membrane tension (133, 134) and that these processes are blocked with spironolactone (132). There is accumulating evidence that ANG II and mineralocorticoids have interactive effects on the vasculature (Fig. 1, A and B). Mineralocorticoids upregulate ANG II receptors in VSMCs (211), and signaling of ANG II is amplified by exposure to mineralocorticoids (210, 211). Both ANG II and aldosterone stimulate vascular growth and remodeling (82, 116, 121), perhaps mediated through MAPK and ROS signaling (116, 121, 155). Furthermore, blockade of both MRs and AT\(_1\)Rs protects against the generation of excess ROS and the resultant vascular remodeling (26). Other studies (26, 99) have demonstrated that aldosterone may interfere with insulin signaling in various tissues, although the effects of mineralocorticoids alone and in conjunction with ANG II on insulin signaling in vascular tissue remain to be elucidated.

Mineralocorticoids in the Heart

There is considerable evidence that mineralocorticoids contribute to abnormal cardiac remodeling, including fibrosis and perivascular inflammation (21, 39, 55, 56, 59, 60, 73, 90, 93, 110, 157, 167–171, 181, 196, 197, 238) (Fig. 2). Both cardiomyocytes and fibroblasts express MRs with high affinity for both corticosterone and aldosterone (110, 181, 157). The inflammatory effects of corticosterone and aldosterone in the heart are partly mediated by an interaction with the RAAS as well as effects directly mediated through MR activation (39, 55, 56, 73, 90, 167, 168, 170, 196, 238). For example, in rats treated with aldosterone and high dietary salt, the AT\(_1\)R expression and ventricular density of the AT\(_1\)R have been observed to increase (90). Additionally, mineralocorticoids increase the expression of angiotensin-converting enzyme in cardiomyocytes from adult rat primary cardiomyocytes (196) and in cultured rat fetal cardiomyocytes (73). Recent data from several laboratories have suggested that MR activation may potentiate the proinflammatory/fibrotic effects of AT\(_1\)R signaling by enhancing the cardiac oxidative stress induced by ANG II (90, 93, 196, 238). Furthermore, animal studies (55, 56, 168) have shown that MR antagonism reduces oxidative stress, inflammation, and fibrosis independent of blood pressure effects. These beneficial effects of MR blockade are mediated, in part, through the inhibition of NADPH oxidase activity (97, 100, 142) (Fig. 2).

Mineralocorticoids and Insulin Sensitivity

There are accumulating data from human and animal studies showing that excess mineralocorticoids impair insulin signaling in a number of tissues. For example, an aldosterone excess in patients with primary aldosteronism is related to impaired glucose homeostasis (52) as well as insulin resistance (28). Several recent publications (99, 194), as well as recent data from our laboratory (102), have suggested that these detrimental effects on insulin signaling are mediated by inflammatory/oxidative stress effects of mineralocorticoids. Indeed, in the TG(mRen2)22 rat, which manifests insulin resistance (18), in vivo MR antagonism with subpressor doses of spironolactone substantially improve ex vivo insulin stimulated increases in glucose uptake in skeletal muscle, a phenomenon that is linked to reductions in NADPH oxidase activity and attenuation of ROS in soleus muscle tissue (102). Future work will focus on the impact of MR and glucocorticoid receptor antagonism and their impact on insulin and IGF-1 signaling in cardiovascular tissue.

Conclusions

In summary, activation of the RAAS contributes to altered insulin/IGF-1 signaling pathways that lead to ROS formation, endothelial dysfunction, and pathological growth and remodeling. Both AT\(_1\)R and MR activation contribute to downstream signaling pathways that attenuate insulin signaling mechanisms in the heart, vasculature, and skeletal muscle that collectively alter the physiological regulation of transcriptional and translational maintenance of cell metabolism. Collectively, these changes contribute to CVD as seen in CMS.

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