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Molecular mechanisms of the antagonistic action between AT₁ and AT₂ receptors

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Abstract

Although angiotensin II (Ang II) binds to Ang II type 1 (AT₁) and type 2 (AT₂) receptors, AT₁ and AT₂ receptors have antagonistic actions with regard to cell signaling. The molecular mechanisms that underlie this antagonism are not well understood. We examined AT₁ and AT₂ receptor-induced signal cross-talk in the cytoplasm and the importance of the hetero-dimerization of AT₁ receptor with AT₂ receptor on the cell surface. AT₁ and AT₂ receptors showed antagonistic effects toward inositol phosphate production. AT₁ receptors mainly formed homo-dimers, rather than hetero-dimers with AT₂ receptor, on the cell surface as determined by immunoprecipitation, and subsequently induced cell signals. AT₂ receptor mainly formed homo-dimers, rather than hetero-dimers with AT₁ receptor, on the cell surface. The expression levels of homo-dimerized AT₁ receptor or AT₂ receptor on the cell surface did not change after treatment with Ang II, the AT₁ receptor antagonist telmisartan or the AT₂ receptor antagonist PD123319. Finally, AT₁ and AT₂ receptor-induced signals antagonized phospholipase C- β 3 phosphorylation. In conclusion, Ang II-induced AT₁ receptor signals may be mainly blocked by AT₂ receptor signals through their negative cross-talk in the cytoplasm rather than by the hetero-dimerization of both receptors on the cell surface. The proper balance of the expression levels of AT₁ and AT₂ receptors might be critical for the antagonistic action between these receptors.

Keywords

Angiotensin II receptors; Signal cross-talk; Dimerization; Inositol phosphate production; Phospholipase C

Introduction

Angiotensin II (Ang II) plays a central role as a major regulator of blood pressure, electrolyte balance, and endocrine function related to cardiovascular disease. Two distinct subtypes of Ang II receptors, type 1 (AT₁) and type 2 (AT₂), have been identified, and both belong to the G-protein coupled receptor superfamily (GPCRs) [1]. Most of its physiological effects are mediated through the AT₁ receptor [2]. The idea that AT₁ and AT₂ receptors

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Disclosures

None.

exert antagonistic activity is supported by the observation of Ang II-induced cell growth and anti-growth effects, respectively [3,4].

GPCRs are able to both homo-dimerize and hetero-dimerize. For example, β_2 -adrenergic, muscarinic, dopamine D₂, and opioid receptors undergo homo-dimerization following agonist stimulation [5–8]. The presence of homo-dimerization suggests that it may be possible to modulate receptor function through intermolecular interactions. On the other hand, several recent reports demonstrate that GPCRs in monomeric form effectively activate G proteins [9–11], and that receptor dimerization is actually less effective. Although the dimerization of GPCRs is a highly controversial issue, a detected AT₁ and AT₂ receptor hetero-dimerization that led to AT₁ receptor signal inhibition was independent of the binding of Ang II to AT₂ receptor [12], and AT₂ receptor has constitutive activity [13,14], which suggests that the constitutive activity of AT₂ receptor itself might induce hetero-dimerization independent of Ang II stimulation. This hetero-dimerization may explain why the functions of AT₁ and AT₂ receptors are antagonistic [12]. Another possible mechanism may explain why the functions of AT₁ and AT₂ receptors are antagonistic, i.e., Ang II-induced AT₁ and AT₂ receptor signals show cross-talk in the cytoplasm. In fact, Horiuchi et al. reported that the stimulation of AT₂ receptor in AT₂-receptor-transfected vascular smooth muscle cells (SMC) inhibited AT₁ receptor-mediated tyrosine phosphorylation of signal transducers and activators of transcription (STAT)-1 α/β , STAT2 and STAT3 [15]. AT₁ and AT₂ receptors regulate STAT activation by negative cross-talk.

