

REVIEW

Signaling Crosstalk Angiotensin II Receptor Subtypes and Insulin

MASATSUGU HORIUCHI, MASAKI MOGI AND MASARU IWAI

Department of Molecular and Cellular Biology, Division of Medical Biochemistry and Cardiovascular Biology, Ehime University School of Medicine, Shitsukawa, Tohon, Ehime 791-0295, Japan

Key words: Angiotensin II, Diabetes, Hypertension, Insulin

(Endocrine Journal 53: 1–5, 2006)

ANGIOTENSIN II (Ang II) seems to be involved in the pathogenesis of both hypertension and insulin resistance, though few studies have examined the direct relationship between the two. Crosstalk between insulin and Ang II signaling has received great attention, since hypertension and insulin resistance often coexist and are leading risk factors for cardiovascular disease.

Recent studies have suggested that Ang II might negatively modulate insulin-mediated actions by regulating multiple levels of the insulin signaling cascade such as the insulin receptor, IRS and PI3K [1, 2]. The major cardiovascular actions of Ang II have been reported to be mediated by a seven transmembrane-spanning, G-protein-coupled receptor (GPCR) which is termed the AT₁ receptor, and a second receptor subtype known as AT₂ receptor. Based on the restricted expression of AT₂ receptor in fetal tissues as well as in disease states such as myocardial infarction and vascular injury, this receptor is thought to be involved in growth, development and/or differentiation, while both AT₁ and AT₂ receptor belong to the seven-transmembrane, GPCR family and share 32% primary sequence homology [3]. Evidence has revealed that the functions of the AT₁ and AT₂ receptors are mutually antagonistic. The effect of AT₁ receptor blockers (ARB) may not be entirely due to blockade of the AT₁ receptor. When the AT₁ receptor is blocked and unbound Ang II can act on the AT₂ receptor, stimulation of the AT₂ receptor might be involved in the effects of ARB.

However, the detailed mechanism of direct cross-talk of insulin and Ang II-mediated signaling via the AT₁ and AT₂ receptor subtypes in glucose metabolism and vascular remodeling is still unclear. Here, we reviewed the potential crosstalk of Ang II receptor subtypes and insulin especially focusing on glucose metabolism.

Insulin sensitivity and angiotensin II

Recent large clinical trials revealed that new-onset diabetes arose in significantly fewer hypertensive patients on an ARB, valsartan, than in those on calcium channel blocker [4]. Moreover it has been reported that treatment with an ARB, losartan, was associated with less peripheral vascular hypertrophy/rarefaction and higher insulin sensitivity in hypertensive patients with a lower incidence of new-onset diabetes compared to those on β -blocker [5]. In animal studies, the insulin sensitivity of fructose-fed rats has been reported to be improved by treatment with an ARB, due to changes in muscle fiber composition and a decrease in TNF- α expression in skeletal muscle [6]. An ARB has been reported to improve glucose tolerance in the obese Zucker rat, at least in part through enhancement of skeletal muscle glucose transport, with an increase in GLUT4 protein expression [7]. These results suggest that Ang II might regulate insulin sensitivity at multiple sites in various diabetic animal models.

However, it remains unclear whether Ang II has a direct effect on the insulin-mediated pathway of glucose metabolism in addition to changes in local blood flow in insulin-sensitive organs such as skeletal mus-

Correspondence to: Masatsugu HORIUCHI, M.D., Ph.D., Department of Molecular and Cellular Biology, Division of Medical Biochemistry and Cardiovascular Biology, Ehime University School of Medicine, Shitsukawa, Tohon, Ehime 791-0295, Japan

cle. Change in blood flow is one important factor in insulin-mediated glucose uptake in peripheral tissues. Ogiwara *et al.* [8] reported that chronic Ang II infusion decreased insulin-induced glucose uptake in rat soleus muscle. We demonstrated that Ang II infusion decreased insulin sensitivity in diabetic and non-diabetic mice, and administration of a non-hypotensive dose of valsartan increased insulin sensitivity in diabetic KK-Ay mice [9]. Opposite evidence, that Ang II increases insulin sensitivity, has also been reported in humans. This potential discrepancy could be due to different experimental procedures and/or species difference, and additional appropriately designed studies are required.

