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ORIGINAL ARTICLE

Multivalent ligand–receptor interactions elicit inverse agonist activity of AT_1 receptor blockers against stretch-induced AT_1 receptor activation

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Type 1 angiotensin II (AT_1) receptor has a critical role in the development of load-induced cardiac hypertrophy. Recently, we showed that mechanical stretching of cells activates the AT_1 receptor without the involvement of angiotensin II (AngII) and that this AngII-independent activation is inhibited by the inverse agonistic activity of the AT_1 receptor blocker (ARB), candesartan. Although the inverse agonist activity of ARBs has been studied in terms of their action on constitutively active AT_1 receptors, the structure–function relationship of the inverse agonism they exert against stretch-induced AT_1 receptor activation has not been fully elucidated. Assays evaluating *c-fos* gene expression and phosphorylated extracellular signal-regulated protein kinases (ERKs) have shown that olmesartan has strong inverse agonist activities against the constitutively active AT_1 receptor and the stretch-induced activation of AT_1 receptor, respectively. Ternary drug–receptor interactions, which occur between the hydroxyl group of olmesartan and Tyr^{113} and between the carboxyl group of olmesartan and Lys^{199} and His^{256}, were essential for the potent inverse agonist action olmesartan exerts against stretch-induced ERK activation and the constitutive activity of the AT_1-N111G mutant receptor. Furthermore, the inverse agonist activity olmesartan exerts against stretch-induced ERK activation requires an additional drug–receptor interaction involving the tetrazole group of olmesartan and Gln^{257} of the AT_1 receptor. These results suggest that multivalent interactions between an inverse agonist and the AT_1 receptor are required to stabilize the receptor in an inactive conformation in response to the distinct processes that lead to an AngII-independent activation of the AT_1 receptor.
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INTRODUCTION

The type 1 angiotensin II (AT_1) receptor is a member of the G protein-coupled receptor (GPCR) family and mediates most of the actions that angiotensin II (AngII) exerts on the cardiovascular system.[1] AT_1 receptor blockers (ARBs) are non-peptide compounds that selectively bind to the AT_1 receptor and inhibit AngII-induced receptor activation. At present, several ARBs are clinically available as a highly effective and well-tolerated class of drugs for the management of hypertension. In addition, clinical trials have indicated that ARBs provide cardiovascular protection that extends beyond blood pressure lowering.[2] Treatment with ARBs effectively prevents cardiac hypertrophy and improves cardiovascular outcomes in patients with hypertension.[2,3] Structurally, most ARBs have a common biphenyl-tetrazole ring and unique side chains, which contribute to drug-specific differences in their pharmacokinetic and pharmacodynamic properties.[2,4] These structural and pharmacological differences among ARBs may have an impact on long-term cardiovascular outcomes, although the clinical significance of these differences remains to be determined in large-scale trials.

Recent studies have shown that most GPCRs, including the AT_1 receptor, show spontaneous activity even in the absence of an agonist.[5] The AT_1 receptor is also activated by the mechanical stress of cellular stretching without the involvement of AngII.[6,7] A ligand capable of suppressing the agonist-independent activities of a receptor is defined as an inverse agonist.[5,8] We have previously reported that pressure overload induces cardiac hypertrophy in angiotensinogen-deficient mice as well as in wild-type (WT) mice and that hypertrophy is significantly attenuated by the inverse agonist, candesartan.[6] Therefore, the inverse agonist activities of ARBs have potential therapeutic benefits, at least in the prevention of load-induced cardiac hypertro-

