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Oxidative stress-mediated effects of angiotensin II in the cardiovascular system

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Abstract

Angiotensin II (Ang II), an endogenous peptide hormone, plays critical roles in the pathophysiological modulation of cardiovascular functions. Ang II is the principle effector of the renin-angiotensin system for maintaining homeostasis in the cardiovascular system, as well as a potent stimulator of NAD(P)H oxidase, which is the major source and primary trigger for reactive oxygen species (ROS) generation in various tissues. Recent accumulating evidence has demonstrated the importance of oxidative stress in Ang II-induced heart diseases. Here, we review the recent progress in the study on oxidative stress-mediated effects of Ang II in the cardiovascular system. In particular, the involvement of Ang II-induced ROS generation in arrhythmias, cell death/heart failure, ischemia/reperfusion injury, cardiac hypertrophy and hypertension are discussed. Ca^{2+}/calmodulin-dependent protein kinase II is an important molecule linking Ang II, ROS and cardiovascular pathological conditions.

Keywords

Angiotensin II; Oxidative stress; Mitochondria; Arrhythmias; Ischemia-reperfusion; Hypertrophy; Hypertension

INTRODUCTION

Synthesis and metabolism of angiotensin II

Angiotensin (Ang) II, since it was first found in bovine brain in 1985[1], has been well recognized as an endogenous vasoactive octapeptide with a wide biological profile in the

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development of various cardiovascular disorders, such as hypertension, hypotrophy, arrhythmias, coronary ischemia and congestive heart failure[2–4]. Ang II is a critical component of the renin-angiotensin system (RAS) for the regulation of blood pressure and cardiovascular homeostasis. It derives from an enzymatic cascade[5] and is traditionally considered as a systemic hormone. Renin is released primarily from the juxtaglomerular cells of the kidney into the blood, where it proteolytically cleaves angiotensinogen to form the decapeptide Ang I. Ang I can be subsequently cleaved by angiotensin converting enzyme (ACE) and generates the octapeptide Ang II within the pulmonary circulation[6,7]. In addition to the circulating RAS, many other tissues are also shown to synthesize Ang II in the presence of angiotensinogen, renin and ACE, including heart, vasculature, kidney and brain, which implies the paracrine and intracrine effects of Ang II[6–9].

Ang I is thought to be physiologically inactive. As the major effector for RAS, Ang II can be further degraded to Ang III[10] and Ang IV[11] under the action of aminopeptidase, and both of them exhibit much less biological activity than Ang II. On the other hand, Ang I converting enzyme 2 was later identified from a heart failure ventricle cDNA library and was shown to be a converter for Ang II to Ang (1–7)[12], which can be further cleaved to Ang (1–5) and Ang (1–4)[13]. Ang (1–7) has functional roles to counteract many effects of Ang II. For example, it can lower the blood pressure by directly acting on endothelium cells to promote the synthesis and releasing of nitric oxide (NO) and PGI2[14].

Ang II receptors and cellular signal transduction

Angiotensin receptors[15] with seven transmembrane domains belong to the G-protein coupled receptor superfamily. Four angiotensin receptors ($AT_{1–4}R$) have been identified so far. They are sensitive to angiotensinogen metabolites and responsible for their signal transductions. Before the cloning of the cDNAs, they were initially defined on their pharmacological and biochemical basis.

AT_1R is the best-elucidated angiotensin receptor and is involved in most of the well-known effects of Ang II, such as cell growth, oxidative stress generation and vasoconstriction, *etc*[16]. AT_1R can be found ubiquitously in the cardiovascular system, e.g., the heart, blood vessels, liver, kidney and adrenal gland[17]. It is selectively blocked by losartan or candesartan and couples to various signal transduction pathways that involve G-proteins, intracellular second messengers and protein kinases[18–20]. While it is well accepted that AT_1R stimulation promotes NAD(P)H oxidase activation, the detailed mechanism(s) has not been completely understood yet. Some studies have shown that NAD(P)H oxidase activation requires upstream PKC in various tissues, such as brain, vascular smooth muscle (VSM) and mesangial cells[21,22]. In our recent study, however, we did not observe any inhibitory effect of chelerythrine or *GF 109203X* (selective PKC blockers) on Ang II-induced early after deplorizations (EADs), suggesting PKC may not be involved in NAD(P)H oxidase activation in cardiac myocytes[23]. Consistent with this notion, a novel signal transduction pathway of Ang II-induced reactive oxygen species (ROS) production in cardiomyocytes was reported by Nishida *et al*[24], who demonstrated that AT_1R stimulation by Ang II activates $G_{\alpha 12/13}$ proteins, which in turn cause Rho/ROCK-mediated Rac1 activation. Rac, one of the small GTP-binding proteins, promotes the production of ROS by activating NAD(P)H oxidase. In addition, another recent study has revealed that the epidermal growth factor receptor (EGFR) kinase and PI-3 kinase may serve as upstream activators for NAD(P)H oxidase in cardiac myocytes[25].

