jraas

Journal of the Renin-Angiotensin-Aldosterone System
12(4) 634–636

DOI: 10.1177/1470320311407232
jra.sagepub.com
SAGE

Does p63RhoGEF, a new key mediator of angiotensin II signalling, play a role in blood pressure regulation and cardiovascular remodelling in humans?

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Angiotensin II (Ang II) is a major regulator of a broad spectrum of important biological processes ranging from vasoconstriction to inflammatory processes including atherosclerosis and vascular ageing, which proceeds, in part, via phosphoinositide-specific phospholipase C (PLC)-generated second messengers.[1-3] Ang II type 1 receptors couple first to PLCβ1 via heterotrimeric Gαq/11βγ and Gαq/12βγ and then to PLCγ via tyrosine kinase activity.[4] Ang II also induces phosphorylation of growth-signalling kinases by redox-sensitive regulation of protein tyrosine phosphatases (PTPs)[5] via oxidation/inactivation and blunted phosphorylation of the PTP, SHP-2. In addition, the activation of the monomeric G protein RhoA and its effector Rho kinase, the RhoA/Rho kinase pathway, downstream of Ang II type 1 receptor (AT1R) stimulation, leads to both vasoconstriction and cardiovascular remodelling.[6,7]

Rho GTPases are monomeric G proteins involved in the control of different vascular cell processes including contraction, proliferation and migration. They are activated by guanine nucleotide exchange factors (RhoGEFs) that catalyse the exchange of GDP to GTP,[8] which leads to conformational change of the G protein allowing interaction with and regulation of downstream effectors. In addition, they can act as sensor for signals coming from activated G protein-coupled receptors, including those coming from Ang II AT1R stimulation, which are coupled to the heterotrimeric Gq protein.[9]

Evidence has been recently provided for the first time, via in vitro loss of function and functional responses experiments, that p63RhoGEF, one of the RhoGEFs, plays a major role in Ang II-mediated early RhoA activation[10] via binding of the α subunit of Gq protein and transducing the signal to activate RhoA[11] and its effector Rho kinase. This, in addition to the slower and later-occurring Ang II-mediated RhoA activation via p115RhoGEF,[12] leads to the subsequently triggered cellular processes such as vascular contraction, proliferation and cardiovascular remodelling, the effects of Ang II-mediated activation of RhoA/Rho kinase on the cardiovascular system,[7] as shown by the impaired Ang II-dependent contraction and proliferation of rat aortic smooth muscle cells (RASMCs) upon p63RhoGEF depletion.[10]

The demonstration of this pathway has not only filled the gap of the lack of a specific mediator able to transduce the Ang II message from activated AT1R via Gq protein to RhoA/Rho kinase activation, but also significantly expands the number and complexity of the signalling pathways through which Ang II signals, including those leading to blood pressure regulation and cardiovascular remodelling via RhoA/Rho kinase activation, highly relevant in human pathophysiology.

However, while the participation of p63RhoGEF in Ang II-induced RhoA activation in humans could be hypothesised based on the results obtained in vitro,[10] no direct supporting evidence is currently available in humans. Such a demonstration should be given, and the human model of Bartter's/Gitelman's (BS/GS) syndromes offers a good opportunity for such an investigation.

Data from patients with BS/GS syndromes, a human model characterised by normotension or hypotension and activation of anti-atherosclerotic and anti-remodelling defences might, in fact, provide indirect support from data in humans for the reported evidence of Ang II-induced, p63RhoGEF-mediated direct RhoA/Rho kinase activation[10] and consequent effects on vascular contraction and cardiovascular remodelling.[7]

BS/GS syndromes, rare diseases caused by gene defects in specific kidney transporters and ion channels, have increased plasma levels of Ang II and aldosterone, activation of the renin–angiotensin–aldosterone system, yet normotension or hypotension, reduced peripheral resistance, and hyporesponsiveness to pressor agents.[13] Patients with BS/GS syndromes likely represent a human model of endogenous Ang II type 1 receptor antagonism. BS/GS