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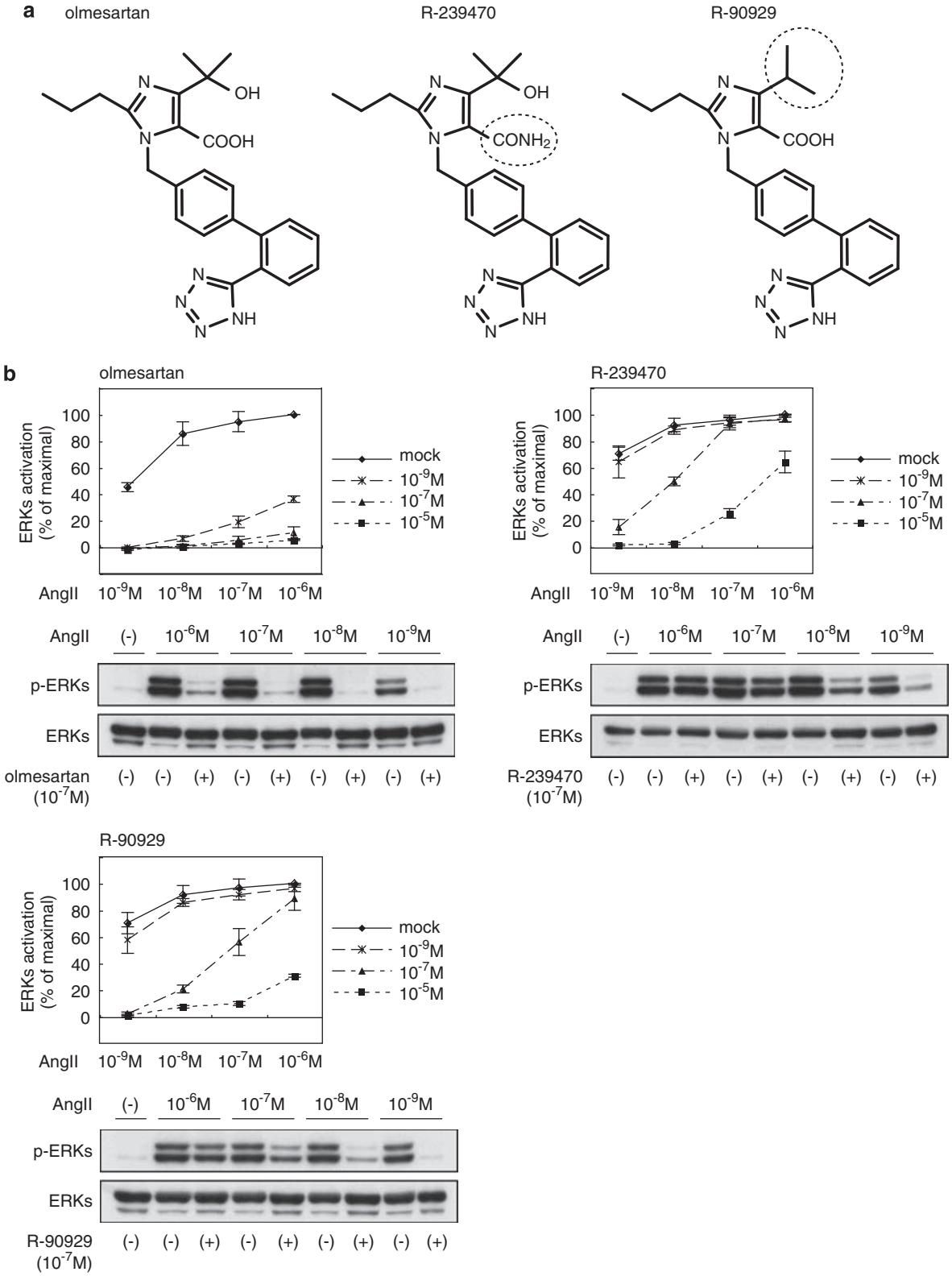
phy. The structural features that are required for the inverse agonist properties of some ARBs have been studied in constitutively active AT_1 receptors that have an Asn^{111} mutation. For example, the ternary interactions between the hydroxyl group of the imidazole ring and Tyr^{113} of the AT_1 receptor and between the carboxyl group and Lys^{199} and His^{256} of the AT_1 receptor were required for the inverse agonist activity that olmesartan exerts on GTPase-stimulating activity in a constitutively active AT_1-N111G mutant containing an Asn^{111} to Gly mutation.[9] However, studies using substituted cysteine accessibility mapping (SCAM) showed that conformation of the AT_1 receptor



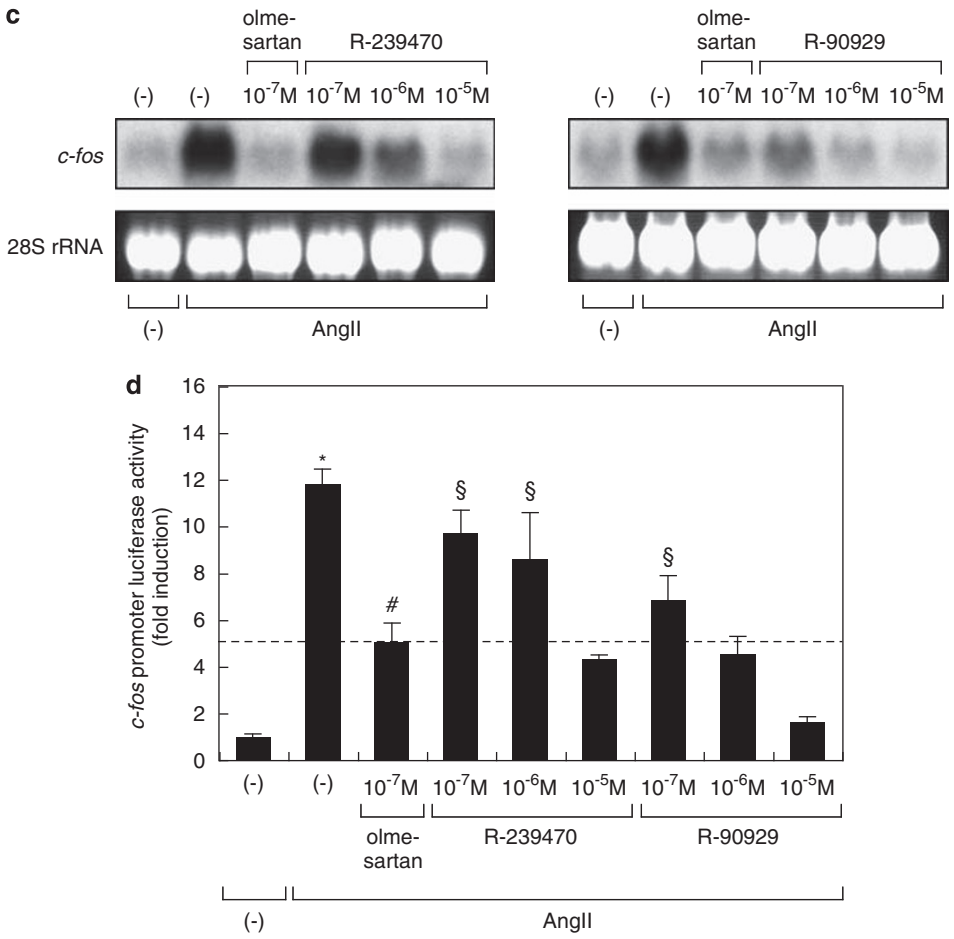


Figure 1 Continued.

