

Forum Minireview

Malfunction of Vascular Control in Lifestyle-Related Diseases: Mechanisms Underlying Endothelial Dysfunction in the Insulin-Resistant State

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Abstract. It is tempting to speculate that increased vasoconstriction and loss of endothelium-dependent vasodilation might be etiological factors of elevated blood pressure in the insulin-resistant state. Vascular contraction induced by angiotensin II and the expression of NAD(P)H oxidase were increased in the aorta of insulin-resistant mice. In addition, both angiotensin II type 1 receptor expression and superoxide anion production were up-regulated in these mice. Another mechanism for impairing endothelial function is the uncoupling of endothelial nitric oxide synthase (eNOS). It has become clear from studies on the aorta of insulin-resistant rat that insulin resistance may be a pathogenic factor for endothelial dysfunction through impaired eNOS activity and increased oxidative breakdown of NO (nitric oxide) due to an enhanced formation of superoxide anion (NO/superoxide anion imbalance), which are caused by relative deficiency of tetrahydrobiopterin, a cofactor of NOS, in vascular endothelial cells. Supplementation of tetrahydrobiopterin restored endothelial function and relieved oxidative tissue damage through activation of eNOS in those rats. These results indicate that generation of superoxide anion from NAD(P)H oxidases and an uncoupled eNOS may be pathogenic factors for impaired endothelial function and hypertension in the insulin-resistant state.

Keywords: insulin resistance, endothelial dysfunction, renin-angiotensin system, nitric oxide, pteridine

Introduction

Accumulated evidence indicates that vascular insulin resistance has a pathogenic role in endothelial dysfunction (1–5). Binding of insulin to its receptor results in an activation of insulin receptor tyrosine kinase, which in turn, phosphorylates tyrosine residues of insulin receptor substrates (IRS) (6). The previous study demonstrated that IRS-mediated signaling in response to ex vivo exposure to insulin was attenuated in the aorta of genetically obese diabetic Zucker fatty rats (2). We have recently reported that tetrahydrobiopterin (BH₄), the natural and essential cofactor of nitric oxide synthases (NOS), plays a crucial role not only in increasing the

rate of nitric oxide (NO) generation by NOS but also in controlling the formation of superoxide anion (O₂^{•−}) in the endothelial cells (7). In vasculature, insulin stimulates BH₄ synthesis through activation of GTP cyclohydrolase I, the rate-limiting enzyme in de novo synthesis of BH₄, and either activity or mRNA expression of GTP cyclohydrolase I is suggested to be decreased in the insulin-resistant state (7). The BH₄ treatment was associated with a 2-fold increase in eNOS activity as well as a 70% reduction of endothelial O₂^{•−} production compared to those in fructose-induced insulin-resistant rats (fructose-fed rats). Moreover, BH₄ treatment of the fructose-fed rats markedly reduced the lipid peroxide content which is increased in fructose-fed rats. Furthermore, increased binding activity of two redox-sensitive transcription factors, nuclear factor- κ B and activating protein-1, in fructose-fed rats was also

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prevented by the treatment with BH₄. However, BH₄ treatment did not normalize blood pressure in the insulin-resistant state (8). These findings suggest that an additional element to regulate vascular tone is therefore likely to be present in the insulin-resistant state.

The vascular tone is regulated by a variety of autocrine and paracrine systems localized in the endothelial and smooth muscle cells. It has been reported that blockade of the renin-angiotensin system with angiotensin converting enzyme (ACE) inhibitors and angiotensin II (Ang II) type 1 (AT1)-receptor antagonists have beneficial effects on patients with insulin resistance (9–11). The GISSI-3 study demonstrated that diabetic patients benefited more from ACE inhibitors than nondiabetic patients did, independent of other risk factors for elevated mortality (12). Recent comparative trials in patients with type 2 diabetes and hypertension have suggested that for the prevention of cardiovascular events, ACE inhibitors may be superior to alternative antihypertensive agents (13, 14). Reduction in cardiovascular events with ACE inhibitors was much greater (almost 50%) than that expected from blood pressure reduction alone compared with placebo, supporting the view that additional mechanisms contribute to the prevention of cardiovascular events with ACE inhibition.

In this review, we are going to address the molecular mechanisms responsible for the modulation of the renin-angiotensin system in the insulin-resistant state.

The NAD(P)H oxidases as major sources of vascular O₂⁻ in the insulin-resistant state

Among many enzymatic systems that are capable of producing reactive oxygen species (ROS), NAD(P)H oxidase and uncoupled eNOS have been extensively studied in vascular cells. Of note, eNOS constitutively produces both NO and O₂⁻, suggesting that the effective release of NO from the vascular endothelium depends on the relative concentrations of these two radical species. It is generally recognized that the NAD(P)H oxidases are predominant sources of O₂⁻ in the vasculature. We measured the vascular O₂⁻ production in the absence of EDTA using intact aortic segments. As a result, aortic segments in the absence of EDTA showed a 1.7-fold elevation of the basal O₂⁻ production compared with those in the presence of EDTA (15). Thus, both calcium-independent and -dependent (primarily eNOS-derived) enzymatic systems are likely to be sources of O₂⁻ production.