It is well known that Ang II receptors couple to the $G_{\alpha q}$ protein-phospholipase C (PLC) pathway, in which multiple second messengers such as phosphatidylinositol 4,5-bisphosphate (PIP_2), inositol-1,4,5-trisphosphate (IP_3), diacylglycerol and Ca^{2+} are included. This pathway activates PKC and thereby causes phosphorylation of membrane ion

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channels, for example the $I_{Ca,L,}$ as shown by Aiello *et al*[26] in cat. Whereas, another study carried out by Endoh's group showed that Ang II may increase $I_{Ca,L}$ *via* AT_1R in adult rabbit ventricular myocytes in a PKC-independent manner[27]. Zhao *et al*[23] have also examined this hypothesis and shown that blocking PKC by chelerythrine or GF 109203X had no effect on Ang II-induced EADs, suggesting direct phosphorylation of $I_{Ca,L}$ or I_{Na} plays less important roles under our experimental conditions. Although we do not have a ready explanation for these discrepancies, animal species or heart regions might be the potential reasons. Indeed, the wide range species-related differences of Ang II in inotropic effects have been revealed in mammalian cardiac muscle[28].

The intracellular C-terminal of AT_1R has been highlighted for receptor signaling and internalization[29]. Previous studies suggested that the C-terminal domain of AT_1R provides a binding site for receptor dimerization[30] and also directly interacts with signaling molecules JAK2 and PLCγ 1[31,32]. Specific binding proteins, such as AT_1R-associated protein[33] and AT_1R C-terminal tail-associated proteins (GLP and ARAP1), were also identified[34,35].

In contrast, the roles of AT_2R have not been well established. It was distinguished from AT_1R for its high affinity to PD123319, PD123177 and poor affinity to losartan and candesartan[20]. AT_2R is highly clustered in the developing fetus, while its expression level is very limited in the adult[17]. However, the expression of AT_2R can be up-regulated during pathological states when the tissue remodeling and inflammation takes place[36]. Although the roles of AT_2R are still under debate, accumulating evidence implied AT_2R may play beneficial roles to counterbalance the deleterious actions of AT_1R. For example, as a vasodilator[37], AT_2R was hypothesized to couple with bradykinin B_2 receptor (B_2R) and modulate NO production[38–40]. Abadir *et al*[41] has recently demonstrated that both NO and cGMP levels were enhanced while the functional heterodimer formed between AT_2R and B_2R.

AT_2R stimulates specific serine/threonine phosphatases such as protein phosphatase 2A, MAPK phosphatase and tyrosine phosphatase SHP-1 in different cell types[37]. As the activation of growth factor requires the phosphorylation of tyrosine kinases, AT_2R can attenuate the AT_1R-derived effects by phosphorylation at those serine/threonine sites *via* dephosphorylation at tyrosine. AT_1R-induced effects can be antagonized *via* direct binding of agonist when co-expressed with AT_2R subunit[42] and the $AT_{1/2}R$ heterodimers have been detected in fetal fibroblasts and myometrial biopsies.

Other angiotensin receptors showed less pathophysiological significance. AT_3R was first obtained by adrenal cortex library screening in 1992[43] but its physiological significance remains unclear. AT_4R has been identified in a wide range of tissues, including heart, kidney and VSM cells. Ang IV has a high binding affinity to AT_4R[44], while Ang II shows 1000-times lower affinity[45].

Electrophysiological effects on cardiac cells

Ang II plays roles in atrial fibrillation (AF) and other types of cardiac arrhythmias. Zankov *et al*[46] have studied the effect of Ang II on action potentials (APs) and the slow component of delayed rectifier potassium current (I_{ks}) in guinea pig atrial myocytes. Ang II increases the I_{ks} and shortens AP duration in a concentration-dependent manner and Ang II-potentiated I_{ks} is attenuated by the AT_1 receptor blocker valsartan and PKC inhibitors, indicating that the enhanced I_{ks} is mediated *via* a PKC signaling cascade[46]. Furthermore, selective AT_1 receptor blocker losartan attenuates Ether-A-Go-Go Related Gene (HERG) currents (or I_{Kr}), as well as prolongs the duration of APs and affects QT dispersion[18].

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Ang II also activates other potassium currents in ventricular myocytes. AT_1R blocker irbesartan induces time-dependent and concentration-dependent blockage of hKv1.5 and Kv4.3 channels, which are thought to form transient outward potassium currents (I_{to})[47]. Yu *et al*[48] made an interesting observation on the transmural gradient of I_{to} in dog ventricle. A notch due to the presence of I_{to} was seen in a typical AP measured on epicardium but not endocardium. However, this notch can be induced in endocardium by a pretreatment with losartan without altering the AT_1R expression level, suggesting the up-regulation of I_{to} at the endocardial layer.

In addition to the potassium currents, outward rectifying Cl^- current is also activated by Ang II in the rabbit sino-atrial node[49] and ventricular myocytes[50]. Ang II-activated Cl^- current is blocked by losartan and shown to be mediated by the PKC pathway[49]. It has been demonstrated that Ang II may inhibit cardiac protein kinase A-dependent Cl^- conductance through inhibition of adenylate cyclase *via* pertussis toxin-sensitive G proteins[51].