during stretch-induced activation is quite different from that of the AT_1-N111G receptor.[7,10] Transmembrane domain 7 (TM7) of the AT_1 receptor undergoes a counterclockwise rotation and a shift toward the ligand-binding pocket in response to mechanical stretch,[7] but it shifts away from the ligand-binding pocket in the AT_1-N111G receptor.[10]

In this study, we show that, as an inverse agonist, olmesartan strongly inhibits the stretch-induced activation of the AT_1 receptor, as well as the constitutive activity of the AT_1-N111G receptor. In addition to the ternary interactions involving the hydroxyl group and the carboxyl group of the imidazole ring of olmesartan, a specific drug–receptor interaction between the tetrazole group of olmesartan and Gln^{257} of the AT_1 receptor is also important for the potent inverse agonist activity olmesartan exerts against stretch-induced AT_1 receptor activation. These results provide new insights into the structure–function relationship of AT_1 receptor inverse agonists.

METHODS

Materials

Olmesartan and its derivatives (R-88145, R-90929 and R-239470) were synthesized at the Research Laboratories of Daiichi Sankyo (Tokyo, Japan). The chemical structures of these compounds are shown in Figures 1a and 6b. AngII was purchased from Sigma-Aldrich (St Louis, MO, USA).

Cell culture and transfection

Cardiomyocytes obtained from ventricles of 1-day-old Wistar rats were plated at a field density of 1×10^5 cells per cm^2 on collagen-coated silicone rubber

Figure 1 The carboxyl group and the hydroxyl group are critical structural characteristics of olmesartan that lead to its insurmountable inhibition of angiotensin II (AT_1) receptors. (**a**) The chemical structures of olmesartan and its derivative compounds, R-239470 and R-90929, are shown. Olmesartan contains a carboxyl group and a hydroxyl group on its benzimidazole ring. R-239470 has a non-acidic carbamoyl group (circled $CONH_2$) instead of the carboxyl group, and R-90929 has no hydroxyl group (circled). (**b**) Response curves of AngII-mediated extracellular signal-regulated protein kinase (ERK) activation (upper panels). HEK293-AT_1 cells were pretreated with 10^{-7} M olmesartan, R-239470 or R-90929, and stimulated by AngII at indicated concentrations (lower panels). The activation of ERKs was determined using a polyclonal antibody against phosphorylated ERKs (p-ERKs). (**c**) The inhibitory effects of olmesartan and its derivative compounds, R-239470 and R-90929, on AngII-induced *c-fos* gene expression in HEK293 cells expressing the AT_1 receptor were examined by northern blot analysis of *c-fos* mRNA. (**d**) The inhibitory effects of olmesartan and its derivative compounds, R-239470 and R-90929, on AngII-induced *c-fos* gene expression in HEK293 cells expressing the AT_1 receptor were examined using a luciferase assay examining *c-fos* promoter activation. $^*P<0.01$ *vs.* that with no stimulation, $^{\#}P<0.01$ *vs.* that with AngII stimulation with no treatment, $^{\S}P<0.05$ *vs.* that with AngII stimulation with olmesartan (10^{-7} M) treatment.

dishes.[6] Cardiomyocytes and HEK293 cells were cultured in DMEM supplemented with 10% fetal bovine serum and nutrient-starved under serum-free conditions for 48 h before AngII or stretch stimulation. The expression vector for AT_1-WT and AT_1-mutant receptors[9] was transfected using FuGENE 6 Transfection Reagent (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions.[7]

Western blot analysis

Total cellular proteins (20 μg) were fractionated by SDS-PAGE and transferred to Hybond membranes (GE Healthcare, Piscataway, NJ, USA). The blotted membranes were incubated with a polyclonal antibody recognizing phospho-extracellular signal-regulated protein kinase 1/2 (ERK1/2) (Cell Signaling, Beverly, MA, USA) or ERK1/2 (Zymed Laboratories, South San Francisco, CA, USA). Horseradish peroxidase-conjugated anti-rabbit IgG (immunoglobulin G) antibody was used as secondary antibody, and signals were detected using the ECL detection kit (GE Healthcare).

RNA extraction and northern blot analysis

Total RNA was isolated from AT_1 receptor-transfected COS7 cells using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, and 20 mg of total RNA was hybridized with a cDNA probe for *c-fos*.

Luciferase assay

The *c-fos* luciferase reporter plasmid, with or without the expression vector for the AT_1-WT or AT_1-N111G receptor, was transfected using FuGENE 6 Transfection Regent (Roche Diagnostics) according to the manufacturer's instructions. pRL-SV40 (Promega, Madison, WI, USA) was co-transfected as an internal control. Luciferase activity was measured 24 h after transfection using the Dual-Luciferase Reporter Assay System (Promega). Experiments were repeated at least in triplicate, and representative data are shown. The *c-fos* luciferase reporter plasmid was a generous gift from Dr M Tsuda (Toyama Medical and Pharmaceutical University, Toyama, Japan).

Statistical analysis

Statistical analyses comparing three or more independent experiments were carried out using one-way ANOVA (analysis of variance) and Dunnett's *t*-test. *P*-values <0.05 were considered statistically significant.