Although the antagonistic effect of AT₁ and AT₂ receptors through their cross-talk is well-known, we determined whether their signal cross-talk mainly occur in the cytoplasm (one molecule interacts AT₁ receptor-signaling with AT₂ receptors-signaling) or on the cell surface (direct interaction between AT₁ receptor and AT₂ receptor by hetero-dimerization). Therefore, we hypothesized that Ang II-induced AT₁ receptor signals were blocked by AT₂ receptor signals in the cytoplasm rather than by the hetero-dimerization of both receptors on the cell surface.

Methods

Materials

The following antibodies and reagents were purchased: anti-phospholipase (PL) C- β_3 , γ_1 and γ_2 antibodies (Abs), anti-phospho(p)-PLC- β_3 , γ_1 and γ_2 Abs, anti-inositol 1,4,5-trisphosphate (IP₃) receptor Ab and anti- β -actin Ab (Cell Signaling Technology Japan, K.K.), anti-AT₁ and AT₂ receptor Abs (Santa Cruz Biotechnology, Inc., CA, USA), Ang II analogues (Bachem, Bubendorf, Switzerland), the AT₁ receptor antagonist telmisartan (Toronto Research Chemical, Canada) and the AT₂ receptor antagonist PD123319 (Sigma–Aldrich Inc., USA).

Cell cultures and transfection

HepG2 cells and human embryonic kidney 293 (HEK) cells (American Type Culture Collection, Rockville, MD) were cultured using Dulbecco's modified essential medium (DMEM) with 10% fetal bovine serum (FBS) (HyClone) and penicillin/streptomycin in 5% CO₂ at 37 °C. Human coronary artery SMCs were purchased from Clonetics. SMCs were cultured and used from 3 to 5 passages in media supplemented with 5% FBS, penicillin/streptomycin and SMC growth supplement (Takara Co., Osaka, Japan) in 5% CO₂ at 37 °C.

The synthetic rat AT₁ and AT₂ receptor genes were used for expression [13,16]. To express AT₁ and AT₂ receptor protein, 10 μ g of purified plasmid DNA per 10⁷ cells were used in transfection. The DNAs were transfected into HEK cells using the Lipofectamine 2000

liposomal reagent according to the manufacturer's instructions (Invitrogen). Cell viability was >95% by trypan blue exclusion analysis in control experiments.

Receptor binding assay

The K_d and B_{max} values of the receptor binding were determined by ^{125}I -[Sar¹, Ile⁸]Ang II-binding experiments under equilibrium conditions, as previously described [16,17]. Cell membranes were prepared by the nitrogen Parr bomb disruption method in the presence of protease inhibitors. The membranes were incubated with 100 pM ^{125}I -[Sar¹, Ile⁸]Ang II for 1 h at 22 °C in a volume of 125 μl . Binding was stopped by filtering the incubation mixture through Whatman GF/C glass-fiber filters, and the residues were extensively washed further with binding buffer. The bound ligand fraction was determined by the counts per minute (cpm) remaining on the membrane. Binding kinetics values were determined as previously described [16,17].

Inositol phosphate (IP) formation studies

Total soluble IP was measured by the perchloric acid extraction method, as described previously [16,17]. Briefly, semi-confluent cells in 60-mm Petri dishes were labeled for 24 h with [^3H]-myoinositol, specific activity 22 Ci/mmol (Amersham), at 37 °C with or without antagonist in medium containing 10% serum. On the day of the functional assay, the labeled cells were washed with Hank's Balanced Salt solution (HBSS) three times and incubated with HBSS containing 10 mM LiCl for 20 min; agonist was added and incubation continued for another 45 min at 37 °C with or without antagonist. At the end of incubation, the medium was removed, and total soluble IP was extracted from the cells by the perchloric acid extraction method.