As mentioned above, $I_{Ca,L}$ is activated by Ang II in either a PKC-dependent[26] or -independent manner[27]. We have determined that Ang II-induced activation of $I_{Ca,L}$ is most likely mediated by the activation of Ca^{2+}/Calmodulin- Dependent Protein Kinase II (CaMKII) oxidized by ROS[23].

Ang II and oxidative stress

Oxidative stress describes an imbalance state while the production of ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydoxyl radicals (OH), exceeds antioxidant defenses. There are several enzyme systems contributing to the formation of ROS, including NAD(P)H oxidase, xanthine oxidase and mitochondrial electron leakage from electron transport chain. ROS are normally generated as a natural byproduct of oxygen metabolism and play important roles in cell signaling. However, ROS levels can be increased dramatically under oxidative stress conditions, such as heart failure, ischemia-reperfusion and aging.

NAD(P)H oxidase, which has seven NOX isoforms, is a membrane-bound, heteromeric enzyme complex distributed throughout the endothelial cells, VSM cells[52] and cardiac myocytes[53]. NAD(P)H oxidase-generated ROS was initially recognized as a major source for vasculature ROS[54] and later for cardiac ROS as well[53]. Previous studies have demonstrated that the Ang II can double the vascular oxidants (ROS) production *in vivo* in a NAD(P)H-dependent manner[55]. It is well accepted that AT_1R activation stimulates NAD(P)H oxidase. Using intracellular NAD(P)H and NADH as electron donors, the activated NAD(P)H oxidase will catalyze the conversion of extracellular molecular oxygen to O_2^-[52,56]. O_2^- is unstable; it will be rapidly modified by superoxide dismutase (SOD) and generate a more stable and membrane permeable form as H_2O_2[52].

It is interesting that the NAD(P)H oxidase-generated ROS was proposed to serve as an initial triggering for further ROS generation by other sources, such as mitochondria. This process is referred to as mitochondrial “ROS-induced ROS release” (RIRR)[57]. Mitochondria are the major source of ROS in cells. Both complex I and complex III of the mitochondrial electron transport chain (METC) are involved in the generation of O_2 to O_2^-. Two principles in regard to the increasing of mitochondrial ROS formation have been suggested as (1) increased O_2^- generation at the METC; and (2) decreased elimination of O_2^- and H_2O_2 in mitochondrial matrix[58]. At least three mechanisms have been proposed to be involved in RIRR: (1) increased triggering of ROS causes mitochondrial depolarization *via* activation of the mitochondrial permeability transition pore (mPTP) and subsequently gives rise to a short-lived burst of ROS originating from METC; (2) increased ROS reaches a threshold level that triggers opening of the requisite mitochondrial membrane anion

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channels, which causes a brief increase in METC-derived ROS; and (3) mitochondrial ROS can be produced under the stimulation of cytosolic ROS by opening redox sensitive mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channels[59–61].

Furthermore, recent studies have hypothesized that Ang II may promote the ROS generation in mitochondria *via* the RIRR mechanism[59,61]. The NAD(P)H oxidase-derived ROS stimulated by Ang II may serve as a trigger to induce mitoK_{ATP} channel opening and mPTP formation, depolarize mitochondrial membrane potential ($\Delta\psi_M$) and lead to the mitochondrial ROS burst[62].

Although the detailed molecular mechanisms for the Ang II signaling on NAD(P)H oxidase are still under investigation, a range of possible upstream mediators for NAD(P)H activation have been hypothesized. For instance, PLA2, PKD, PKC, EGFR, PI3K, Rac and c-Src are in the candidate lists. Ang II can activate the PLA2, PLD and PKC phosphorylation pathway *via* binding to AT_1R and subsequently activate a cytosolic oxidase subunit $p47^{phox}$, which may migrate to the plasma membrane to participate in the oxidation process[63]. Mollnau *et al*[64] discovered the importance of the PKC regulation pathway. Their results showed the Ang II-derived ROS production was reduced by the treatment with the PKC inhibitor chelerythrine, which also dramatically inhibited the up-regulation of NAD(P)H oxidase subunits.

Previous evidence has shown that Ang II stimulates the activation of MAPK *in vivo*[65]. Kimura *et al*[66] further suggested that cardiac mitochondrial RIRR may be mediated by JNK and MAPK. In addition, a recent study[67] revealed that SS-31, a mitochondria targeted antioxidant peptide, reduces mitochondrial ROS in neonatal cardio-myocytes and subsequently inhibits the downstream signaling for fibrosis and apoptosis with reduced activation of MAPK. It has also been reported that Rac GTPase directly modulates NAD(P)H oxidase activity by interacting with its components (e.g., $gp91^{phox}$, $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ subunits) and forming an active enzyme complex[68].