RESULTS

Inhibitory effects of olmesartan and its derivative compounds on AngII-induced activation of the AT_1 receptor

We first determined the inhibitory effects of olmesartan and its derivative compounds, namely R-239470 and R-90929 (Figure 1a), on AngII-induced ERK activation. As previously reported, stimulation with AngII for 8 min induced a significant increase in the phosphorylation level of ERKs in HEK293 cells expressing the AT_1 receptor (Figure 1b).[7] Pretreatment with 10^{-7} M olmesartan strongly inhibited ERK activation induced even by 10^{-6} M AngII. The concentration–response curve of AngII-induced ERK activation in the presence of olmesartan (10^{-6} to 10^{-9} M) showed that olmesartan produced an insurmountable inhibitory effect on the AT_1 receptor, because it decreased the maximal response to AngII (Figure 1b). In contrast, R-239470 and R-90929, which lack the carboxyl or hydroxyl group possessed by olmesartan, respectively, showed surmountable inhibitory effects and led to a rightward shift of the concentration–response curve rather than a decrease in maximal response (Figure 1b).

We have further confirmed that these side-chain structures are crucial for the insurmountable inhibitory effect olmesartan exerts on AngII-induced *c-fos* gene expression. Stimulation with 10^{-6} M AngII significantly increased the expression level of *c-fos* mRNA, which was suppressed significantly by pretreatment with olmesartan but only partially by pretreatment with R-239470 or R-90929 (Figure 1c). Similarly, stimulation with 10^{-6} M AngII for 24 h induced a 12-fold increase in *c-fos* promoter activity, which was suppressed significantly by pretreatment with olmesartan but only partially suppressed by pretreatment with R-239470 or R-90929 (Figure 1d).

Collectively, these results suggest that the carboxyl group and the hydroxyl group on the imidazole ring of olmesartan are required for

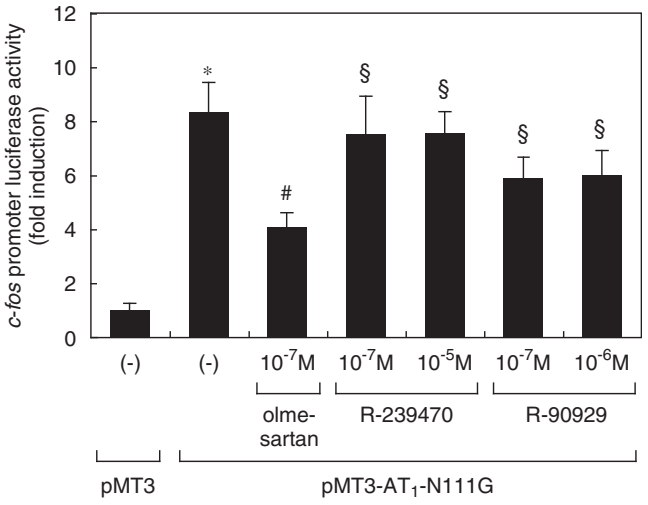


Figure 2 The carboxyl group and the hydroxyl group are critical structures in olmesartan's inverse agonist activity that allow it to suppress basal *c-fos* promoter activity. The basal activities of the AT_1-N111G mutant receptor were evaluated by a luciferase assay examining *c-fos* promoter activity in HEK293 cells expressing AT_1-N111G. Cells were treated with indicated concentrations of olmesartan, R-239470 or R-90929. $^*P<0.01$ *vs.* that of pMT3-transfected cells, $^{\#}P<0.01$ *vs.* that of untreated AT_1-N111G-transfected cells, $^{\S}P<0.05$ *vs.* that of AT_1-N111G-transfected cells treated with olmesartan (10^{-7} M). AT_1, angiotensin II type 1.

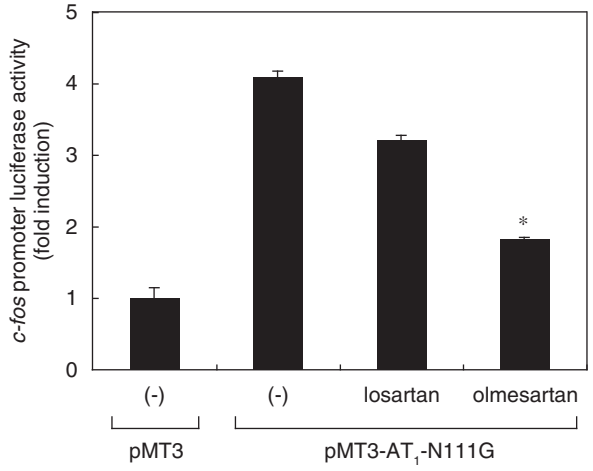


Figure 3 Comparison of the inverse agonist activities of olmesartan and losartan and their ability to suppress basal *c-fos* promoter activity. The basal activities of the AT_1-N111G mutant receptor were evaluated by a luciferase assay examining *c-fos* promoter activity in HEK293 cells expressing AT_1-N111G. The inhibitory effect of 10^{-7} M of olmesartan on basal *c-fos* promoter activity was much stronger than the inhibitory effect exerted by 10^{-7} M losartan. $^*P<0.01$ *vs.* that of losartan. AT_1, angiotensin II type 1.

the insurmountable inhibition of AngII-induced activation of the AT_1 receptor.

Inhibitory effects of olmesartan and its derivative compounds on stretch-induced ERK activation

A recent study showed that olmesartan suppresses the basal production of inositol phosphate (IP) in cells expressing WT AT_1 receptor (AT_1-WT) and a constitutively active mutant AT_1 receptor (AT_1-N111G).[9] We also found that basal *c-fos* promoter activity was suppressed by olmesartan in HEK293 cells expressing AT_1-N111G (Figure 2). The inhibitory effect of olmesartan on basal *c-fos* promoter activity was significantly stronger than that of losartan (Figure 3). Olmesartan is therefore defined as an inverse agonist of the AT_1 receptor because it decreases the basal activity level of the receptor in the absence of the agonist.