Polyacrylamide gel electrophoresis (PAGE), Western blotting, and immunoprecipitation

Cell membrane and cytoplasmic fractions were prepared as described previously [14]. For Western blotting, equal amounts of samples on a protein basis as determined using the Bradford reagent (Bio-Rad) were resolved on nonreducing 7–10% SDS-PAGE. Cell membrane was incubated at room temperature without boiling for 30 min with loading buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.2 mM sodium orthovanadate, 0.2 mM phenylmethylsulfonyl fluoride, 0.5% NP-40). Western blot analysis was performed with primary antibodies as specified in each case. Horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescent substrate system (Amersham) were used for detection. The signal was independently quantified by a digital image-analysis system.

Statistical analysis

The results are expressed as the mean $\pm$ standard error of three or more independent determinations (Figs. 1–4). The significance of differences between measured values was evaluated by unpaired Student's *t* test and an analysis of variance with Fisher's test. Statistical significance was set at a level of $p < 0.05$.

Results

AT₁ and AT₂ receptors showed antagonistic effects toward IP production

First, we used HepG2 cells because they endogenously expressed AT₁ and AT₂ receptors (B_{max} , 7.9 ± 0.7 and 6.8 ± 1.4 fmol/mg protein, respectively). [Sar¹]Ang II (0.1 μM) did not induce significant IP production (Fig. 1A). In the presence of 1 μM PD123319, the same concentration of [Sar¹]Ang II significantly stimulated IP production. On the other hand, in the presence of 1 μM telmisartan, 0.1 μM [Sar¹]-Ang II significantly suppressed IP

production. Since the clinical plasma concentration of telmisartan ($C_{\max}$) is about 1 μM [18], the concentration of telmisartan used in the experiments was 1 μM . When both receptors were blocked by telmisartan and PD123319, $[\text{Sar}^1]\text{Ang II}$ did not induce significant IP production.

Since HepG2 is a perpetual cell line which was derived from the human liver tissue with a well differentiated hepatocellular carcinoma, we also used native SMCs which endogenously expressed AT_1 and AT_2 receptors ($B_{\max}$, 19 ± 8 and 17 ± 4 fmol/mg protein, respectively). As shown in Fig. 1B, the results of IP production using SMCs showed the similar to those using HepG2 cells. In this way, AT_1 and AT_2 receptors showed antagonistic action against IP production.

Expression levels of AT_1 receptor or AT_2 receptor on the cell surface with telmisartan and PD123319

Since HepG2 cells were incubated with telmisartan and PD123319 for 24 h before the measurement of Ang II-induced IP production (Fig. 1), we analyzed the expression levels of AT_1 and AT_2 receptors on the cell surface with the indicated concentrations of telmisartan or PD123319 for the indicated incubation times. There were no differences in the expression levels of AT_1 and AT_2 receptors throughout the experimental period under the no-treatment condition using immunoblotting (Fig. 2A and B). Although 1 μM telmisartan transiently decreased the expression level of AT_1 receptor and increased the level of AT_2 receptor after 8 h of incubation, the expression levels of both receptors returned to the basal levels after 24 h. In addition, PD123319 did not alter the expression level of AT_2 receptor under different concentrations and incubation times (data not shown).

To confirm the expression levels of Ang II receptors, we also analyzed the receptors expression by receptor binding assay (Fig. 2C and D). One μM telmisartan transiently decreased the expression level of AT_1 receptor and increased the level of AT_2 receptor after 8 h of incubation, the expression levels of both receptors returned to the basal levels after 24 h.

AT_1 receptor mainly formed homo-dimers, rather than hetero-dimers with AT_2 receptor, on the cell surface

