Rock ‘n’ Rho: regulation of ion channels

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The small GTPase Rho has three isoforms, RhoA, RhoB, and RhoC, of which RhoA has been widely studied. When the Rho gene was first cloned in 1980s, the physiological function of Rho was thought to be similar to its relatives, the Ras proteins. Unexpectedly, Rho was soon found to be crucial in the regulation of the actin cytoskeleton (10, 11). In the following years, many Rho downstream targets were discovered by using the yeast two-hybrid system. One of the most studied Rho effectors is ROCK, also known as Rho kinase or ROK, which has two isoforms, ROCKI and ROCKII. To date, Rho and ROCK have been shown to mediate a broad range of cellular responses, such as stress fiber formation, cell proliferation and migration, contraction, and apoptosis (1, 14).

The activity of small GTPase Rho is tightly controlled by guanine nucleotide exchange factors (RhoGEF), GTPase-activating proteins (RhoGAP), and GDP-dissociation inhibitors (RhoGDI). Once activated, GTP-bound Rho migrates to the cell membrane, where it activates ROCK. In 1996, Kimura et al. (6) first demonstrated that ROCK mediated the Rho inhibitory effect on myosin light chain phosphatase (MLCP). ROCK, a serine/threonine kinase, phosphorylates the myosin-targeting subunit of MLCP. This leads to the inhibition of MLCP and increases myosin light chain phosphorylation, thereby increasing smooth muscle contraction. Since these discoveries, most attention has focused on the role of the Rho/ROCK/MLCP signaling pathway in mediating vascular smooth muscle contraction. However, emerging evidence suggests that Rho and ROCK can regulate smooth muscle contraction by controlling activities of ion channels such as potassium channels.

Potassium channels are the main determinant of membrane potential, playing a significant role in the regulation of smooth muscle tone. Closure of potassium channels prevents potassium from effluxing and thus depolarizes the cell plasma membrane and activates voltage-gated calcium channels, leading to calcium entry. The effects of Rho and ROCK have been investigated on several types of potassium channels. Cachero et al. (3) were the first to report that the voltage-dependent delayed rectifying channel (KDR) activity was inhibited by RhoA/ROCK. Electrophysiology experiments showed that both wild-type and constitutively active RhoA reduced activity of Kv1.2, a main pore-forming subunit of the KDR channel. Coimmunoprecipitation of RhoA and Kv1.2 in both neuroblastoma cells and Kv1.2 overexpressing kidney 293 cells provided evidence that RhoA physically interacts with Kv1.2. Using the yeast hybrid analysis, they found that RhoA bound to the N terminus of Kv1.2. Additionally, inhibition of RhoA by the C3 exoenzyme prevented the M1 muscarinic receptor from suppressing of Kv1.2 activity, suggesting that RhoA links the signal transduction between the G protein-coupled receptor and ion channels. In cerebral smooth muscle cells, Luynkenaar et al. (7) observed that RhoA and ROCK inhibitors abolished the inhibitory effect of uridine triphosphate (UTP) on KDR channel activity in smooth muscle cells and decreased UTP-induced vasoconstriction in cerebral arterioles. Subsequently, they showed that activation of protein kinase A or protein kinase G attenuated RhoA-dependent suppression of KDR activity (8).

Luynkenaar and colleagues (8A) now further delineate the molecular mechanisms by which Rho/ROCK regulates KDR channels. Their key finding is that actin polymerization is required for ROCK-mediated suppression of KDR channel activity in response to UTP stimulation. In this study, they confirm their previous observations that KDR is negatively regulated by ROCK with two selective but structurally different ROCK inhibitors, Y-27632 and H-1152. They further demonstrate that pretreatment of H-1152 prevented UTP-induced actin polymerization, which was indicated by a reduced F-actin-to-G-actin ratio. When smooth muscle cells were treated with cytochalasin D and latrunculin A, agents that disrupt actin polymerization, UTP-induced suppression of KDR was obstructed. Altogether, the data suggest that ROCK modulates KDR activity through interaction with the actin cytoskeleton.