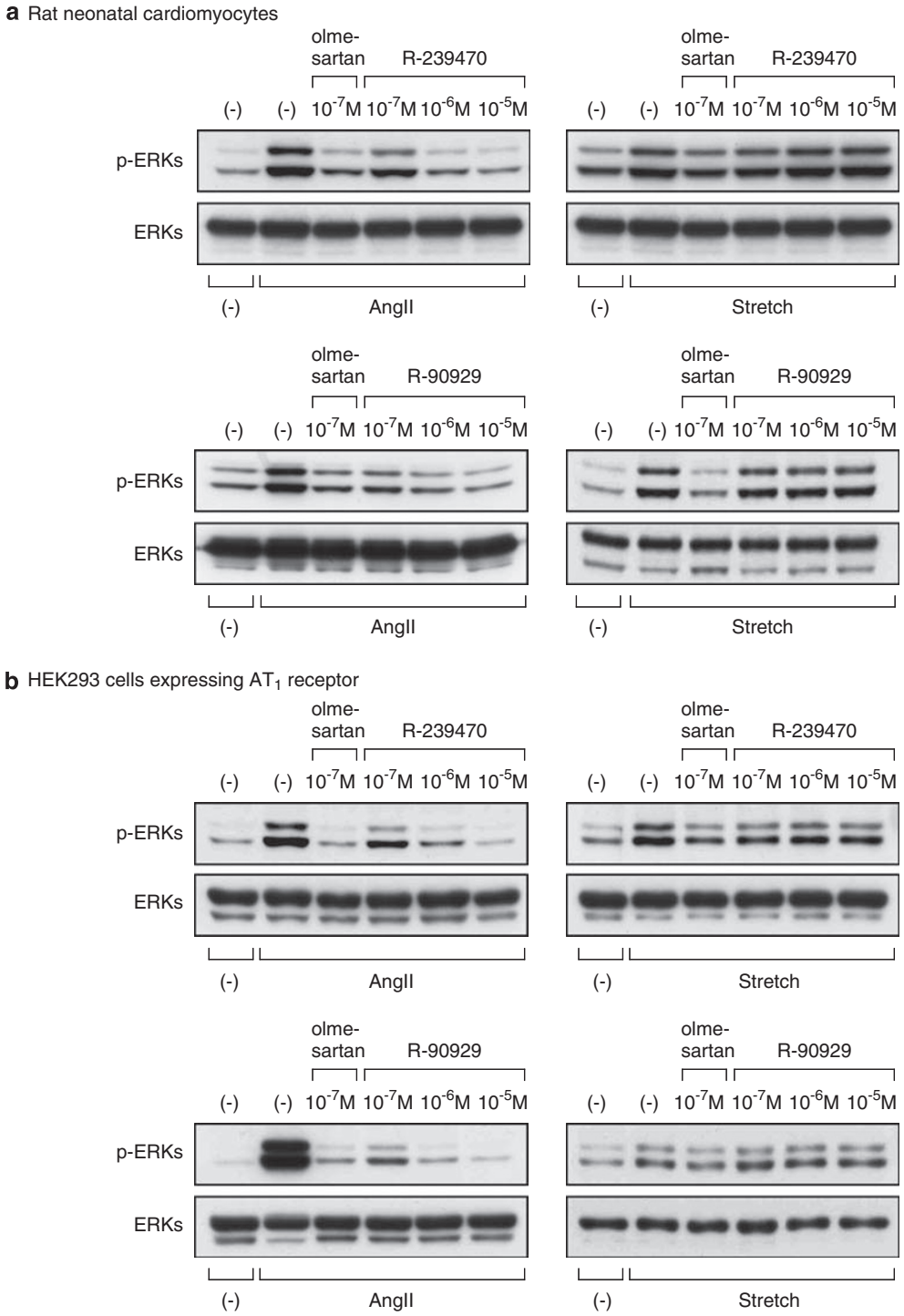


Figure 4 The carboxyl group and the hydroxyl group as critical structures for olmesartan's inverse agonist activity against stretch-induced ERK activation. Rat neonatal cardiomyocytes (**a**) or HEK293-AT_1 cells (**b**) were pretreated with indicated concentrations of olmesartan, R-239470 or R-90929, and stimulated by 10^{-7} M AngII (left) or by mechanical stretch (right). The activation of extracellular signal-regulated protein kinase (ERKs) was then determined. AT_1, angiotensin II type 1.

We recently reported that mechanical stress activates the AT_1 receptor independently of AngII and that this AngII-independent activation of AT_1 receptor is inhibited by the inverse agonist, candesartan.[6] Therefore, we next examined the inhibitory effects of olmesartan on stretch-induced ERK activation in cardiomyocytes cultured from neonatal rats. We found that the stretch-induced phosphorylation of ERKs in cultured cardiomyocytes was largely dependent on the direct activation of AT_1 receptor and that AngII, even if secreted from cardiomyocytes, had only a marginal role in the stretch-induced activation of ERKs.[6] We found that the activation of ERKs in response to mechanical stretch was significantly attenuated by a pretreatment with 10^{-7} M olmesartan (Figure 4a). Furthermore, to exclude the effect of secreted AngII on stretch-induced ERK activation, we imposed stretch stimulation on HEK293 cells that showed no detectable expression of angiotensinogen.[6] Neither stimulation with AngII nor mechanical stretch activated ERKs in HEK293 cells, but mechanical stretching did activate ERKs in these cells when the AT_1 receptor was overexpressed[6] (Figure 4b). Similar to the results in cardiomyocytes, pretreatment with olmesartan significantly inhibited stretch-induced ERK activation in HEK293 cells expressing the AT_1 receptor (HEK293-AT_1 cells) (Figure 4b). Furthermore, the inhibitory effect of olmesartan on stretch-induced ERK activation was significantly stronger than that of losartan (Figure 5). These results suggest that olmesartan, as a potent inverse agonist, strongly suppresses stretch-induced ERK activation, as well as the basal activity of the AT_1 receptor.