To analyze the dimerization of AT_1 receptor and AT_2 receptor on the cell surface, we performed immunoprecipitation using total cell lysate (cytoplasm and cell membrane) and cell membrane (Fig. 3). The ratio of the expression levels of homo-dimerized AT_1 receptors, homo-dimerized AT_2 receptors and hetero-dimerized AT_1 and AT_2 receptors was $4.3 \pm 1.2:8.0 \pm 2.0:1.0 \pm 0.2$ in total cell lysate and $4.1 \pm 0.9:12.2 \pm 2.9:1.0 \pm 0.1$ on the cell surface in SMCs, respectively. In addition, the ratio of the expression levels of homo-dimerized AT_1 and AT_2 receptors and hetero-dimerized receptors was $6.9 \pm 0.9:13.2 \pm 1.6:1.0 \pm 0.1$ in total cell lysate and $4.7 \pm 1.2:10.7 \pm 2.5:1.0 \pm 0.2$ on the cell surface in HepG2, respectively. Thus, AT_1 receptor mainly formed homo-dimers, rather than hetero-dimers with AT_2 receptor, on the cell surface, and AT_2 receptor mainly formed homo-dimers, rather than hetero-dimers with AT_1 receptor, on the cell surface. Next, we examined the expression levels of the homo-dimerization of AT_1 or AT_2 receptor on the cell surface with 0.1 μM $[\text{Sar}^1]\text{Ang II}$ after incubation for 40 min, and with 1 μM telmisartan and 1 μM PD123319 after incubation for 24 h. Neither $[\text{Sar}^1]\text{-Ang II}$, telmisartan, nor PD123319 altered the expression levels of the homo-dimerization of both receptors.

AT_1 and AT_2 receptor-induced signals antagonize $\text{PLC-}\beta_3$ phosphorylation

Since AT_1 and AT_2 receptors showed antagonistic action toward IP production and the mechanism was not the hetero-dimerization of both receptors, we analyzed the mechanism

of signal cross-talk in the cytoplasm (Fig. 4). Since PLC are located immediately downstream from the receptor, we analyzed 3 isoforms (PLC- β_3 , γ_1 and γ_2) which may be important for Ang II-induced IP production in AT₁ and AT₂-receptor-transfected HEK cells. Since HEK cells did not endogenously express either receptor by our receptor binding assay, we used these cells as surrogate models to link the *de novo* expression of the receptors to PLC phosphorylation. The expression levels of AT₁ and AT₂ receptors were similar ($B_{\max}$, 8.4 ± 1.3 and 7.9 ± 2.1 pmol/mg protein, respectively). Neither [Sar¹]Ang II, telmisartan nor PD123319 significantly altered the ratio of phosphorylated to total protein levels of PLC- γ_1 , γ_2 (Fig. 4A and B) and β -actin (data not shown). Although [Sar¹]Ang II induced phosphorylation of PLC- β_3 , [Sar¹]Ang II induced additional phosphorylation in the presence of PD123319 (Fig. 4C). On the other hand, in the presence of telmisartan, [Sar¹]Ang II did not induce the phosphorylation of PLC- β_3 . When both receptors were blocked by telmisartan and PD123319, PLC- β_3 was not phosphorylated by [Sar¹]Ang II.

Next, we examined the expression levels of IP₃ receptor with or without Ang II, telmisartan and PD123319 in AT₁ and AT₂-receptor-transfected HEK cells because the down-regulation of IP₃ receptor represents a reversible adaptive response to protect cells against the adverse effects of constitutively active AT₁-N111G mutant receptor [19]. IP₃ receptor was similarly expressed in the presence or absence of Ang II, telmisartan and PD123319 (data not shown). AT₁ and AT₂ receptor-mediated signals did not change the expression level of IP₃ receptor. Therefore, AT₁ and AT₂ receptor-induced IP production may directly regulate diverse physiological functions through regulation of the phosphorylation of PLC- β_3 .

Discussion

The main finding of the present study is that AT₁ receptor mainly formed homo-dimers, rather than hetero-dimers with AT₂ receptor, on the cell surface, and AT₂ receptor also mainly formed homo-dimers, rather than hetero-dimers with AT₁ receptor, on the cell surface. Furthermore, AT₁ receptor signals may be blocked by AT₂ receptor signals through their negative cross-talk in PLC- β_3 activation, rather than by hetero-dimerization of both receptors on the cell surface.