In fructose-fed rats, vascular O₂⁻ production measured in the presence of EDTA was increased 1.5-fold compared with control rats. Incubation of vessels with either apocynin or diphenyliodonium (DPI) markedly

attenuated the O₂⁻ production, whereas oxypurinol, rotenone, indomethacin, nordihydroguaiaretic acid (NDGA), or N^G-nitro-L-arginine (L-NA) were ineffective. Therefore, it appears that NAD(P)H oxidase is a major source of O₂⁻ generation in these vessels, whereas the yield of O₂⁻ and lipid hydroperoxide from the lipoxygenase and/or cyclooxygenase pathway is much less than that from NAD(P)H oxidase. NAD(P)H oxidase has recently been demonstrated to be the predominant O₂⁻ source in both endothelial and smooth muscle cells (16, 17). Endothelial removal produced no significant reduction in NAD(P)H oxidase activities in homogenates from control rats, while this caused marked reduction of the enzyme activities in homogenates from fructose-fed rats. These findings indicate that the insulin-resistant state results in enhanced NAD(P)H oxidase activities only in the presence of endothelium.

Modulation of vascular renin-angiotensin system in the insulin-resistant state

There are two major subtypes of Ang II receptors, AT1, AT2; and AT1 receptors are further subdivided into AT1a and AT1b receptors in the rodent (18). ACE inhibitors and AT1-receptor antagonist have been noted to improve vascular reactivity in the insulin-resistant state (15, 19). These evidence suggest that AT1-mediated Ang II signaling is essential for the maintenance of systemic blood pressure in the insulin-resistant state.

Ang II is the major effector substance of the renin-angiotensin system and has effects in the CNS, heart, vasculature, and kidney (20). G-protein-coupled membrane oxidases are known to be responsible for the generation of O₂⁻ at the cell surface such as activation of NADPH oxidase by an amyloidogenic peptide in neurons (21) or by Ang II in vascular endothelial and smooth muscle cells (22). To gain insights into how insulin resistance increases the oxidase activity, Ang II-induced vasoconstricting responses were assessed in vivo and in vitro. The contractile response to Ang II was enhanced in the fructose-fed rats compared with the control rats (15). We could not find any difference in vasoconstrictor response to L-phenylephrine. In addition, in the present study, AT1-receptor blockade inhibited NAD(P)H oxidase activities and the Ang II-induced vasoconstriction and in parallel improved endothelial dysfunction in the insulin-resistant state. Thus, it is reasonably speculated that the insulin-resistant state is related to overfunction of Ang II, probably due to upregulation of AT1-receptor number and affinity for the agonist.

Since the lack of discriminatory pharmacological antagonists made it impossible to define the individual functions of the two AT1-receptor subtypes (AT1a and AT1b), we examined the pathophysiological roles of AT1 receptor using AT1a-receptor knockout (AT1a KO) mice. We found that AT1a-receptor expression was dramatically increased in aortic tissues from insulin-resistant mice (15). The increase in Ang II-receptor expression was entirely due to an increase in AT1a expression, because expression of both AT1b and AT2 receptor was unaltered. In the vascular wall, Ang II induces vasoconstriction by a direct action on smooth muscle cells, but this effect might be modulated by Ang II interaction with endothelial cells. We demonstrated that endothelial removal produced a slight reduction of O_2^- levels in vessels from control rats, while the marked reduction of O_2^- production was found in vessels from fructose-fed rats. In addition, endothelial removal produced no significant reduction in NAD(P)H oxidase activities in homogenates from control rats, while there was marked reduction of these enzyme activities in homogenates from fructose-fed rats. These data indicate that the insulin-resistant state

may stimulate the generation of O_2^- through the activation of NAD(P)H oxidase in aortic endothelial cells under in vivo conditions.

AT1-receptor blockade normalized both NAD(P)H oxidase activities and vascular O_2^- production in fructose-fed rats. In addition, losartan treatment resulted in markedly elevated plasma Ang II compared with control rats (15). AT1-receptor blockade interferes with the negative feedback of Ang II on the release and synthesis of renin from the kidneys, leading to an increase in Ang II levels (23). A recent in vitro study has shown that AT2 receptor functionally antagonizes the AT1 receptor-induced endothelial O_2^- production by a pathway involving tyrosine phosphatases (24). Consequently, increased stimulation of AT2 receptors during AT1-receptor blockade may inhibit endothelial O_2^- production in these rats.