We further examined the inhibitory effects of R-239470 and R-90929 on stretch-induced ERK activation, both in cardiomyocytes and in HEK293-AT_1 cells. As shown in Figure 4, 10^{-7} M R-239470 or R-90929 could not inhibit ERK activation induced either by 10^{-7} M AngII or by mechanical stretch, although 10^{-7} M olmesartan inhibited ERK activation. Interestingly, AngII-induced ERK activation was inhibited by 10^{-5} M R-239470 and 10^{-6} M R-90929, but stretch-induced ERK activation was not inhibited by the same concentrations of these compounds (Figure 4). These results suggest that the carboxyl and the hydroxyl groups present in olmesartan are responsible for the potent inverse agonist activity olmesartan exerts against stretch-induced ERK activation. Similar to the results of experiments evaluating stretch-induced ERK activation, 10^{-5} M R-239470 and 10^{-6} M R-90929 failed to suppress basal *c-fos* promoter activity in HEK293 cells expressing AT_1-N111G (Figure 2).

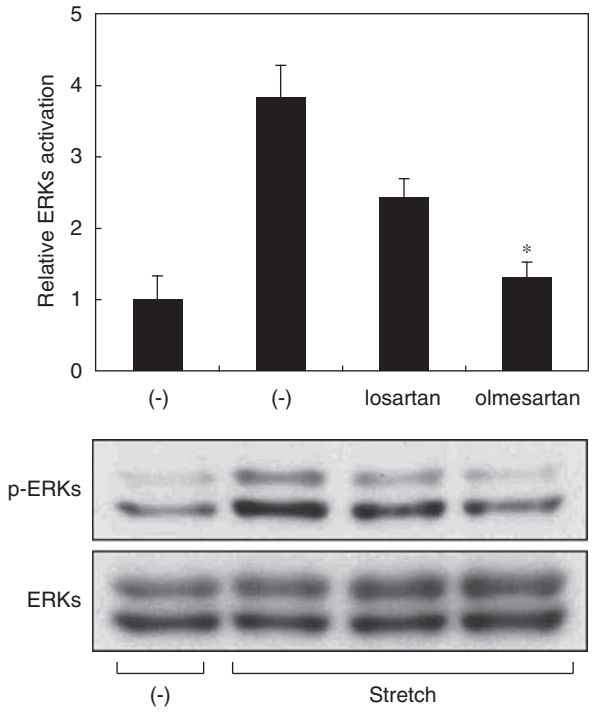


Figure 5 Comparison of the inverse agonist activities of olmesartan and losartan against stretch-induced ERK activation. HEK293-AT_1 cells were stimulated by mechanical stretch, and the activation of extracellular signal-regulated protein kinase (ERKs) was determined. The inhibitory effect of 10^{-7} M olmesartan on stretch-induced ERK activation was much stronger than that of 10^{-7} M losartan. $*P<0.01$ *vs.* that of losartan. AT_1, angiotensin II type 1.

Inhibitory effects of olmesartan on stretch-induced ERK activation in mutated AT_1 receptors

Structure–function analyses have shown that ternary interactions between the hydroxyl group of olmesartan and Tyr^{113} of the AT_1 receptor and between the carboxyl group of olmesartan and Lys^{199} and His^{256} of the AT_1 receptor are essential for the inverse agonist activity that olmesartan exerts on basal IP production in both AT_1-WT and AT_1-N111G receptors.[9] The tetrazole group of olmesartan also interacts with Gln^{257} of the AT_1 receptor, but its binding is not required to reduce the basal activity level of the AT_1 receptor.[9] We first examined the effect of olmesartan on stretch-induced ERK activation in HEK293 cells overexpressing AT_1-WT or an AT_1 mutant receptor harboring one of the following mutations: Y113F, K199Q, H256A or Q257A. As shown in Figure 6a, mechanical stretch-induced phosphorylation of ERKs occurred in AT_1-Y113F, AT_1-K199Q, AT_1-H256A and AT_1-Q257A cells in degrees equivalent to AT_1-WT cells. Interestingly, the inhibitory effects of olmesartan on stretch-induced ERK activation were abolished in cells expressing AT_1-Y113F, AT_1-K199Q, AT_1-H256A or AT_1-Q257A (Figure 6a). These results suggest that the interactions between olmesartan and Gln^{257}, Tyr^{113}, Lys^{199} and His^{256} are required for the potent inverse agonism olmesartan exerts on stretch-induced activation of the AT_1 receptor.

As the tetrazole ring of olmesartan interacts with Gln^{257} of the AT_1 receptor,[9] we next examined the inhibitory effect that R-88145 (in which the tetrazole group was replaced with a carboxyl group, Figure 6b) had on stretch-induced ERK activation in HEK293 cells overexpressing AT_1-WT. Although 10^{-7} M R-88145 did not inhibit ERK activation induced by 10^{-7} M AngII, 10^{-5} M R-88145 could inhibit ERK activation to an extent equivalent to 10^{-7} M olmesartan (Figure 6c). However, stretch-induced ERK activation was not significantly inhibited by 10^{-5} M R-88145 (Figure 6c). These results suggest that the interaction between the tetrazole group of olmesartan and Gln^{257} of the AT_1 receptor is also responsible for the potent inverse agonist activity olmesartan exerts against stretch-induced ERK activation.

