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Triple twist theory of Rho inhibition by the AT_2 receptor

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Although there are some exceptions[1,2], numerous publications support the counter-regulatory roles of the angiotensin II (Ang II) type-2 receptor (AT_2) against the AT_1 receptor functions, such as inhibition of vascular contraction and hypertrophy[3–5]. However, it is still very uncertain as to how the AT_2 receptor signals interfere with those of the AT_1 receptor in the cardiovascular system[5,6]. Past findings suggest that the signal transduction of AT_1 inhibition by the AT_2 receptor may involve multiple distinct mechanisms. Some of these mechanisms appear to be indirect, such as production of nitric oxide through bradykinin opposing the vasoconstrictor actions of the AT_1 receptor[3]. The direct inhibitory cross-talk of the two receptors occurs proximal to the receptor hetero-dimerization, as well as downstream from the receptors between AT_1-activated protein kinases, epidermal growth factor (EGF) receptor kinase and extracellular signal-regulated kinase (ERK1/2)/p42/44 mitogen activated protein kinase (MAPK), etc, and AT_2-activated protein phosphatases, protein phosphatese 2A (PP2A), SHP-1, and MAPK phosphatase-1 (MKP-1)[7,8]. The activation of the protein phosphatases by the AT_2 receptor may or may not require hetero-trimeric G proteins (G_i or G_s) and/or the recently identified AT_2 receptor C-terminal tail interacting proteins[4–6].

Given that induction of hypertrophy of vascular smooth muscle cells (VSMCs) via the AT_1 receptor appears to require a “triple-membrane-passing signal” involving a metalloprotease-dependent EGF receptor transactivation[9,10], the article by Guilluy et al in this issue of *Circ Res*[11] may not be so surprising, as it suggests the requirement of rather “twisty” three sequential phosphorylation/dephosphorylation events between a phosphatase, SHP-1, and two protein kinases for RhoA inhibition by the AT_2 receptor (see Figure 7 in the article). Although negative regulation of RhoA through its Ser188 phosphorylation by the AT_2 receptor has been demonstrated[12,13], the two kinases—caseine kinase II (CK2) and Ste20-related kinase SLK—are novel downstream elements of the AT_2 receptor.

By using multiple distinct molecular approaches, a novel signal transduction cascade for inhibition of RhoA via the AT_2 receptor, which is expected to counter-regulate RhoA activation by the AT_1 receptor in VSMCs, becomes apparent[11] (Figure). Rho-kinase (ROCK), the best-characterized effector of the small G protein RhoA, contributes to vascular contraction via Ca^{2+} sensitization. Moreover, the Rho/ROCK pathway has been implicated in a wide variety of cardiovascular pathogenic conditions including hypertension, atherosclerosis and cardiovascular hypertrophy[14–17]. It should be noted that both heterotrimeric G protein-

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Disclosures
None.

dependent and -independent signal transductions have been proposed to mediate AT_1 receptor function[4,18,19]. In addition to the production of reactive oxygen species[4] and enhanced VSMC contraction, hypertrophy, as well as migration induced by the AT_1 receptor, seem to require at least two parallel signal transduction cascades mediated through G_q and $G_{12/13}$. The latter is primarily implicated in the Rho/ROCK cascade activation via RGS (regulator of G protein signaling)-domain containing Rho guanine nucleotide exchange factors (RhoGEFs)[20–23]. Inhibition of either cascade appears to block those pathogenic functions induced by the AT_1 receptor[20–23], and the study by Guilluy et al has further demonstrated that the RhoA inhibition mechanism via the AT_2 receptor in VSMCs results in vasodilation[11]. The findings also indicate a strong support of this potential "triple twist" RhoA inhibition theory to explain the multiple tissue protective effects of AT_1 receptor blockers beyond the expected AT_1 inhibition, since the AT_2 receptors could be strongly stimulated under these treatments.

In addition, identification of the novel key components of the AT_2 signal transduction will aid in exploring the molecular insight regarding the dynamic regulation of cardiovascular remodeling via the AT_1 versus AT_2, which likely involves far more additional cross-talk. Both cyclic AMP and cyclic GMP dependent kinases have been shown to phosphorylate RhoA at Ser188[24], which in part explains the vasodilatory properties of these kinases in VSMCs. The study by Guilluy et al has identified SLK as a novel RhoA Ser188 kinase[11]. Interestingly, SLK has been shown to be able to activate apoptosis signal-regulated kinase-1 (ASK1) and p38 MAPK, leading to cell apoptosis[25]. This fits well with the past findings that AT_2 mediates apoptosis in VSMCs via p38 MAPK activation[26]. Regarding CK2, activation of CK2 has been recently shown to mediate p27 degradation by Ang II likely through the AT_1 receptor, which leads to cardiac hypertrophy[27]. The p27 degradation has also been implicated in vascular hyperplasia[28]. Therefore, inhibition of CK2 activity by the AT_2 receptor may also antagonize the AT_1 mediated detrimental effects through stabilization of p27 in addition to the RhoA inhibition.