Recent studies by Montezano *et al*[69] have identified the roles of Nox5, a homolog of $gp91^{phox}$, in endothelial cells for Ang II and endothelium-1 signaling, which is calcium/calmodulin-dependent but Rac-1-independent. These data link the calcium/calmodulin regulation pathway to the ROS generation and imply its potential importance in various cardiovascular conditions. Palomeque *et al*[70] postulated that the Ang II-induced ROS production is dependent on the activity of CaMKII; the presence of Ang II or ROS can reset the activation of CaMKII for apoptotic cascade signaling at subdiastolic calcium concentrations. We have also shown that either exogenously applied ROS (H_2O_2) or endogenously generated ROS by Ang II stimulation can induce ventricular arrhythmias *via* the CaMKII signaling pathway[23,71]. By the treatment with the CaMKII inhibitor KN-93, the occurrence of Ang II-induced EADs is attenuated.

In addition to its direct action on the activity of enzymes, Ang II facilitates the nitric oxide synthase (NOS) uncoupling by enhancing the superoxide production and the uncoupled NOS further up-regulates the superoxide level in vasculature and accelerates the endothelial dysfunction[64].

Therefore, Ang II has been recognized as a potent activator for NAD(P)H oxidase. NAD(P)H oxidase-derived ROS further promotes the ROS generation from mitochondria (i.e., RIRR). In the following sections, we will discuss the involvement of Ang II in detail, especially *via* oxidative stress activation in several severe cardiovascular disorders.

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ARRHYTHMIAS

Compelling evidence has suggested that the ROS are arrhythmogenic factors[72]. AF is the most common cardiac arrhythmia with high morbidity and mortality risk and chronic AF causes myocardial ischemia with abnormal calcium handling and ventricular perfusion[73]. In patients with chronic AF, the densities of $I_{ca,L}$, I_{to} and I_{kur} were markedly reduced[74]. A recent study further pointed out that AT_1R is the key factor for arrhythmia generation, as the major arrhythmic effects usually occur followed by the activation of AT_1R[75]. Zankov *et al*[46] exhibited the activation of AT_1 receptor led to marked potentiation on I_{Ks} current in atrial myocytes. Such potentiation can be induced by Ang II at nM range and indicates a potential mechanism for Ang II-induced AF. In another study, Goette *et al*[76] noted the occurrence of AF involves the down-regulation of AT_1R and up-regulation of AT_2R, suggesting AT_2R may also be involved. Goette *et al*[77] revealed the importance of RAS as a potential molecular basis to link oxidase stress and microvascular flow abnormalities in ventricles. In their study, atrial tachyarrhythmia was induced by rapid atrial pacing in pigs and abnormal coronary flow reserve was measured. The changes in microcirculatory blood flow was accompanied with the elevation in ventricular expression levels for Nox2, lectinlike oxidized low-density lipoprotein receptor-1 (LOX-1) and F2-isoprostane levels; these increases cannot be found in animals treated with AT_1R inhibitor irbesartan, suggesting the roles of oxidative stress in abnormal flow regulation. Evidence showed beneficial effects of selective AT_1R blocker candesartan and ACE inhibitor captopril on atrial electrical remodeling caused by rapid atrial pacing[78].

We have also shown the potential mechanisms for abnormal afterdepolarizations and triggered activities induced by oxidative stress in rabbit ventricular myocytes[23,71]. The correlation between ROS generation by NAD(P)H oxidase and EADs induction by Ang II has been clearly demonstrated[23]. The EADs were induced after the application of Ang II (1–2 μmol/L) and were eliminated after exposure to NAD(P)H oxidase inhibitor (aposanin) and antioxidants trolox or MnTMPyP, supporting the hypothesis that the NAD(P)H oxidase-derived ROS are involved in EAD generation. The involvement of Ca^{2+}/Calmodulin-sensitive CaMKII was examined using a CaMKII inhibitor and the data showed that Ang II generated EADs were suppressed by KN-93 and inhibitory peptide AIP but not KN-92, an inactive analogue of KN-93. $I_{Ca,L}$ and late sodium current were suggested to be the essential factors for the generation of ROS-induced EADs. Ang II potentiates the amplitudes of both $I_{Ca.L}$ and late I_{Na} significantly and these enhancements can be attenuated by application of antioxidants, e.g., MnTMPyP and trolox. These pieces of evidence clearly link the activation of AT_1R to endogenous generation of ROS, oxidation of CaMKII, activation of $I_{Ca,L}$ and late I_{Na} and the EAD genesis.

Other studies have revealed that Ang II activates $I_{Ca,L}$ through stimulating PKC regulation pathway and may be involved in the genesis of reperfusion-induced ventricular arrhythmias by elevating cellular Ca^{2+} loading[78–80]. Application of ACE antagonist TCV116 also reduced the reperfusion induced ventricular arrhythmia[81]. Touyz *et al*[82] further examined the cellular Ca^{2+} concentration levels in atrial and ventricular myocytes and disclosed that the calcium overload caused impairment on atrial myocytes is more severe. This damage may also be attributed to the higher expression level of AT_1R in atria under heart failure conditions[83].