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syndromes, in fact, have been shown to have a blunted Ang II signalling via AT1R as shown by the reduced Gαq gene and protein expression upon Ang II stimulation,[14] in addition to the upregulation of the regulator of G protein signalling (RGS)-2,[15] which regulates Ang II AT1R function via downregulation of Gαq signaling,[16] including the negative regulation of Gαq-induced p63RhoGEF direct activation,[17] and downregulation of the RhoA/Rho kinase pathway.[7] Moreover, altering RGS-2 via its silencing[18] produces in these patients, on Ang II signalling, effects that mirror those seen in hypertensive patients.[19] In BS/GS syndromes it has also been recently demonstrated that Ang II signalling via AT2R is activated, as shown by the increased Ang II-induced expression of the mitogen-activated protein kinase phosphatase 1.[20] The activation of Ang II signalling via AT2R in these patients mainly contributes to the blunted Ang II signalling via AT1R and related pathways, including downregulation of Ang II-induced RhoA/Rho kinase pathway,[7,21,22] reduced oxidative stress alongside increased heme oxygenase expression,[23,24] upregulation of the nitric oxide (NO) system and increased NO-dependent vasodilation,[14,25] lack of endothelial dysfunction and cardiovascular remodelling,[25,26] which produce a mirror image of hypertension. This, in fact, fits with widely recognised counter-regulatory roles of the Ang II type 2 receptor (AT2R) signalling against the AT1R functions, such as inhibition of vascular contraction and hypertrophy.[2,27] These roles may involve multiple distinct mechanisms including the activation of the protein phosphatases by the AT2R receptors,[28] production of NO[29] and the negative regulation of the RhoA/Rho kinase pathway.[30]

Of particular relevance to the report of p63RhoGEF role in Ang II-mediated activation of the RhoA/Rho kinase pathway is, in these patients, the downregulation of the RhoA/Rho kinase pathway, which has been documented by the reduced expression of the Ang II-induced p115RhoGEF and Rho kinase.[7,21,22] In fact, although the role of p63RhoGEF has not been explored in these patients, a blunted response to Ang II stimulation on RhoA via p63RhoGEF might be expected in BS/GS syndrome patients, which indirectly supports a role of p63RhoGEF in the fast Ang II-mediated RhoA activation shown on the basis of in vitro data.[10] The following evidence documented in patients with BS/GS syndromes, all closely related with p63RhoGEF, in fact, points toward reduced p63RhoGEF-mediated effects: 1) the blunted response of the Ang II signalling via AT1R, such as the reduced Ang II-stimulated increase of cytosolic calcium,[14,25] a known early response upon Ang II stimulation; 2) the reduced Gαq gene and protein expression upon Ang II stimulation,[7,14] whose bond with p63RhoGEF is essential for transducing the Ang II signal to activate RhoA;[11] 3) the increased RGS-2 expression,[15] whose upregulation negatively regulates Gαq-induced p63RhoGEF activation;[17] and 4) the downregulation of the RhoA/Rho kinase pathway.[7,21,22] In addition, given the demonstration of Ang II direct activation of RhoA/Rho kinase via AT1R stimulation/p63RhoGEF/Gαq actions, the combination of the indirect evidence from BS/GS patients with data provided in vitro, including the impaired Ang II-dependent contraction of RASMCs upon p63RhoGEF depletion,[10] might offer additional support to the proposed role of Ang II AT2R signalling in the cardiovascular protective effects of Ang II type 1 receptor blockers beyond Ang II type 1 receptor blockade[31] also via inhibition of Ang II-mediated, p63RhoGEF/Gαq-induced RhoA/Rho kinase activation.

The analysis of the relevance of p63RhoGEF-mediated signalling directly performed in vivo in humans remains, however, a high priority in order to clarify the Ang II-mediated p63RhoGEF signalling in processes involved in the regulation of blood pressure and long-term complications of high blood pressure such as cardiovascular remodelling. It may likely give the chance for new potential targets of therapy for diseases such as hypertension, diabetes and cardiovascular disease, in which Ang II plays a major role. The model of BS/GS syndrome patients may offer a useful tool for such an investigation.

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