Editorial

The RhoA/Rho kinase pathway in the myocardium

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The small GTPase RhoA and its downstream target Rho kinase (Rho-associated coiled-coil protein kinase or ROCK) are involved in cell contraction and a variety of other cellular processes via modulation of actin cytoskeletal assembly. Several studies, including investigations in humans, have clearly shown an important pathophysiological role of this pathway in the cardiovascular system and in disease states such as hypertension, heart failure, stroke, and diabetes [1]. The RhoA/ROCK pathway is best described in smooth muscle cells, where it mediates calcium sensitization and thereby enhances and sustains contraction. However, the role of the RhoA/ROCK pathway in the myocardium is less well understood. Most studies on RhoA/ROCK in the heart address its involvement in cardiac hypertrophy and remodelling, and chronic inhibition of the pathway may prevent these long-term effects. In the current issue of *Cardiovascular Research* Lin et al. [2] describe a possible role of the RhoA/ROCK pathway in the contractile function of diabetic rat heart. These authors report that the decreased contractile function of hearts of diabetic animals was associated with an increased expression and activity of RhoA and ROCK. Interestingly, acute inhibition of ROCK improved cardiac performance in vivo and in vitro. These findings show an involvement of the RhoA/ROCK pathway in diabetic cardiomyopathy and that this system is also involved in myocardial contraction.

1. The RhoA/ROCK pathway

The small GTPase RhoA can cycle between an active state where it is GTP bound and an inactive state where it is GDP bound (for review see [3]). Several proteins regulate the activity of RhoA: guanine nucleotide exchange factors (GEFs) can activate RhoA by stimulating the exchange of GDP for GTP; GTPase-activating proteins (GAPs) inactivate RhoA by accelerating the hydrolysis of GTP to GDP. Moreover, guanine dissociation inhibitors (GDIs) prevent the activation of RhoA via several mechanisms. Several receptor systems (e.g. angiotensin II, α-adrenoceptors, sphingosine-1-phosphate receptors) have been shown to activate GEFs and thereby activate RhoA. Many downstream targets for RhoA have been described, and ROCK is most likely the most prominent and best studied target [3]. The two isoforms of ROCK (ROCK-1 and ROCK-2) are serine/threonine kinases that can be inhibited by fasudil, Y-27632, or H-1152, all of which target the ATP-dependent kinase domain of ROCK-1 and ROCK-2. ROCK can phosphorylate a variety of downstream targets, as will be discussed briefly for cardiovascular cell types.

2. RhoA/ROCK pathway in vascular cells

The activation of the RhoA/ROCK pathway by vasoactive substances such as angiotensin II plays an essential role in maintaining vascular tone. One of the main downstream targets of ROCK in the vascular smooth muscle cell is myosin light chain phosphatase (MLCP). This enzyme is involved in the dephosphorylation of myosin light chain, resulting in a decreased contraction. Since phosphorylation of MLCP by ROCK decreases its activity, activation of the RhoA/ROCK pathway will increase smooth muscle cell contraction at steady calcium concentrations [3]. Moreover, myosin light chain itself is also a target of ROCK. In most vessel types nitric oxide (NO), produced after activation of endothelial NO synthase, acts as an important vasodilator and is therefore a crucial factor in maintaining vascular tone. It has been shown recently that ROCK activity is inversely correlated with endothelial NO

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synthase expression and activity [1]. Thus, in the vasculature increased ROCK activity is associated with an increased vascular contraction.

3. RhoA/ROCK pathway in the myocardium

Although cardiac myocytes express RhoA, ROCK-1, and ROCK-2, their role in the myocardium is less well understood than in the vasculature. Earlier experiments have shown that RhoA/ROCK is involved in myofibril formation and organisation. Angiotensin II, α-adrenoceptor stimulation, and also mechanical stress can activate the cardiac RhoA/ROCK pathway. All of these are involved in cardiac hypertrophy and, accordingly, chronic administration of ROCK inhibitors has been shown to prevent cardiac hypertrophy in a variety of animal models [4–6]. While early studies were based on the use of ROCK inhibitors that do not distinguish between the two kinase isoforms, experiments with ROCK-1$^{-/-}$ and ROCK-1$^{+/-}$ mice revealed that ROCK-1 deficiency does not prevent cardiac hypertrophy but inhibits fibrosis of the heart in different animal models of cardiac hypertrophy and remodelling, most likely by a reduced expression of profibrotic cytokines [7,8]. This may indicate a differential role for ROCK-1 and -2 in cardiac hypertrophy and fibrosis.

Moreover, it has been shown that inhibition of ROCK protects the heart against ischemia/reperfusion injury [6,9,10]. Treatment with Y-27632 or fasudil reduced infarct size and enhanced cardiac function after ischemia/reperfusion. The infarct size-limiting effect may be achieved via inhibition of myocyte apoptosis as evidenced by a decrease of TUNEL-positive cells in these models. Indeed, it has been shown recently that ROCK induces cardiomyocyte apoptosis via activation of a mitochondrial death pathway [11].

However, the above described effects of RhoA/ROCK cannot explain the effects of acute ROCK inhibition as shown in the diabetic heart by Lin et al. [2]. The role of ROCK in cardiac contraction is still unclear, but similar mechanisms as in smooth muscle cells have been proposed, i.e. ROCK activity increasing contraction and hence a reduction of contraction by ROCK inhibition [12,13]. However, this is in contrast with the studies that show enhanced cardiac function after ROCK inhibition in infarction and diabetic models. Thus, the mechanism by which acute ROCK inhibition improves cardiac function awaits further study.

4. RhoA/ROCK as potential drug target

Although the exact role of ROCK in cardiac contraction remains elusive, it may be an interesting drug target to treat cardiovascular diseases. The fact that in most cases ROCK inhibition has no major effects in healthy subjects or preparations, as also shown in the study of Lin et al., makes it an even more promising drug target. Several clinical studies have already shown possible beneficial effects of ROCK inhibition, e.g. in hypertension, chronic heart failure and stroke [1]. In this context it is of interest to note that statins are also inhibitors of the RhoA/ROCK pathway and, therefore, a part of their pleiotropic effects may be attributable to ROCK inhibition [14]. Further research is warranted to investigate the role of ROCK in the cardiovascular system and its value as a drug target.

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