A recent study has demonstrated that Ang II plays roles in increasing transmural dispersion of repolarization in the ventricles, which promotes the re-entrant ventricular arrhythmias[84]. In the transgenic rats harboring the human renin and angiotensinogen genes, the mRNA expressions for Kv4.3 subunit and gap junction protein connexin 43 were predominantly reduced compared to the controls. Cardiac magnetic field mapping further revealed the

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lengthening in depolarization and repolarization and enhancement in APD inhomogeneity and application of the AT_1R blocker losartan ameliorated the disturbances.

Although AT_1Rs are suggested to be predominantly involved in most of the well-known effects of Ang II, a recent study by Gopinathannair *et al*[85] discovered a potential role of AT_2R in ischemic focal ventricular tachycardia. In a dog model with coronary artery occlusion, Ang II infusion caused sustained focal ventricular tachycardia from Purkinje origin. Ang II enhanced the *in vitro* triggered activities in Purkinje cells, which were blocked by selective AT_2R PD-123319 but not AT_1R blocker losartan. These results imply the significant role of AT_2R in arrhythmogenesis during ischemia and the protective effect of AT_2R blocker in myocardial ischemia.

The “cross-talk” between Ang II and catecholamine was also hypothesized. Evidence indicates the interactions between the adrenergic system and RAAS, as norepinephrine (NE) can be released from sympathetic nerves *via* activation of prejunctional AT_1R[86]. This indicates that Ang II might increase ectopic automaticity of atria and ventricles through stimulating the production of catecholamine.

CELL DEATH/APOPTOSIS AND HEART FAILURE

Ang II-induced ROS generation has been suggested to promote heart failure[87]. Erickson *et al*[88,89] showed that Ang II stimulates NAD(P)H oxidase and activates CaMKII by oxidation on $Met_{281/282}$. CaMKII is initially activated by binding to calcified calmodulin (Ca^{2+}/CaM) but Met oxidation permits persistent CaMKII activity even in the absence of Ca^{2+}/CaM. Ang II-induced oxidation of CaMKII promotes myocardial dysfunction and heart failure in part by increasing apoptosis in ventricular myocytes[88,89]. Supporting the finding of Erickson *et al*[88,89], Palomeque *et al*[70] revealed that the Ang II-induced apoptosis is mediated by p38MAPK activation induced by ROS-dependent CaMKII activation. They also suggested the presence of Ang II or ROS can reset the activation of CaMKII for apoptotic cascade signaling at subdiastolic calcium concentrations.

A very recent study by Swaminathan *et al*[90] implicated the roles of Ang II, ROS and oxidation of CaMKII to the development of sino-atrial node dysfunction (SND). SND is mostly characterized by sinus bradycardia (abnormal slow heart rate). It commonly occurs in the setting of heart failure and hypertension. Swaminathan *et al*[90] found that the patients with heart failure and SND and dogs with pacing-induced SND had higher levels of oxidized CaMKII in right atrial tissue compared to non-SND controls. They also showed elegantly that Ang II infusion in mice causes sino-atrial nodal cell apoptosis/death and SND by activating NAD(P)H oxidase and oxidation of CaMKII. Moreover, the mice lacking functional NAD(P)H oxidase (p47−/−mice) and mice with CaMK II inhibition (AC3-I mice) have higher resistance to sino-atrial nodal cell apoptosis and SND induced by Ang II. These results unambiguously suggested that: (1) the activation of NAD(P)H oxidase, ROS and oxidation of CaMKII are involved in Ang II-induced SND; and (2) the inhibition of CaMKII in the sino-atrial node prevents/ delays the occurrence of SND induced by Ang II.

Evidence consistent with a central role of Ang II in the pathophysiology of heart failure comes from the fact that the Ang II receptor blockers or ACE inhibitors are clinical medications used to treat high blood pressure and heart failure. The recent study by Swaminathan *et al*[90] also suggested that early inhibition of ACE, Ang II receptor or CaMKII in high-risk patients (heart failure) may be beneficial for preventing SND.

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ISCHEMIA/REPERFUSION INJURY AND CARDIAC PROTECTION

Early studies have revealed the involvement of the RAS in ischemia-reperfusion injury of the heart. The RAS is up-regulated during ischemia, infarction and reperfusion[91,92]. Evidence suggests that the binding of Ang II to either AT_1R or AT_2R can enhance ischemia-reperfusion injury. Yang *et al*[93,94] showed that, compared to the control, total myocardial Ang II receptor expression is greater in hearts subjected to the global ischemia-reperfusion. AT_1R accounts for most of this increase, while AT_2R is unaffected. In the hearts suffering ischemia-reperfusion, the AT_1R blocker losartan can attenuate the damage. Consistent with this observation, Flynn *et al*[95] demonstrated that treatment of isolated rat hearts with losartan before global ischemia-reperfusion resulted in significant cardioprotective effect, leading to decrease in end-diastolic pressure and reduction in infarct size. On the other hand, in isolated working rat hearts, Xu *et al*[96] showed that ischemia-reperfusion decreased AT_2R mRNA and protein level. The AT_2R blocker PD-123319 increases AT_2R mRNA and protein levels and improves functional recovery, suggesting a potential link between increased AT_2R protein expression and cardioprotection. On the contrary, the AT_1R blocker losartan increased AT_1R mRNA and impaired functional recovery[96]. Thus, it seems that the AT_1R blockade is not universally beneficial in all ischemia-reperfusion models in all species. Nevertheless, both ACE inhibitors and AT_1R or AT_2R inhibitors have been suggested as cardiac protection strategies.