DISCUSSION

The ARBs share a common mode of action, namely they block AngII-mediated responses, but the antihypertensive potency of ARBs differs by drug.[2,4] Indeed, the pharmacokinetics of ARBs in human bodies, specifically factors such as bioavailability, half-life duration and route of elimination, differ considerably between different ARBs. These different degrees of efficacy possessed by ARBs are based on differences in their chemical structures, which determine their unique pharmacological properties. Insurmountable antagonism is one of the pharmacological parameters that is relevant to antihypertensive efficacy.[11] Insurmountable antagonism reflects tight binding and a slow dissociation of the drug–receptor complex. ARBs with insurmountable

antagonist properties suppress maximal AngII-induced responses.[11] Recently, it was reported that olmesartan showed a higher degree of insurmountable antagonism than did telmisartan against AngII-induced IP accumulation in CHO-K1 cells expressing the AT_1 receptor.[12] In this study, we showed that olmesartan shows insurmountable antagonist activity against the AT_1 receptor and that the carboxyl and hydroxyl groups on the imidazole ring are required for the insurmountable inhibition of AngII-induced ERK activation and *c-fos* gene expression (Figure 1).

The unique side-chain structure olmesartan possesses (its carboxyl group and hydroxyl group) contributes to its specific receptor-binding activity. These drug–receptor interactions cooperate to stabilize the receptor in an inactive conformation and thereby confer inverse agonism against the basal expression of the *c-fos* gene (Figure 2) and the basal production of IP[9] in cells expressing the AT_1-N111G receptor, as well as insurmountable antagonism. The inverse agonist activities that ARBs exert against the constitutive activity of the AT_1 receptor could be an important pharmacological parameter that may be relevant to their efficacy at blood pressure lowering and in preventing end-organ damage. Although it remains unclear whether the subtle constitutive activity of the native AT_1 receptor has a pathophysiological role, the enhancement of its constitutive activity

