Malfunction of Vascular Control in Lifestyle-Related Diseases: Oxidative Stress of Angiotensin II-induced Hypertension: Mitogen-Activated Protein Kinases and Blood Pressure Regulation

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Abstract. The candidate mechanisms for maintaining hypertension in a chronically angiotensin II (Ang II)-infused state include direct vasoconstriction of the vasculature, disturbance of renal water/sodium handling, and central/peripheral sympathetic nerve regulation of hemodynamics. The involvement of reactive oxygen species (ROS) has been studied in these proposed mechanisms and the importance of ROS in progression of Ang II-induced hypertension has been accepted. We recently reported ROS-sensitive blood pressure regulation in chronically as well as acutely Ang II-infused hypertensive rats. The facts suggested that mechanisms for maintaining high peripheral vascular resistance in chronically Ang II-infused hypertensive rats were different from those involved in the acute hypertensive response to Ang II from the perspective of ROS sensitivity and that there must be a time-dependent transition from ROS-non-sensitive to ROS-sensitive vasoconstriction during prolonged Ang II infusion. In this review, we introduced our recent work describing the time transition of ROS sensitivity in Ang II-induced hypertension and activation of cardiovascular mitogen-activated protein kinase (MAPK) in acute and chronic phases Ang II infusion in conscious rats.

Keywords: angiotensin, vasoconstriction, tempol, superoxide anion, mitogen-activated protein kinase

Introduction

Angiotensin II (Ang II) is a powerful vasoconstrictor involved in the pathogenesis of arteriosclerosis and hypertension (1, 2), and reactive oxygen species (ROS) have been recognized as intracellular signaling mediators of Ang II. The candidate mechanisms for maintaining hypertension in a chronically Ang II infused state include direct vasoconstriction of the vasculature (3), disturbance of renal water/sodium handling (4–7), and central/peripheral sympathetic nerve regulation of hemodynamics (8, 9). The important role of ROS has been demonstrated in these proposed mechanisms and progression of Ang II-induced hypertension (2). As shown in Fig. 1, chronic treatment of Ang II induces cardiac hypertrophy accompanied by increased oxidative stress in the heart and vasculature.

In this review, we introduce our recent works describing the ROS sensitivity of Ang II-induced hypertension and activation of cardiovascular mitogen-activated protein kinase (MAPK) in acute and chronic phase Ang II infusion in conscious rats.

ROS-dependent vasoconstriction in chronically but not acutely Ang II-infused hypertension

We found that lipid peroxidation levels in plasma and cardiovascular tissue were increased within a few minutes after commencing a pressor dose of Ang II infusion (200 ng/kg per min). Treatment with 4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl (tempol), which is a membrane-permeable superoxide dismutase mimetic that exhibits potent antioxidant activity against superoxide as well as hydroxy radicals (10, 11), completely normalized the enhanced levels of lipid peroxidation in acutely as well as chronically Ang II infused states. Concerning blood pressure, pre- and simultaneous treat-
ment of tempol did not produce any significant modulation of the hemodynamics elicited by acutely-infused vasopressor doses of Ang II, whereas in chronically Ang II-infused hypertensive rats (200 ng/kg per min for 14 days, s.c.) blood pressure was efficiently normalized by tempol. 3-Carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (3-CP), an inactive compound of tempol, did not affect lipid peroxidation and hemodynamics in acutely or chronically Ang II-infused rats. These facts suggested that the mechanisms for maintaining high peripheral vascular resistance in chronically Ang II-infused hypertensive rats were different from those involved in the acute hypertensive response to Ang II from the perspective of ROS sensitivity and that there must be a time-dependent transition from ROS-non-sensitive to ROS-sensitive vasoconstriction during prolonged Ang II infusion.