Rac1 GTPase is important for ischemia-reperfusion injury induced NAD(P)H oxidase activation, superoxide production and oxidative stress[97,98]. The NAD(P)H oxidative activity and superoxide level rapidly increased and reached a peak at 3 h post-reperfusion, whereas this effect can be significantly attenuated by treatment with Rac GTPase inhibitor NSC23766 before or after ischemia/reperfusion[97]. This observation is supported by an *in vivo* study using the ischemia-reperfusion mouse model[98]. The size of infarct in Rac-1 knockout mice is much smaller than in control mice. This study further showed that the disruption of Rac1 signaling inhibits the cellular and mitochondria ROS production and exerts protective effects to myocardium during ischemia-reperfusion conditions. These findings provide some possible mechanisms for the roles of Ang II in the development of ischemia-reperfusion injury.

CARDIAC HYPERTROPHY

Cardiac hypertrophy may develop as a physiological adaptive response to heart volume overload and pressure following intense physical training. However, sustained hypertrophy due to intrinsic cardiomyopathic stimuli or extrinsic stimuli (e.g., hypertension and valvular diseases) will lead to severe pathophysiological conditions, with increased risk of morbidity and sudden cardiac death, especially for left ventricular hypertrophy[99]. Cardiac hypertrophy represents a heart enlargement, which involves two components: (1) cardiac myocyte enlargement; and (2) cardiac fibroblast proliferation and collagen formation. Cardiac hypertrophy further leads to an extensive remodeling of ion channels, gap junctions, calcium handling and cytoskeleton. These remodeling changes subsequently reduce the excitability of cardiac myocytes, induce arrhythmias and eventually cause heart pump failure[99].

The renin-angiotensin-aldosterone system (RAAS) has been recognized as one of the most important non-hemodynamic factors for the development of left ventricular hypertrophy, apart from the sympathetic nervous system. The circulating concentrations of Ang II and aldosterone in plasma are suggested to be involved in the development of left ventricular hypertrophy. Previous evidence[100–102] showed that Ang II can increase the cell size, RNA and protein synthesis levels and gene expressions for cardiac myocytes.

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Direct effects of Ang II were shown in cultured embryonic chick myocytes. Aceto *et al*[103] observed that the cellular protein synthesis rate was increased over 25% after 7 d exposure to [Sar1] Ang II (an analogue of Ang II that is an agonist AT_1Rs), which was inhibited in the presence of Ang II receptor antagonist, suggesting the stimulation is receptor-linked. They also performed *in vivo* experiments using rat model with developed pressure-overload cardiac hypertrophy and found the increase in left ventricular mass can be totally prevented in animals fed with ACE inhibitor enalapril maleate[104]. In addition, a fibroblast-derived factor was identified as responsible for the biochemical process by which Ang II stimulates the cardiomyocyte hypertrophy[105]. Ang II stimulates protein synthesis in neonatal myocytes by over 40%, while about 50% of the increase can be blocked using fibroblast proliferation inhibitor bromodeoxyuridine. Further experiments showed that the Ang II stimulated protein synthesis can be abolished by the AT_1R blocker losartan[105], indicating the direct role of Ang II in the production of some fibroblasts factor.

The effect of intracellular Ang II to the development of hypertrophy was examined by over-expressing Ang II peptide in mouse heart using a plasmid-mediated gene delivery system. As such, Ang II was retained intracellularly. Significant cardiac hypertrophy and cardiac gene expression were observed in this model, suggesting Ang II acts as an “intracrine” in stimulating the cellular hypertrophy[106]. A previous study by Mazzolai *et al*[107] also demonstrated the development of ventricular hypertrophy in transgenic mice over-expressing the angiotensinogen gene is independent from fibrosis and hypertension, indicating the significance of local Ang II in mediating hypertrophic response *in vivo*. New evidence has demonstrated the critical role of mitochondrial ROS in the development of Ang II-derived cardiac hypertrophy. The mice over-expressed catalase targeted to mitochondria has shown to be resistant to cardiac hypertrophy, fibrosis and mitochondrial damage induced by Ang II. Therefore, mitochondrial-targeted antioxidants have been suggested as promising agents for prevention and treatment of hypertensive cardiomyopathy[67,108].