Crosstalk between angiotensin II and insulin signaling

Stimulation of the insulin receptor induces a rapid tyrosine autophosphorylation that both activates the receptor kinase and allows transient interaction with intracellular protein substrates including insulin receptor substrate (IRS)-1 and IRS-2 via their phosphotyrosine binding, allowing tyrosine phosphorylation of IRS-1 and IRS-2, which induces their binding to SH-2-containing molecules, including phosphatidylinositol 3-kinase (PI 3K), *etc.* The interaction between the IRS and PI 3-K occurs through the p85 regulatory subunit of PI3K, resulting in an increase in catalytic activity of PI 3-K, thereby enhancing GLUT4 translocation. Folli *et al.* [2] demonstrated in cultured vascular smooth muscle cells (VSMC) that Ang II increases serine phosphorylation of the insulin receptor, inhibits insulin-stimulated tyrosine phosphorylation of IRS-1, increases IRS-1 serine phosphorylation, and inhibits insulin-stimulated docking between IRS-1 and PI 3-K, and that Ang II increases serine phosphorylation of p85 and decreases the intrinsic PI 3-K activity. We examined *in vivo* the possibility that an ARB enhances the effects of insulin on glucose metabolism by activating the insulin signaling cascade, thereby increasing insulin sensitivity in KK-Ay mice as a model of type 2 diabetes [9]. Treatment of KK-Ay mice with ARB, valsartan, significantly increased insulin-mediated 2- ^{3}H deoxy-D-glucose (2- ^{3}H]DG uptake into skeletal muscle, and attenuated the increase in plasma glucose concentration after a glucose load, and plasma concentrations of glucose and insulin. Moreover, we observed that valsartan treatment exaggerated the insulin-induced tyrosine phosphorylation of IRS-1, the association of IRS-1 with p85, PI 3-K activity, and

translocation of GLUT4 to the plasma membrane (Fig. 1). To examine the role of the AT₂ receptor in the regulation of insulin-mediated glucose uptake, we employed AT₂ receptor null (*Agtr2*⁻) mice. No apparent difference in insulin-mediated 2- ^{3}H]DG uptake into skeletal muscle was observed between strains, whereas insulin-induced 2- ^{3}H]DG uptake in white adipose tissues in *Agtr2*⁻ mice was significantly lower than that of wild-type mice. Consistent with these results, we observed AT₁ receptor expression in the soleus skeletal muscle by immunohistochemical staining, whereas AT₂ expression in skeletal muscle was very low.

It has been indicated that reactive oxygen species play a pivotal role in the development of insulin resistance, and that Ang II is involved in the regulation of reactive oxygen species production. Furthermore, recent evidence suggested that an increase in oxidative stress is involved in the effects of Ang II on pressor response, vascular injury and insulin resistance. Superoxide anion production specifically increases in the endothelium of aortic tissue in chronic hyperinsulinemic rats through activation of NAD(P)H oxidase [10]. We observed that superoxide production was greater in soleus muscle of KK-Ay mice compared with that in C57BL/6J mice, and observed that administration of valsartan significantly decreased superoxide production [9]. Ogiwara *et al.* [8] reported that Ang II-induced insulin resistance in the rat cannot be attributed to impairment of early insulin-signaling steps, and that increased oxidative stress, possibly through impaired insulin signaling located downstream from PI 3-K activation, is involved in Ang II-induced insulin resistance. Moreover, an increase in GLUT4 protein expression in the skeletal muscle and heart of obese

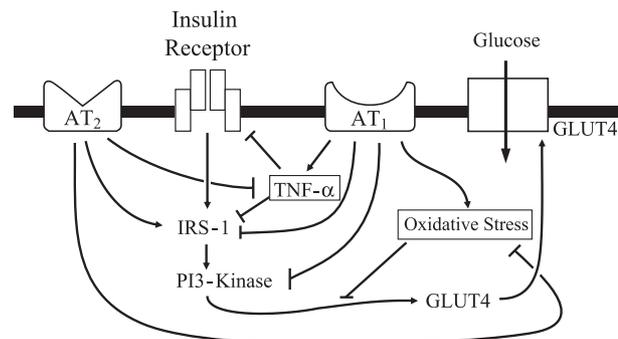


Fig. 1. Stimulation of AT₁ or AT₂ receptor modulates insulin-mediated GLUT translocation to the plasma membrane.

Zucker rats by an ARB [7] has also been reported. These results suggest that Ang II might regulate insulin sensitivity at multiple sites.