Since the findings by Guilluy et al were mostly limited to VSMC culture and a ex vivo desendothelialized contraction assay using rat thoracic aorta rings, the relevance of this novel AT_2 signal transduction in mediating the beneficial AT_2 function in cardiovascular diseases remains unclear. Further expansion of the research in this field by using animal models of cardiovascular diseases has strong potential for future translation of the outcomes, which may lead to prevention of cardiovascular diseases linked to enhanced AT_1 receptor signal transduction.

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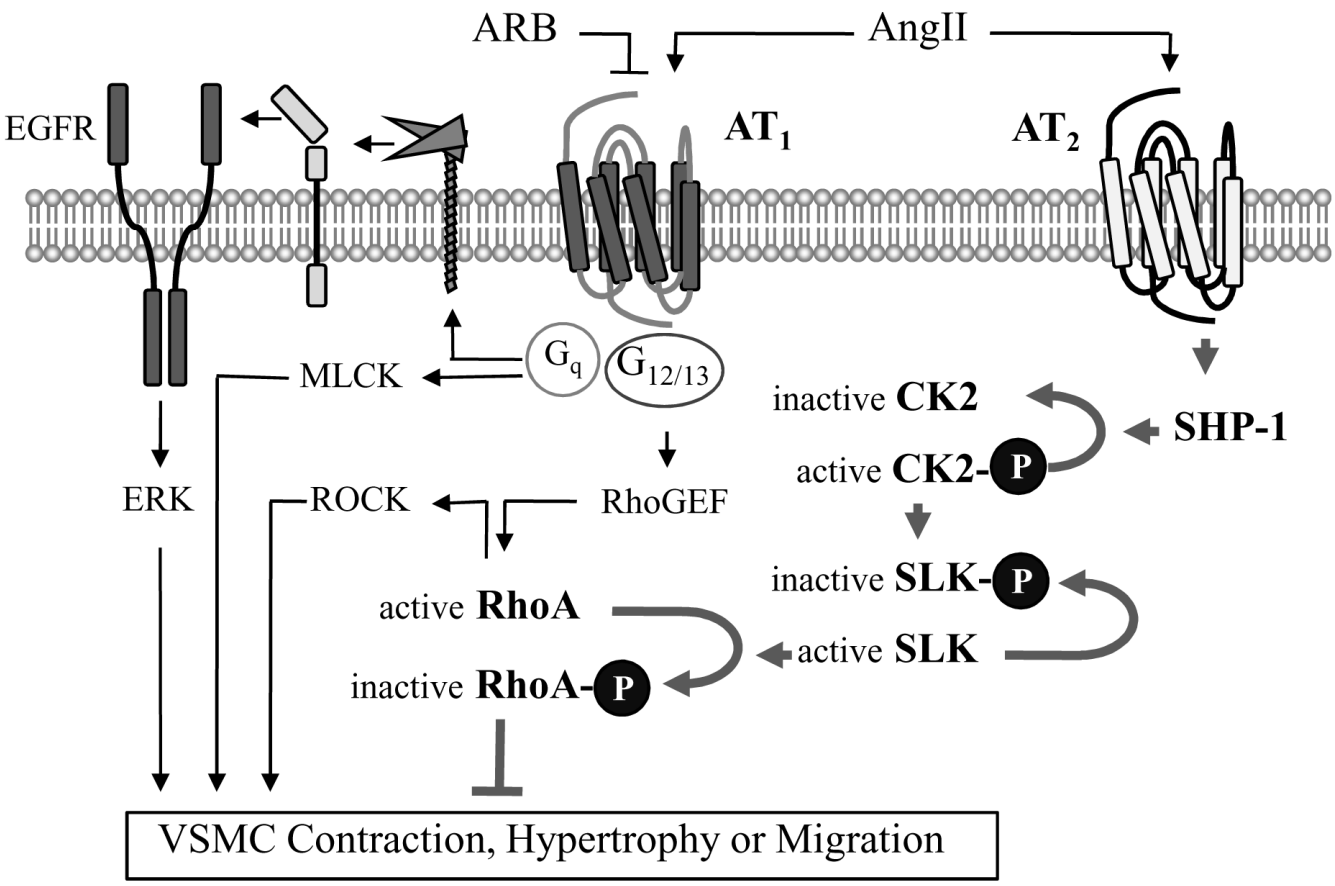

Figure.

Novel signal transduction cross-talk between AT_1 and AT_2 in VSMCs. The Rho/Rhokinase cascade inhibition by the AT_2 receptor via the "triple-twist" theory involving SHP-1, CK2 and SLK not only inhibits AT_1-induced vascular contraction, but also likely inhibits VSMC hypertrophy and migration. In addition, CK2 may be activated through the AT_1 receptor, and AT_2-activated SHP-1 has been shown to directly inactivate EGFR to inhibit AT_1-activated hypertrophic signal transduction.