Members of the GPCRs undergo homo-dimerization to induce cell signaling. Homo-dimers of AT₁ or AT₂ receptor are also clearly important for inducing cell signals. As for AT₁ receptor, intracellular factor XIIIa transglutaminase cross-links Ang II-induced AT₁ receptor homo-dimers through Gln³¹⁵ in the carboxyl-terminal tail of the AT₁ receptor [20]. The elevated levels of AT₁ receptor dimers on monocytes promote atherogenesis in ApoE-deficient mice. In addition, constitutively active homo-dimerization of AT₂ receptor was localized in the cell membrane without Ang II stimulation and induced apoptosis without changes in receptor conformation [14]. The constitutive activity of AT₂ receptor itself might serve to effect hetero-dimerization independent of Ang II stimulation. AbdAlla et al. reported that the AT₂ receptor binds directly to the AT₁ receptor and thereby antagonizes the function of the AT₁ receptor. They also found that the AT₁-specific antagonism of the AT₂ receptor was independent of AT₂ receptor activation and signaling [12]. Unexpectedly, the present study indicated that Ang II-induced AT₁ receptor signals may be blocked by AT₂ receptor signals through their negative cross-talk, rather than through the hetero-dimerization of AT₁ and AT₂ receptors. The discrepancy between our data and their study may be due to the use of different types of cells and expression levels of AT₁ and AT₂ receptors. Since transglutaminase cross-links Ang II-induced AT₁ receptor homo-dimers in monocytes [20], AT₁ and AT₂ receptors also may hetero-dimerize by an unknown enzyme. It is possible that HepG2 cells, but not PC12 cells, may lack such an enzyme. In addition, HepG2 cells equally express AT₁ and AT₂ receptors. It has been reported that AT₁-receptor hetero-dimerized bradykinin (BK) type B₂ (B₂) receptor [21], dopamine receptor [22],

endothelin type B receptor [23] and Mas receptor [24]. Hetero-dimerization of AT₁ receptor and B₂ receptor significantly increased in preeclampsia [21]. In this case, the hetero-dimerization in preeclampsia correlated with a 4- to 5-fold increase in B₂ receptor protein levels compared to AT₁-receptor levels. Moreover, since we also reported that constitutively active homo-dimerization, which was due to disulfide bonding between Cys³⁵ in one AT₂ receptor and Cys²⁹⁰ in another AT₂ receptor [14], was localized in the cell membrane, this bonding might not be easy to disrupt. If the expression level of AT₂ receptor is much higher than that of AT₁ receptor, AT₂ receptor may more easily hetero-dimerize with AT₁ receptor and induce an antagonistic action of AT₁ receptor as a dominant negative receptor. In fact, AbdAlla et al. analyzed protein levels of the heterodimerization of AT₁ and AT₂ receptor on myometrial biopsies from pregnant and nonpregnant women [12]. Although the heterodimerized level in nonpregnant women was much higher than that in pregnant women, the expression level of AT₂ receptor in nonpregnant women was also about 10-fold higher. The proper balance of expression levels of AT₁ and AT₂ receptors may be critical for antagonistic action through hetero-dimerization between these receptors.

AT₂ receptor may have anti-proliferative and apoptotic effects. In fact, overexpression of the AT₂ receptor downregulates AT_{1a} receptor expression in rat SMC in an Ang II-independent manner that is mediated by the BK/nitric oxide pathway [25]. In our study, there were no differences in the expression levels of AT₁ and AT₂ receptors under the no-treatment condition, and the expression levels of AT₁ and AT₂ receptors with telmisartan or PD123319 returned to the basal levels after 24 h when IP production was performed. The expression levels of AT₁ and AT₂ receptors may not affect IP production in our cell system.