It is known that TNF- α inhibits insulin signaling linked to GLUT translocation to the plasma membrane [11], and activation of the renin-angiotensin system is thought to upregulate skeletal muscle tumor necrosis factor- α (TNF- α) [6], suggesting that inhibition of local production of TNF- α in skeletal muscle contributes at least partly to enhancement of insulin sensitivity by ARB. We demonstrated that the expression of TNF- α was upregulated in the injured artery in wild-type mice and was further enhanced in *Agtr2*- mice [12], suggesting possible antagonistic effects of the AT₁ and AT₂ receptors on TNF- α expression in skeletal muscle and adipose tissue, although future analysis in these tissues has to be waited.

Renin-angiotensin system in adipose tissues

Obesity is a major risk factor for the development of hypertension and is further complicated by the concomitant presence of dyslipidemia and insulin resistance [13]. Adipose tissue is another target organ influencing glucose metabolism, and both AT₁ and AT₂ receptors are expressed in adipocytes. We demonstrated that valsartan increases insulin-induced 2-[³H]DG uptake in brown and white adipose tissue, whereas insulin-induced 2-[³H]DG uptake in white adipose tissue in *Agtr2*- mice was significantly lower [9], suggesting that AT₁ and AT₂ receptor stimulation are mutually antagonistic in glucose uptake in adipose tissue. Moreover, we could speculate that AT₁ receptor stimulation seems to increase adipose cell size, whereas stimulation of the AT₂ receptor induces pre-adipocyte differentiation. Adipose tissue in obesity and type 2 diabetes tends to be abnormally hypertrophied, and secretes Ang II and several cytokines, producing insulin resistance and hypertension [14]. It has been demonstrated that angiotensinogen produced by adipose tissue plays a role in both local adipose tissue development and in the endocrine system, which supports a role of adipose angiotensinogen in hypertensive obese patients [15]. The roles of the AT₁ and AT₂ receptor subtypes in adipose tissue are still an enigma and need to be investigated for further understanding the pathogenesis of metabolic syndrome.

There is now evidence that the pancreas may contain an *in situ* active renin-angiotensin system, which

appears to be upregulated in an animal model of type 2 diabetes. Thus, angiotensin converting enzyme (ACE) inhibitors and ARB may act by attenuating the deleterious effect of Ang II on vasoconstriction, fibrosis, inflammation, apoptosis and beta-cell death in the pancreas, thereby protecting a critical beta-cell mass essential for insulin production [16].

Vascular remodeling insulin resistant state

Alterations in insulin's action in the vasculature, mediated via both the insulin and IGF-1 receptors, have been proposed to contribute to vascular remodeling and atherosclerosis, although the pathways of insulin signaling in vascular cells are not well known. The growth-promoting effect of insulin may be the most important factor in increased cardiovascular risk. It has been demonstrated that insulin activates vascular angiotensinogen and Ang II production, and upregulates AT₁ receptor expression [17]. Ang II and IGF have been shown to exert synergistic growth-promoting effects on VSMC [18]. One of the earliest events triggered by AT₁ receptor stimulation is activation of c-Src, which has been shown to participate in the PLC γ and Ras/MAPK signaling pathways. In VSMC, AT₁ receptor stimulation activates the EGF receptor, and in turn activates the ERK pathway. It could be possible that insulin receptor stimulation acts in concert with AT₁ receptor-mediated growth-promoting ERK signals (Fig. 2).

AT₂ receptor stimulation inhibits AT₁ receptor- or receptor tyrosine kinase (RTK)-mediated MAPK via

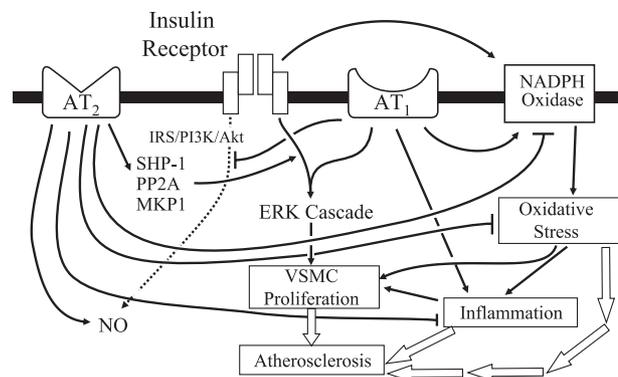


Fig. 2. Stimulation of AT₁ or AT₂ receptor regulates vascular remodeling and atherosclerosis in concert with insulin receptor signaling in insulin-resistant state.