In addition to KDR channels, studies have shown that Rho/ROCK also negatively regulates other types of potassium channels, such as ether-a-go-go-related gene potassium channels in rat pituitary tumor cells (16). Some evidence suggests that RhoA/ROCK affects the inward rectifying potassium channels (KIR). Transfection with constitutively active RhoA decreased basal KIR2.1 activity in human embryonic kidney 293 cells, whereas dominant negative RhoA abolished the inhibitory effect of carbachol, a M1 muscarinic receptor agonist, on KIR2.1 (4). Interestingly, this effect was not mediated by ROCK because Y-27632 did not block the inhibitory effect of carbachol on KIR2.1. Further studies by the same group suggest that all three types of the KIR2 channels are negatively regulated by RhoA (12).

It seems that the influence of the Rho/ROCK pathway is not limited to potassium channels. For example, L-type calcium channel activity was decreased in ventricular myocytes over-expressing RhoGDI-α (17). Overexpression of dominant negative RhoA displayed a similar reduction of calcium channel activity, whereas other dominant negative mutants of Rho family members Rac-1 or Cdc42 failed to inhibit the calcium channels, suggesting that RhoA mediates the inhibitory effect of RhoGDI-α on the calcium channels. Staruschenko et al. (15) demonstrated that RhoA and ROCK increased epithelial sodium channel activity by activating phosphatidylinositol 4-phosphate 5-kinase. In pulmonary artery endothelial cells, RhoA and ROCK were found to activate Cl– efflux via volume-regulated anion channels in response to cell swelling (9). These data indicate that Rho/ROCK can regulate different...
types of ion channels and that the effect of Rho/ROCK is channel specific, either negative or positive.

Taken together, the accumulating evidence suggests that Rho/ROCK mediates G protein-coupled receptor or mechano-sensitive signaling to regulate ion channels. Although the underlying molecular mechanisms are still not clear, it has been speculated that Rho/ROCK may modulate channel activity through actin reorganization. Luukenaar et al.’s recently published work (8A) confirms this hypothesis and at the same time raises more interesting questions, such as how the actin reorganization induced by Rho/ROCK changes the activities of ion channels, what signaling pathways are involved in this process, and whether Rho/ROCK changes ion channel membrane targeting and recycling. Studies by Stuaruschenko and colleagues (15) suggest that RhoA/ROCK increased sodium channel activity via increasing channel insertion into the plasma membrane. However, KIR2.1 channel trafficking was not regulated by RhoA; instead, it was regulated by Rac1 (2). In contrast, Yatani et al. (17) reported that Rho/ROCK reduced voltage-dependent calcium channel activity without changing the expression level or channel density on the membrane.

Previous studies have shown that ROCK phosphorylates ezrin/radixin/moesin proteins, which are involved in actin filament-plasma membrane interaction (11a). ROCK also phosphorylates LIM kinases, leading to the inhibition of cofillin-mediated actin filament disassembly. Whether Rho/ROCK modulates ion channels through modifying these proteins requires further investigation. An added complexity to the regulation of ion channels by Rho is that ROCK may not be the only downstream effector, since some studies did not observe any effect of ROCK inhibitors on the ion channels (4, 17). RhoA has been shown to regulate the activities of other kinases, such as mammalian diaphanous protein and protein kinase N, whose functions are involved in actin polymerization and endosomal trafficking (13). It remains a question whether these proteins mediate the effect of RhoA on ion channels.

Interactions with actin cytoskeleton are probably only one aspect of the complex regulation of ion channels by Rho/ROCK. Ion channel subunits have direct phosphorylation sites for protein kinases and Rho/ROCK can cross talk with intracellular signaling pathways involving protein kinases (5, 9a, 15). Thus it raises the questions whether ROCK can directly phosphorylate channel subunits, whether Rho/ROCK modulate the ion channel activity through other protein kinases, or whether ROCK acts on protein phosphatases to regulate ion channel activities. It also will be interesting to determine the contributions of Rho and ROCK isoforms to the regulation of ion channels. So far most of the studies use pharmacological inhibitors such as C3 exoenzyme and Y-27632, which cannot distinguish the Rho/ROCK isoforms. The augmented Rho/Rho kinase activity has been implicated in many cardiovascular diseases such as hypertension, cerebral and coronary spasm, atherosclerosis, and cardiac hypertrophy. Thus understanding how Rho/ROCK regulates ion channels is a crucial step toward developing therapeutic targets for the treatment.

REFERENCES
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