Finally, we analyzed the possibility that Ang II-induced AT₁ receptor signals were blocked by AT₂ receptor signals through their cross-talk in the cytoplasm. Three families of PLC isozymes, PLC- β , - γ and - δ have been described [26]. PLC phosphorylation is one of the earliest events in Ang II signaling [27]. Ang II binding to AT₁ receptor induces a rapid phosphorylation of PLC to produce IP₃. AT₁ receptors sequentially couple to PLC- β_1 via a heterotrimeric G protein and to PLC- γ via a downstream tyrosine kinase in SMC [28]. In this study, although we did not analyze PLC- β_1 , PLC- β_3 but not PLC- γ_1 or - γ_2 is important for Ang II-induced IP production. Since we examined these PLC phosphorylations after 1 min of stimulation by Ang II, since IP₃ formation is markedly increased in a few seconds [27], we should also examine IP formation in an early phase. AT₁ receptor blockade decreased PLC- β_3 phosphorylation, and AT₂ receptor blockade increased phosphorylation. AT₁ receptor signals may be mainly blocked by AT₂ receptor signals through negative cross-talk in PLC- β_3 phosphorylation.

AT₁ receptor-induced signaling can counteract the function of AT₂ receptors. Although AT₁ receptor antagonists that prevent Ang II-induced signaling are clinically available, a new drug may be discovered that resembles a dimeric ligand formed by two monovalent ligands for AT₁ and AT₂ receptors. In this study, we indicated that hetero-dimerization did not explain why the functions of AT₁ and AT₂ receptors are antagonistic. Therefore, discovery of AT₂ receptor agonist, but not a dimeric ligand for AT₁ and AT₂ receptors, may be more useful to block AT₁ receptor-induced signaling in their negative cross-talk in cytoplasm.

In conclusion, AT₁ receptor signals may be mainly blocked by AT₂ receptor signals through their negative cross-talk in PLC- β_3 phosphorylation rather than by the hetero-dimerization of both receptors on the cell surface. The balance of the expression levels of AT₁ and AT₂ receptors may be critical for the antagonistic action between these receptors.

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References

1. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International Union of Pharmacology. XXIII. The angiotensin II receptors. *Pharmacol. Rev.* 2000; 52:415–472. [PubMed: 10977869]
2. Miura S, Fujino M, Saku K. Angiotensin II receptor blocker as an inverse agonist: a current perspective. *Curr. Hypertens. Rev.* 2005; 1:115–121.
3. Hiroi Y, Hiroi J, Kudoh S, Yazaki Y, Nagai R, Komuro I. Two distinct mechanisms of angiotensin II-induced negative regulation of the mitogen-activated protein kinases in cultured cardiac myocytes. *Hypertens. Res.* 2001; 24:385–394. [PubMed: 11510751]
4. Horiuchi M, Hamai M, Cui TX, Iwai M, Minokoshi Y. Cross talk between angiotensin II type 1 and type 2 receptors: cellular mechanism of angiotensin type 2 receptor-mediated cell growth inhibition. *Hypertens. Res.* 1999; 22:67–74. [PubMed: 10487321]
5. Jordan BA, Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature.* 1999; 399:697–700. [PubMed: 10385123]
6. Hebert TE, Loisel TP, Adam L, Ethier N, Onge SS, Bouvier M. Functional rescue of a constitutively desensitized beta2AR through receptor dimerization. *Biochem. J.* 1998; 330:287–293. [PubMed: 9461522]
7. Maggio R, Barbier P, Corsini GU. Functional role of the third cytoplasmic loop in muscarinic receptor dimerization. *J. Biol. Chem.* 1996; 271:1055–1160.
8. Ng GY, O'Dowd BF, Lee SP, Chung HT, Brann MR, Seeman P, George SR. Dopamine D2 receptor dimers and receptor-blocking peptides. *Biochem. Biophys. Res. Commun.* 1996; 277:200–204. [PubMed: 8858125]
9. Ernst OP, Gramse V, Kolbe M, Hofmann KP, Heck M. Monomeric G protein-coupled receptor rhodopsin in solution activates its G protein transducin at the diffusion limit. *Proc. Natl. Acad. Sci. USA.* 2007; 104:10859–10864. [PubMed: 17578920]
10. Whorton MR, Bokoch MP, Rasmussen SG, Huang B, Zare RN, Kobilka B, Sunahara RK. Monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. *Proc. Natl. Acad. Sci. USA.* 2007; 104:7682–7687. [PubMed: 17452637]
11. Bayburt TH, Leitz AJ, Xie G, Oprian DD, Sligar SG. Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins. *J. Biol. Chem.* 2007; 282:14875–14881. [PubMed: 17395586]
12. AbdAlla S, Lothar H, Abdel-tawab AM, Quitterer U. The angiotensin II AT2 receptor is an AT1 receptor antagonist. *J. Biol. Chem.* 2001; 276:39721–39726. [PubMed: 11507095]
13. Miura S, Karnik SS. Ligand-independent signals from the angiotensin II type 2 receptor induce apoptosis. *EMBO J.* 2000; 19:4026–4035. [PubMed: 10921883]
14. Miura S, Karnik SS, Saku K. Constitutively active homo-oligomeric angiotensin II type 2 receptor induces cell signaling independent of receptor conformation and ligand stimulation. *J. Biol. Chem.* 2005; 280:18237–18244. [PubMed: 15746093]
15. Horiuchi M, Hayashida W, Akishita M, Tamura K, Daviet L, Lehtonen JY, Dzau VJ. Stimulation of different subtypes of angiotensin II receptors, AT1 and AT2 receptors, regulates STAT activation by negative crosstalk. *Circ. Res.* 1999; 84:876–882. [PubMed: 10222333]
16. Miura S, Feng YH, Husain A, Karnik SS. Role of aromaticity of agonist switches of angiotensin II in the activation of the AT1 receptor. *J. Biol. Chem.* 1999; 274:7103–7110. [PubMed: 10066768]
17. Miura S, Karnik SS. Constitutive activation of angiotensin II type 1 receptor alters the orientation of transmembrane Helix-2. *J. Biol. Chem.* 2002; 277:24299–24305. [PubMed: 11983705]
18. Stangier J, Su CA, Roth W. Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. *J. Int. Med. Res.* 2000; 28:149–167. [PubMed: 11014323]

