Letter to the Editor

Rho Kinase Inhibition and Vascular Protection: Support From Studies in Bartter and Gitelman Syndrome

To the Editor:

In a recent article, Wolfrum et al.1 have shown that in human cells in culture, inhibition of Rho kinase (ROK) activates Akt pathway, which they contend leads to cardiovascular protection via activation of eNOS. ROK (a downstream effector of RhoA G protein) involvement has been advanced in the pathogenesis of hypertension and atherosclerosis.2 This is based on its modulation of regulatory chain phosphorylation of myosin II which contributes to smooth muscle Ca2+ sensitization,3 increased expression of NAD(P)H oxidase,4 and induction of oxidative stress.

We would like to suggest that recent results from our ongoing studies in patients with Bartter and Gitelman syndrome (BS/GS)5 provide additional support for Wolfrum and colleagues’ conclusions as well as additional evidence for the importance of ROK in cardiovascular protection. Of direct relevance to the report of Wolfrum and coworkers6 is our recent demonstration in BS/GS patients that RhoA/Rho Kinase pathway is blunted6 and that the expression of p22phox, a subunit of the multienzymatic complex NADPH oxidase, is reduced.7 BS/GS, caused by gene defects in specific kidney transporters and ion channels, presents a puzzling clinical picture characterized by hypokalemia, sodium depletion, activation of the ren-in-angiotensin-aldosterone system (RAAS), with increased plasma levels of Ang II and aldosterone, yet normohypotension, reduced peripheral resistance, and hyporesponsiveness to pressor agents.7 Therefore, understanding why patients with BS/GS do not develop hypertension, in spite of high Ang II and activation of RAAS, sheds considerable light on the cellular basis of hypertension. In BS/GS specifically, the short-term Ang II signaling pathway is blunted as documented by the increased regulator of G-protein signaling-2 (RGS-2) in BS/GS patients.5 The reduced RhoA/ROK expression in BS/GS patients and their collection of biochemical characteristics present a mirror image of those found in hypertension. The reduced RKO and p22phox expression noted in recent studies6,7 occurred in the context of the increased level of the endothelial subunit of NO synthase (eNOS) mRNA14 alongside elevated urinary NO metabolites and cGMP levels.7 This, we suggest, indicates that the RKO activity of BS/GS patients is reduced with induction of Akt pathway in response to RKO downregulation.1 Our findings of reduced RKO expression in the face of increased ecNOS expression and increased NO level present in BS/GS8,9,14 exactly parallel the upregulation of NO system on ROK inhibition as shown by Wolfrum et al.1 Moreover, the induction of the Akt pathway reported by Wolfrum after ROK inhibition is also mirrored in BS/GS, as these patients have an increased expression of alpha subunit of Gq protein in monocytes of Bartter and Gitelman syndromes: relevance for vascular hyporeactivity.1,3

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In Response:

Calò and coworkers suggest that signal pathways in patients experiencing Bartter and Gitelman syndrome may be similar to those activated by the acute inhibition of Rho-kinase as observed in our study.1 Calò and colleagues have shown that the expres-
sion of Rho-kinase mRNA is reduced in mononuclear cells of these patients. This is associated with a decreased expression of p22^phox^, a subunit of the NADPH oxidase, but an increased expression of eNOS and heme-oxygenase-1.\(^2^\)\(^-^\)\(^4^\) Therefore, decreased activity of Rho-kinase in these patients may lead to the chronic activation of Akt and eNOS.

However, in our recent work, we investigated the short-term effects of Rho-kinase inhibition on Akt activity. The effects were observed within minutes after Rho-kinase inhibition. This time course supports a posttranslational modification, eg, phosphorylation of eNOS, by Akt, which has also been seen by Ming and coworkers.\(^5^\) This, however, does not exclude chronic effects of Rho-kinase inhibition on eNOS expression. Indeed, we have previously reported that eNOS mRNA is negatively regulated by Rho GTPase and Rho-kinase. We showed that activation of Rho destabilizes eNOS mRNA stability and that inhibition of Rho-kinase by hydroxyfasudil blocks hypoxia-induced downregulation of eNOS in endothelial cells.\(^6^\)\(^-^\)\(^7^\) This upregulation of eNOS expression was not mediated by activation of Akt, as demonstrated by Ming et al.\(^5^\) Therefore, we believe that stabilization of eNOS mRNA by long-term Rho-kinase inhibition may explain the changes in eNOS expression observed by Calò and coworkers in patients with Bartter and Gitelman syndrome.

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