activation of a series of phosphatases including the protein tyrosine phosphatase, SHP-1, MKP-1, and the serine/threonine phosphatase 2A (PP2A) [3] and attenuates AT₁ receptor-mediated vascular remodeling and atherosclerosis formation at least partly due to its anti-inflammatory effect and inhibition of oxidative stress [12, 19, 20]. These results suggest complex interaction between the Ang II receptor subtypes and RTK signaling. A recent report has shown that AT₂ receptor stimulation inhibits ERK activation and cell proliferation induced by insulin, through impairing insulin-induced autophosphorylation of the insulin receptor β subunit and phosphorylation of IRS-1 [21]. We demonstrated that AT₂ receptor activation inhibits the insulin-mediated IRS-2-associated PI 3-K activation, Akt phosphorylation and anti-apoptotic effect in PC12W cells, and that stimulation of the AT₂ receptor attenuates insulin-induced p85 α phosphorylation, via recruitment of SHP-1 to the complex of IRS-2 and PI 3-K [22]. These results suggest that AT₂ receptor stimulation negatively modulates the growth promoting signaling of insulin.

Scherrer *et al.* [23] reported that insulin's vasodilator effects are mediated by stimulation of NO release, and that abnormalities in insulin-induced NO release could contribute to altered vascular function and hypertension in insulin-resistant states. It is known that AT₂ receptor stimulation in VSMC induces the production of bradykinin, which stimulates the NO/cGMP

system to promote vasodilatation [24]. It could be possible that AT₂ receptor-mediated NO production contributes in part to ARB's vasoprotective effects. Taken together, these results suggest that stimulation of the AT₁ and AT₂ could exert antagonistic effects on insulin sensitivity, glucose metabolism and moreover modulate vascular remodeling (Fig. 2) in concert with insulin.

Summary

Insulin-resistant states are often associated with hypertension, and the accumulated data indicate that ARB decrease new-onset of diabetes with vasoprotective effects. Recent evidence suggests that activation of Ang II receptor subtypes could regulate insulin sensitivity at multiple sites of insulin signaling in various diabetic animal models and regulate vascular remodeling in concert with insulin in potentially distinct fashions. Moreover, the roles of Ang II receptor subtypes have been highlighted in insulin resistance in obesity, which is one of the major risk factors for the development of hypertension. More detailed analysis of the crosstalk of Ang II and insulin-mediated signaling in various tissues would provide further information to understand the clinical relevance of the effect of ARB on insulin resistance, thereby preventing cardiovascular events associated with insulin resistance.

References

1. Velloso LA, Folli F, Sun XJ, White F, Saad MJA, Kahn CR (1996) Cross-talk between the insulin and angiotensin signaling systems. *Proc Natl Acad Sci USA* 93: 12490–12495.
2. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP (1997) Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. *J Clin Invest* 100: 2158–2169.
3. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T (2000) International Union of Pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 52: 415–472.
4. Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, Hua T, Laragh J, McInnes GT, Mitchell L, Plat F, Schork A, Smith B, Zanchetti A, VALUE trial group (2004) Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. *Lancet* 363: 2022–2031.
5. Olsen MH, Fossum E, Hoiegggen A, Wachtell K, Hjerkin E, Nesbitt SD, Andersen UB, Phillips RA, Gaboury CL, Ibsen H, Kjeldsen SE, Julius S (2005) Long-term treatment with losartan versus atenolol improves insulin sensitivity in hypertension: ICARUS, a LIFE substudy. *J Hypertens* 23: 891–898.
6. Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K (2000) The contribution of skeletal muscle tumor necrosis factor- α to insulin resistance and hypertension in fructose-fed rats. *J Hypertens* 18: 1905–1910.
7. Henriksen EJ, Jacob S, Kinnick TR, Teachey K, Krekler M (2001) Selective angiotensin II receptor antagonism reduces insulin resistance in obese Zucker rats. *Hypertension* 38: 884–890.
8. Ogiwara T, Asano T, Ando K, Chiba Y, Sakoda H, Anai M, Shojima N, Ono H, Onishi Y, Fujishiro M,