19. Auger-Messier M, Arguin G, Chaloux B, Leduc R, Escher E, Guillemette G. Down-regulation of inositol 1,4,5-trisphosphate receptor in cells stably expressing the constitutively active angiotensin II N111G-AT(1) receptor. *Mol. Endocrinol.* 2004; 18:2967–2980. [PubMed: 15331757]
20. AbdAlla S, Lother H, Langer A, el Faramawy Y, Quitterer U. Factor XIIIa transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis. *Cell.* 2004; 119:343–354. [PubMed: 15507206]
21. AbdAlla S, Lother H, el Massiery A, Quitterer U. Increased AT1 receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. *Nat. Med.* 2001; 7:1003–1009. [PubMed: 11533702]
22. Zeng C, Yang Z, Wang Z, Jones J, Wang X, Altea J, Mangrum AJ, Hopfer U, Sibley DR, Eisner GM, Felder RA, Jose PA. Interaction of angiotensin II type1 and D5 dopamine receptors in renal proximal tubule cells. *Hypertension.* 2005; 45:804–810. [PubMed: 15699451]
23. Zeng C, Hopfer U, Asico LD, Eisner GM, Felder RA, Jose PA. Altered AT1 receptor regulation of ETB receptors in renal proximal tubule cells of spontaneously hypertensive rats. *Hypertension.* 2005; 46:926–931. [PubMed: 16144989]
24. Bohlen, Von; und Halbach, O.; Walther, T.; Bader, M.; Albrecht, D. Interaction between Mas and the angiotensin AT1 receptor in the amygdala. *J. Neurophysiol.* 2000; 83:2012–2021. [PubMed: 10758111]
25. Jin XQ, Fukuda N, Su JZ, Lai YM, Suzuki R, Tahira Y, Takagi H, Ikeda Y, Kanmatsuse K, Miyazaki H. Angiotensin II type 2 receptor gene transfer downregulates angiotensin II type 1a receptor in vascular smooth muscle cells. *Hypertension.* 2002; 39:1021–1027. [PubMed: 12019286]
26. Rhee SG, Bae YS. Regulation of phosphoinositide-specific phospholipase C isozymes. *J. Biol. Chem.* 1997; 272:15045–15048. [PubMed: 9182519]
27. Alexander RW, Brock TA, Gimbrone MA Jr, Rittenhouse SE. Angiotensin increases inositol trisphosphate and calcium in vascular smooth muscle. *Hypertension.* 1985; 7:447–451. [PubMed: 2987120]
28. Ushio-Fukai M, Griendling KK, Akers M, Lyons PR, Alexander RW. Temporal dispersion of activation of phospholipase C-beta1 and -gamma isoforms by angiotensin II in vascular smooth muscle cells. Role of alphaq/11, alpha12, and beta gamma G protein subunits. *J. Biol. Chem.* 1998; 273:19772–19777. [PubMed: 9677408]