The activation of CaMKII *via* the Gq/PLC/InsP3 signaling pathway has been believed to contribute to the hypertrophic growth and gene expression in response to Ang II and other G-protein couple receptor stimulations, such as by NE and endothelin (ET-1)[109]. The G-protein couple receptor stimulations alter the expression of a range of hypertrophy-associated genes, including atrial natriuretic peptide, brain natriuretic peptide, myosin light chain-2 and α- and β-myosin heavy chains, which can be prevented by the inhibition of CaMKII[110–112]. The expression and phosphorylation levels of CaMKII are increased in the mouse with transverse aortic constriction induced cardiac hypertrophy[113]. It has also been demonstrated that nuclear CaMKII activated by envelope IP_3 Receptor-mediated Ca release can cause histone deacetylase (HDAC) phosphorylation and nuclear export. This relieves HDAC-dependent suppression of myocyte enhancer factor 2-driven transcriptions and contributes to hypertrophy (Figure 1). However, it remains to be studied whether ROS-activated cytosolic CaMKII can also mediate Ang II-induced hypertrophy.

HYPERTENSION

Accumulating data from molecular, cellular and *in vivo* animal studies implicate a central role of ROS in the pathogenesis of hypertension (refer to a review article by Briones, 2010)[114]. Ang II-induced hypertension is also associated with an increase of ROS production in vasculature[55]. Infused Ang II leads to the increase of systolic blood pressure and marked up-regulation of vascular ROS production, which can be blocked by losartan. The underlined regulation pathway is further characterized as NAD(P)H dependent. Laursen *et al*[115] showed that the Ang II infusion induced hypertension in rats is likely due to the degradation of endothelium-derived NO, as well as increased superoxide levels, since the treatment with liposome-encapsulated SOD decreases blood pressure in Ang II-infused rats

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and had no effect in positive control. A similar phenomenon was revealed by Virdis *et al*[116], who showed that Ang II caused increase in systolic blood pressure and structural alterations in small resistant arteries, which was prevented by the NAD(P)H oxidase inhibitor apocynin. Taking these together, NAD(P)H oxidase activity is essential for vascular structural and functional changes in Ang II-dependent hypertension.

Oxidative stress has critical roles in vascular damages. ROS may affect the vascular tone by altering the bioactivities or signaling of antioxidant and vascular NAD(P)H oxidase. In VSM cells, the NAD(P)H oxidase $p22^{phox}$ has been shown to be important in the arachidonic acid metabolite-mediated hypertrophy[117], as transfection of antisense $p22^{phox}$ can attenuate NAD(P)H oxidase expression and inhibit Ang II-stimulated protein synthesis.

In patients with inherent structural membrane abnormalities in essential hypertension, in which blood cell membranes contain less unsaturated fatty acyl composition, the levels of free radical and SOD were examined. As a result, the untreated patients with severe hypertension have significantly higher levels of oxygen derived free radicals but lower levels of SOD[118]. Berry *et al*[119] further measured the superoxide production in vasculatures and revealed the basal level of superoxide in arteries is greater than those in veins. As there are more VSM cells in arteries, this indicated the role of arteries in ROS generation. They also suggested that Ang II could stimulate superoxide anion production and this Ang II mediated effect can be blocked by either losartan or NAD(P)H inhibitor DPI.

In genetic hypertension models, such as the spontaneous hypertensive rats (SHR) and stroke-prone SHRs (SHRSP), enhanced O_2^- production associated with NAD(P)H were observed in resistance arteries, aorta and kidneys. In SHR 30 wk old, significantly higher levels of $p22^{phox}$ mRNA, NAD(P)H induced superoxide anion production and aorta damage were measured compared to the control WKY rats[120]. Vitamin C and E have shown antioxidant properties. They were used to treat adult SHRSP models and have shown preventive effects on the progress of hypertension[121]. In this study, it was found that both vitamin-treated groups improve the total antioxidant status, with decreased activation of vascular NAD(P)H oxidase and significantly increased activation of SOD.

CaMKII inhibition, which has been proposed as a novel therapy for arrhythmias and heart failure, was also linked to Ang II-mediated VSM hypertrophy and hypertension in a recent study[122].

CONCLUSION

Oxidative stress plays key roles in the development and progression of myocardial dysfunction and cardiovascular diseases (Table 1 and Figure 1). In spite of the clinical relevance of Ang II-induced ROS being unclear, recent evidence strongly implicates the benefit of inhibition on Ang II in reducing the risk for cardiovascular remodeling and inflammation. Several novel ROS modulation pathways on which Ang II exerts direct effects have been identified. Apart from its crucial roles in vasoconstriction modulation, recent studies further emphasized the importance of Ang II in heart function. Knowledge linking oxidative stress to clinical cardiac disorders is potentially of exceptional importance. Besides, there are great interests in developing therapeutic strategies by targeting the AT_1R and Ang II-induced ROS transduction cascades.