- Katagiri H, Fukushima Y, Kikuchi M, Noguchi N, Aburatani H, Komuro I, Fujita T (2002) Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. *Hypertension* 40: 872–879.
9. Shiuchi T, Iwai M, Li HS, Wu L, Min LJ, Li JM, Okumura M, Cui TX, Horiuchi M (2004) Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. *Hypertension* 43: 1003–1010.
 10. Kashiwagi A, Shinozaki K, Nishio Y, Maegawa H, Maeno Y, Kanazawa A, Kojima H, Haneda M, Hidaka H, Yasuda H, Kikkawa R (1999). Endothelium-specific activation of NAD(P)H oxidase in aortas of exogenously hyperinsulinemic rats. *Am J Physiol* 277: E976–E983.
 11. Brindley DN, Wang CN, Mei J, Xu J, Hanna AN (1999) Tumor necrosis factor-alpha and ceramides in insulin resistance. *Lipids* 34: S85–S88.
 12. Wu L, Iwai M, Nakagami H, Li Z, Chen R, Suzuki J, Akishita M, de Gasparo M, Horiuchi M (2001) Roles of angiotensin II type 2 receptor stimulation associated with selective angiotensin II type 1 receptor blockade with valsartan in the improvement of inflammation-induced vascular injury. *Circulation* 104: 2716–2721.
 13. Sharma AM (2004) Is there a rationale for angiotensin blockade in the management of obesity hypertension? *Hypertension* 44: 12–19.
 14. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM (2002) Mature adipocytes inhibit in vitro differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes* 51: 1699–1707.
 15. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulangue A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M (2001) Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J* 15: 2727–2729.
 16. Jandeleit-Dahm KA, Tikellis C, Reid CM, Johnston CI, Cooper ME (2005) Why blockade of the renin-angiotensin system reduces the incidence of new-onset diabetes. *J Hypertens* 23: 463–473.
 17. Kamide K, Rakugi H, Nagai M, Takiuchi S, Matsukawa N, Higaki J, Kawano Y, Ogihara T, Tuck ML (2004) Insulin-mediated regulation of the endothelial renin-angiotensin system and vascular cell growth. *J Hypertens* 22: 121–127.
 18. Gustafsson T, Andersson P, Chen Y, Magnusson JO, Arnqvist HJ (1999) Interaction of angiotensin II and the insulin-like growth factor system in vascular smooth muscle cells. *Am J Physiol* 277: H499–H507.
 19. Suzuki J, Iwai M, Nakagami H, Wu L, Chen R, Sugaya T, Hamada M, Hiwada K, Horiuchi M (2002) Role of angiotensin II-regulated apoptosis through distinct AT1 and AT2 receptors in neointimal formation. *Circulation* 106: 847–853.
 20. Iwai M, Chen R, Li Z, Shiuchi T, Suzuki J, Ide A, Tsuda M, Okumura M, Min LJ, Mogi M, Horiuchi M (2005) Deletion of angiotensin II type 2 receptor exaggerated atherosclerosis in apolipoprotein E-null mice. *Circulation* 112: 1636–1643.
 21. Elbaz N, Bedecs K, Masson M, Sutren M, Strosberg AD, Nahmias C (2000) Functional trans-inactivation of insulin receptor kinase by growth-inhibitory angiotensin II AT2 receptor. *Mol Endocrinol* 14: 795–804.
 22. Cui TX, Nakagami H, Nahmias C, Shiuchi T, Takeda-Matsubara Y, Li JM, Wu L, Iwai M, Horiuchi M (2002) Angiotensin II subtype 2 receptor activation inhibits insulin-induced phosphoinositide 3-kinase and Akt and induces apoptosis in PC12W cells. *Mol Endocrinol* 16: 2113–2123.
 23. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P (1994) Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94: 2511–2515.
 24. Tsutsumi Y, Matsubara H, Masaki H, Kurihara H, Murasawa S, Takai S, Miyazaki M, Nozawa Y, Ozono R, Nakagawa K, Miwa T, Kawada N, Mori Y, Shibasaki Y, Tanaka Y, Fujiyama S, Koyama Y, Fujiyama A, Takahashi H, Iwasaka T (1999) Angiotensin II type 2 receptor overexpression activates the vascular kinin system and causes vasodilation. *J Clin Invest* 104: 925–935.