Fig. 1.

% Inositol phosphate (IP) production in the presence or absence of 0.1 μ M [Sar¹]Ang II, 1 μ M telmisartan and 1 μ M PD123319 in HepG2 cells (A) and SMCs (B). Basal IP production (A, 450 cpm; B, 1150 cpm) without treatment adjusted to 100% IP production. * p < 0.05 vs. no treatment.

Fig. 2.

Expression levels of AT₁ receptor or AT₂ receptor on the HepG2 cell surface with or without 1 μM telmisartan under the indicated concentrations (A) and incubation time (B) by immunoblotting. Proteins were resolved on 10% SDS-containing polyacrylamide gels under reducing conditions, and the receptors were detected by immunoblotting. Expression levels of AT₁ receptor (C) or AT₂ receptor (D) on the HepG2 cell surface with or without 1 μM telmisartan under the indicated concentrations and incubation time by receptor binding assay. After incubation, cell membranes were prepared by the freeze/thaw method. The cell membranes were washed twice with assay buffer and subjected to receptor binding study. * $p < 0.05$ vs. no treatment. # $p < 0.05$ vs. no treatment. NS, not significant; AU, arbitrary unit.

Fig. 3.

Expression levels of homo-dimerized or hetero-dimerized AT_1 receptor and/or AT_2 receptor in SMCs and HepG2 cells. Samples (total cell lysate and membrane fraction) were separated on 7% SDS-containing polyacrylamide gels under nonreducing conditions after immunoprecipitation (IP), and the receptors were detected by immunoblotting (IB). (A) The ratio of the expression levels of homo-dimerized AT_1 receptors (AT_1/AT_1), homo-dimerized AT_2 receptors (AT_2/AT_2) and hetero-dimerized AT_1 and AT_2 receptors (AT_1/AT_2) were quantified by digital image-analysis. * $p < 0.05$ vs. AT_1/AT_2 . (B) Expression levels of homo-dimerized AT_1 receptor and AT_2 receptor with or without 0.1 μM [Sar¹]Ang II, 1 μM telmisartan and 1 μM PD123319 for 24 h in HepG2 cells. NS, not significant; AU, arbitrary unit.

Fig. 4.

Protein expression levels of PLCs [PLC- γ_1 (A), γ_2 (B) and β_3 (C)] were analyzed under stimulation with 0.1 μ M [Sar¹]Ang II (1 min) after pretreatment with 1 μ M telmisartan or 1 μ M PD123319 for 1 h using HEK cells that had been transfected with AT₁ and AT₂ receptors. PLC activities were analyzed by anti-phospho(p)-PLC- β_3 , γ_1 and γ_2 Abs. Proteins were resolved on 7% SDS-containing polyacrylamide gels under reducing conditions, and bands were detected by immunoblotting. * p < 0.05 vs. no treatment. # p < 0.05 vs. [Sar¹]Ang II. NS, not significant; AU, arbitrary unit.