ROS-dependent activation of cardiovascular MAPK by acutely administered Ang II

Augmentation of phosphorylated MAPK in cardiovascular tissues by acutely administered Ang II is a well-established observation. The MAPK pathway is a tyrosine kinase-dependent pathway normally stimulated by growth factors and cellular stress or inflammatory cytokines (12, 13). There is increasing evidence that this pathway is involved in various cardiovascular disorders such as cardiac hypertrophy and atherosclerosis (14, 15). The importance of extracellular signal-regulated kinase (ERK1/2) MAPK in maintaining high blood pressure has been revealed using a specific inhibitor in chronic Ang II-infused rats (16). In cultured vascular smooth muscle cells (VSMC), Ang II rapidly stimulated phosphorylation of the MAPK family; ERK1/2, p38, and c-jun N-terminal kinase (JNK) (17 – 19). We revealed the inhibitory dose-dependency of tempol on cardiovascular MAPK activation by acutely administering Ang II to rats. Low and medium doses of tempol (3 and 10 mg/kg, respectively, i.v.) significantly suppressed aortic p38 and JNK activation by Ang II. Interestingly, aortic ERK1/2 activation by Ang II was relatively resistant to tempol treatment and only significantly suppressed by a high dose of tempol (30 mg/kg, i.v.).
A similar pattern of tempol sensitivity was seen for the inhibition of Ang II-induced MAPK activation in the cardiac left ventricle of rats. The results indicate that in contrast to vasoconstrictor effects, cardiovascular MAPK activation by acutely administered Ang II is mediated through ROS-sensitive mechanisms.

**Transition of ROS-sensitive blood pressure reduction occurs within 12 h of Ang II infusion**

Next, we examined the time transition of tempol-sensitive blood pressure reduction during Ang II infusion. Intravenous infusion of Ang II to conscious normotensive rats at a rate of 200 ng/kg per min increased mean blood pressure by around 50 mmHg, and a high blood pressure level was maintained thereafter. As illustrated in Fig. 2, the magnitude of mean blood pressure reduction as a result of tempol administration increased gradually with time during Ang II infusion and reached a maximum 12 h after the start of Ang II infusion. Li et al. demonstrated that ganglion blockade completely inhibited increases in arterial blood pressure during chronic pressor doses of Ang II infusion and suggested that the vasoconstrictor action of Ang II occurred primarily on vascular smooth muscle during acute and via neural pathways during chronic treatment (20). It was noted that this transition occurred within 10 h of Ang II infusion. Therefore, ROS-sensitive blood pressure regulation during Ang II infusion might be closely related to sympathetic nerve activity, which we recently reported (21).

**Relationship between ROS-sensitive blood pressure elevation and NAD(P)H oxidase activity**

There is increasing evidence supporting the fact that NAD(P)H oxidase is a predominant source of vascular and cardiac superoxides (22–26). The messages of NAD(P)H oxidase components, especially p22phox and a gp91phox analogue, Nox1, have been shown to increase several fold in vascular tissue in chronically Ang II-infused rats (27). Landmesser et al. reported that mice lacking p47phox showed significantly lower blood pressure elevation compared with wild-type mice during chronically Ang II infusion (28). However, in the early phase of Ang II infusion (at the latest until 24 h after starting Ang II infusion), message levels of NAD(P)H oxidase components (p22phox, gp91phox, Nox-1, and Nox-4) in the aorta were unchanged (Fig. 3). On the other hand, for activation of NAD(P)H oxidase, p47phox, a cytosol component, might be phosphorylated within minutes by Ang II stimulation, and translocated to the membrane to form a complex with other components. Thus, although the transition of tempol-sensitive blood pressure elevation is not correlated with the abundance of cardiovascular NAD(P)H oxidase, the relationship between ROS-sensitive blood pressure elevation and neural NAD(P)H oxidase activity requires clarification.

**Effects of superimposed Ang II on chronic phase Ang II infusion**

Interestingly, we found that superimposed Ang II (total, 400 ng/kg per min) equally elevated blood pressure at all time points during Ang II infusion in the presence or absence of tempol. Similar to the case of acute phase Ang II infusion, all MAPKs, including ERK1/2, p38, and JNK, in the cardiac left ventricle and aorta of chronically Ang II-infused rats, which returned to basal levels, were reactivated by superimposed Ang II. Magnitudes of phosphorylated MAPK induction were smaller in the cardiac left ventricle and were equipotent in the aorta compared to acutely administered Ang II in normotensive rats. MAPK activation by superimposed Ang II in chronically Ang II-infused rats was blunted by treatment with tempol but not with...
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3-CP. The results indicate that even after sensitization to tempol, superimposed Ang II still restores the abilities of ROS-independent vasoconstriction and ROS-dependent MAPK activation.

**Conclusion**

Figure 4 summarizes the ROS sensitivity of Ang II-induced vasoconstriction and MAPK activation. In conclusion, the present studies clearly demonstrated the time-dependent transition of ROS sensitive blood.
pressure elevation and cardiovascular MAPK activation during prolonged Ang II hypertension. The results might provide a new insight into vasoconstrictor mechanisms in both the acute developing and chronic maintaining phases of hypertension.

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References