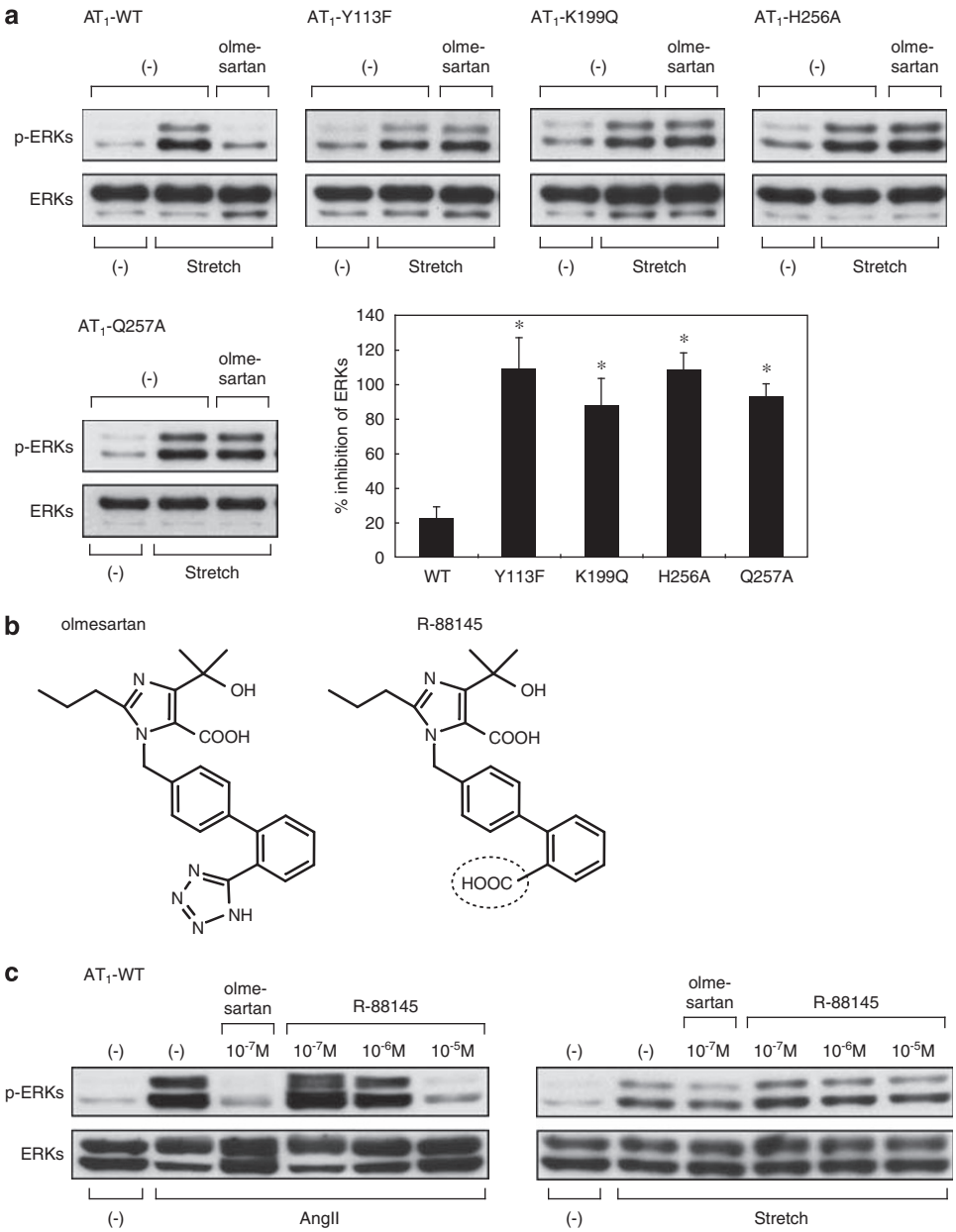


Figure 6 Specific drug–receptor interactions are required for olmesartan's inverse agonist activity against stretch-induced extracellular signal-regulated protein kinase (ERK) activation. (**a**) HEK293 cells expressing AT_1-WT, -Y113F, -K199Q, -H256A or -Q257A mutant receptors were pretreated with 10^{-7} M olmesartan and stimulated by mechanical stretch. The activation of ERKs was then determined. $^*P<0.01$ *vs.* that of wild-type AT_1-WT. (**b**) The chemical structures of olmesartan and R-88145, which has a carboxyl group (circled COOH) instead of a tetrazole group. (**c**) HEK293-AT_1 cells were pretreated with indicated concentrations of olmesartan or R-88145 and were stimulated by 10^{-7} M AngII (left) or mechanical stretch (right). The activation of ERKs was then determined. AT_1, angiotensin II type 1.

through upregulation of receptor expression may promote cardiovascular remodeling. Indeed, the expression level of the AT_1 receptor in vascular cells is upregulated by low-density lipoprotein cholesterol,[13] insulin,[14] glucose,[15] progesterone[16] and inflammatory cytokines, such as interleukin-1α or interleukin-6.[17,18] Analyses of the binding affinity of olmesartan for mutant AT_1 receptors as well as molecular modeling analyses indicated that the ternary interactions between the hydroxyl group and Tyr^{113} and between the carboxyl group and Lys^{199} and His^{256} are critical to the inverse agonist properties of olmesartan, but that the interaction between the tetrazole group and Gln^{257} is dispensable.[9] Interestingly, differential interactions between valsartan and Ser^{105}, and between Ser^{109} and Lys^{199}, are crucial for producing inverse agonism.[19] It has therefore been proposed that ARBs may bind to the AT_1 receptor primarily by docking at Lys^{199} and subsequently through a distinct combination of drug–receptor interactions in a drug-specific manner.[19] According to this model, the spatial pattern of drug–receptor contact points will determine the potency of the inverse agonist activity of a given ARB.

We recently showed that mechanical stretching of cells induces a counterclockwise rotation and a shift of TM7 of the AT_1 receptor toward the ligand-binding pocket.[7] However, TM7 shifts away from the ligand-binding pocket in the AT_1-N111G receptor,[10] implying that the conformation of AT_1 receptor during stretch-induced activation is different from that of the constitutively active AT_1 receptor. In general, GPCRs are structurally flexible and unstable, and multiple conformational states exist during the GPCR activation process.[20–22] In this study, we showed that, aside from the ternary drug–receptor interactions involving the hydroxyl and carboxyl groups of olmesartan, an additional interaction between the tetrazole group of olmesartan and Gln^{257} of the AT_1 receptor is required for its potent inverse agonism against stretch-induced AT_1 receptor activation (Figures 4 and 6). Each of the quaternary interactions involving the hydroxyl group, carboxyl group and tetrazole group contributes to a tight drug–receptor binding,[9] but is not sufficient enough to produce a potent inverse agonism against stretch-induced AT_1 receptor activation. Thus, the quaternary drug–receptor interactions work together to stabilize the receptor in an inactive conformation, even under conditions in which mechanical stretching occurs.

With regard to candesartan, the carboxyl group on the benzimidazole ring is responsible for its inverse agonism and leads to the suppression of both the constitutive activity and the mechanical stress-induced activation of the AT_1 receptor.[7] The SCAM studies showed that the binding of the carboxyl group of candesartan to Gln^{257} of TM6 and Thr^{287} of TM7 forcibly induces a clockwise rotation of TM6 and TM7, and leads to the stabilization of the AT_1 receptor in an inactive conformation.[7] At present, it remains unclear how the helical movement of TM7 induced by mechanical stretch is affected by the presence of olmesartan. According to molecular modeling, Thr^{287} of TM7 is located in a position that would allow it to form a hydrogen bond with His^{256} of TM6.[9] We assume that the helical movements of TM6 and TM7 are coupled and that TM7 may be restricted in motion when TM6 is rigidly bound to olmesartan through the dual interactions between the carboxyl group and His^{256} and between the tetrazole group of olmesartan and Gln^{257}.

Our study shows that olmesartan strongly inhibits both AngII-dependent and AngII-independent activation of the AT_1 receptor. Ternary drug–receptor interactions between the hydroxyl group and Tyr^{113} and between the carboxyl group and Lys^{199} and His^{256} are crucial for olmesartan's inverse agonist activity against the constitutive activity of an AT_1 mutant receptor, AT_1-N111G. In addition, a drug–receptor interaction between the tetrazole group of olmesartan and Gln^{257} of the AT_1 receptor is required for potent inverse agonism against stretch-induced AT_1 receptor activation. These results suggest that multivalent drug–receptor interactions cooperate in combination to stabilize the receptor in an inactive conformation according to the distinct processes of receptor activation. The inverse agonist activity of ARBs has therapeutic benefits in the prevention of load-induced cardiac hypertrophy,[5] and thus has the potential to affect long-term outcomes in patients with hypertension. Elucidation of the molecular basis for the inverse agonist activity of ARBs in relation to their chemical structure will help to categorize ARBs according to their individual efficacies in receptor inactivation and will also help researchers to develop novel ARBs with superb efficacy in terms of blood pressure lowering and end-organ protection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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