Insulin resistance and modulation of vascular NAD(P)H oxidases

Because the stimulation of AT1a receptor by Ang II leads not only to direct activation of the O_2^- -generating

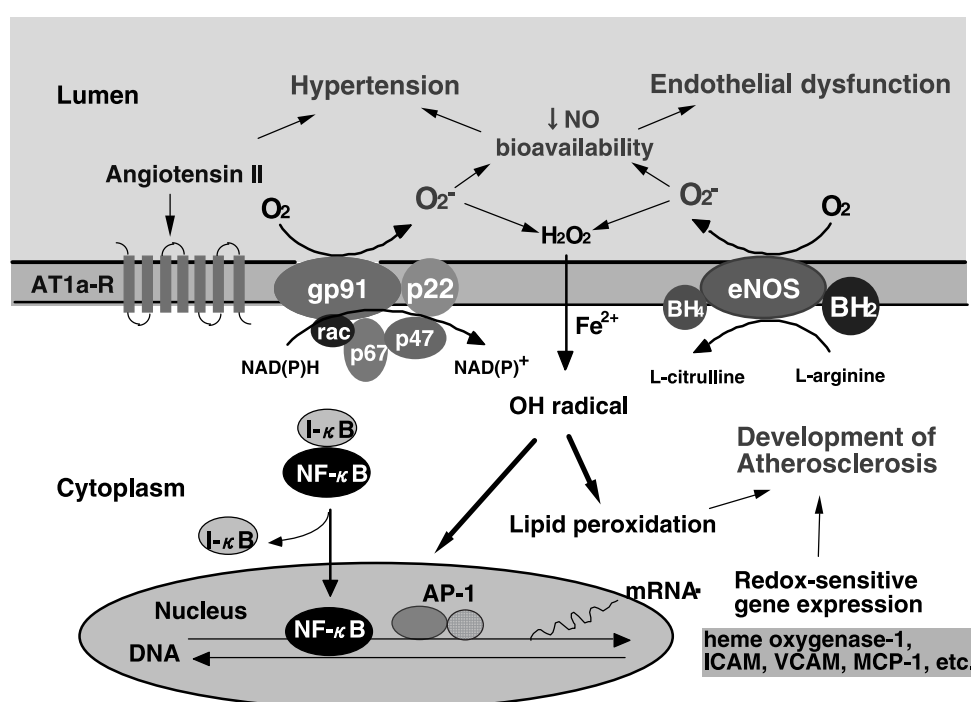


Fig. 1. Schematic model depicting the close association of enhanced formation of superoxide anion (O_2^-) and impaired endothelium-dependent arterial relaxation in the insulin-resistant state. Overactivity of the vascular renin-angiotensin system and uncoupling of endothelial nitric oxide (eNOS) is likely to increase endothelial O_2^- production and contribute in part to the pathogenesis of endothelial dysfunction and hypertension in the insulin-resistant state. AT1a-R, angiotensin II type 1a receptor; NO, nitric oxide; BH₄, tetrahydrobiopterin; BH₂, 6,7(8H)-dihydrobiopterin; H₂O₂, hydrogen peroxide; OH radical, hydroxy radical; NF-κB, nuclear factor-kappaB; I-κB, I-kappaB; AP-1, activating protein-1; ICAM, intercellular adhesion molecule-1; VCAM, vascular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1.

NAD(P)H oxidase but also to an enhanced expression of essential subunits (p22^{phox}, gp91^{phox}, p67^{phox}) of this enzyme, the decreased expression of these subunits in the aorta of fructose-fed AT1a KO mice may well contribute to the observed reduction of vascular O₂⁻ production and NAD(P)H oxidase activities. Using molecular biological approaches, the presence of mRNAs for gp91^{phox}, p22^{phox}, p47^{phox}, and p67^{phox} has been demonstrated in endothelial cells and adventitial cells (25). Vascular smooth muscle cells appear to express p22^{phox} and p47^{phox}, but not gp91^{phox} and p67^{phox} (26). Indeed, gp91^{phox} appears to be crucial for the endothelial O₂⁻ production, because knockout of the gp91^{phox} gene abolished O₂⁻ production in endothelium-intact aortic segments and exhibited a more pronounced endothelium-dependent relaxation than that observed in aorta from WT mice (27). Therefore, our findings in rodent vessels support a potential mechanistic relationship between upregulation of AT1 receptors and NAD(P)H oxidase-dependent endothelial O₂⁻ production, proposed on the basis of similar findings in human blood vessels from diabetic patients (28).

Conclusions

In a series of our recent work, we describe the role of vascular insulin resistance in the pathogenesis of endothelial dysfunction. These results indicate that generation of O₂⁻ from NAD(P)H oxidases and an uncoupled eNOS may be pathogenic factors for impaired endothelial function and increased vascular tone in the insulin-resistant state. Vascular insulin resistance might induce an impaired pteridine metabolism and activated renin-angiotensin system (Fig. 1). Even though the relative importance of the various possible mechanisms leading to the depressed endothelial function in the insulin-resistant state remains to be elucidated, our study shows that BH₄ augmentation and blockade of the renin-angiotensin system results in the restoration of endothelial function and vascular tone in the insulin-resistant states.

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