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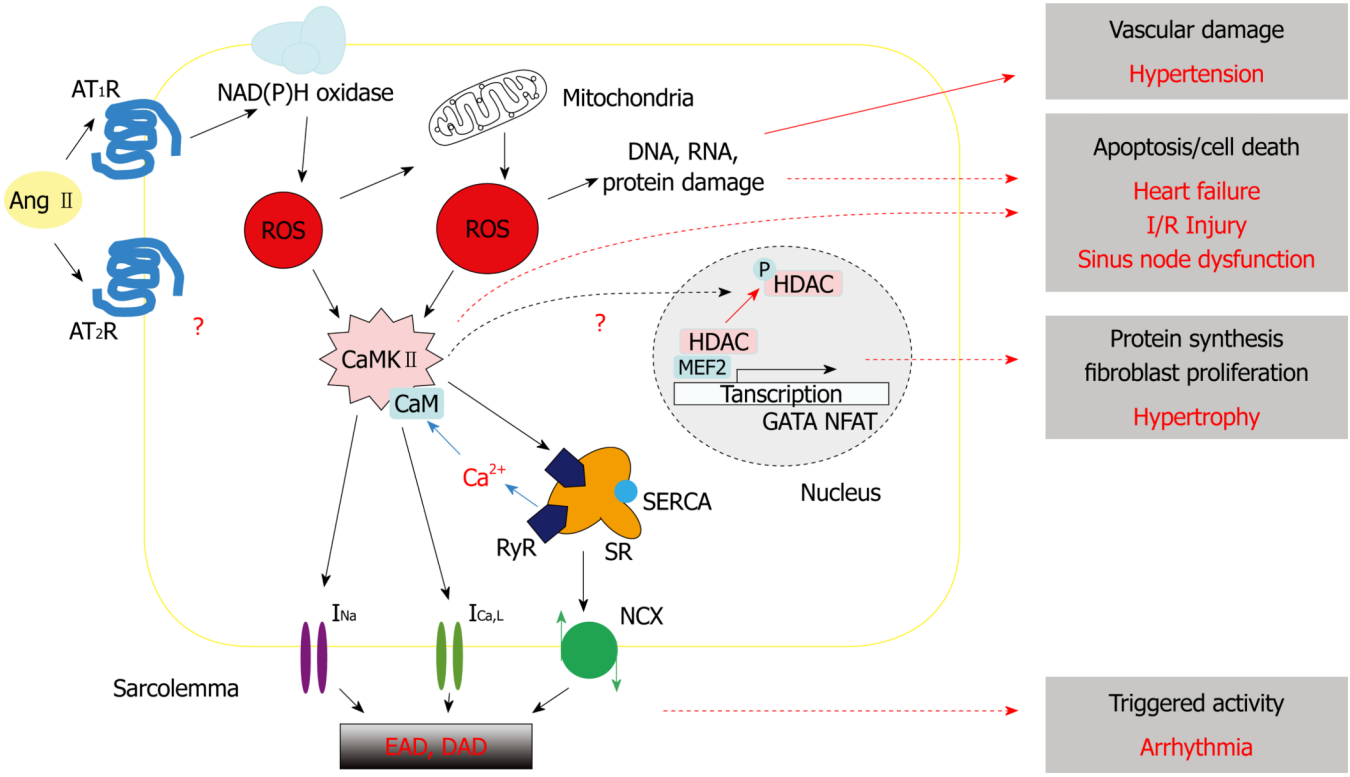

Figure 1. A schematic diagram illustrating the involvement of ROS signaling pathways in numeral Ang II-induced cardiovascular diseases

See main text for details. Ang II: Angiotensin II; AT_1R: Angiotensin II type 1 receptor; AT_2R: Angiotensin II type 2 receptor; ROS: Reactive Oxygen Species; CaMKII: calcium/calmodulin-dependent protein kinase II; I_{Na}: Sodium current; $I_{Ca,L}$: L-type calcium current; NCX: Sodium-Calcium current; SR: Sarcoplasmic reticulum; RyR: Ryanodine receptor; SERCA: SR Ca^{2+} ATPase; EAD: Early afterdepolarization; DAD: Delayed afterdepolarization.

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Table 1

Reactive oxygen species signaling pathways and Ang II-induced cardiovascular diseases

Disease	Receptor(s)	NAD(P)H oxidase	ROS	CaMKII	Other signaling molecules	Ref.
Arrhythmias	AT_1R/AT_2R	+ (Nox2)	+	+	Ca^{2+} handing, RyR, $I_{Ca,L}$, I_{Na}, I_{NCX}	[23,71,74–85]
Cell death/heart failure	AT_1R ?	+ ($p47^{phox}$)	+	+	p38MARK	[70,88–90]
I/R injury	AT_1R/AT_2R	+	+	?	Rac1 GTPase	[91–98]
Hypertrophy	AT_1R	+	+	+, ?	Transcription factors	[67,103–113]
Hypertension	AT_1R	+ ($p22^{phox}$)	+	+	Nitric oxide	[114–122]

+: Involved; ?: Not clear; AT_1R: Ang II type 1 receptor; AT_2R: Ang II type 2 receptor; RyR: Ryanodine receptor; $I_{Ca,L}$: L-type calcium current; I_{Na}: Sodium current; I_{NCX}: Sodium-calcium exchange current; p38MARK: p38 mitogen-activated protein kinase.