The RISK of ROCK

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Since myocardial infarct size is the major contributor to the development of heart failure, interventions aiming to limit irreversible damage of ischemic myocardium are essential. In the clinical setting, reduction of myocardial oxygen demand and restoration of blood flow to the ischemic myocardium remain the primary targets to accomplish this goal, despite experimental evidence that reperfusion by itself might elicit irreversible injury. In the laboratory, phenomena such as ischemic myocardial pre- and postconditioning and ischemic preconditioning of remote organs have all been shown to limit infarct size, independent of the employed animal species. Several groups of investigators have expressed that pharmacological mimicking of these phenomena might also benefit the patient. Especially ischemic postconditioning (a form of graded reperfusion) is clinically attractive because this does not require application before the onset of the ischemic insult. Interestingly, pre- and postconditioning have been proposed to act via similar signaling pathways and target similar end points. Thus both have been shown to involve activation of the reperfusion injury salvage kinase (RISK) pathway and ultimately prevent opening of the mitochondrial permeability transition pore (7). Despite advances in our understanding, the exact mechanisms involved in reperfusion damage and the protection by pre- and postconditioning remain incompletely understood. In this issue of American Journal of Physiology-Heart and Circulatory Physiology, Hamid et al. (6) provide evidence that Rho-associated kinases (ROCKs) contribute to irreversible myocardial damage and that blockade of ROCK during early reperfusion can limit infarct size. These findings suggest that the Rho/ROCK pathway is also an active part in reperfusion injury (Fig. 1).

The ROCKs consist of two isoforms, ROCK-1 and ROCK-2. ROCK-1 is known as ROK-β, whereas ROCK-2 is known as ROK-α or Rho kinase (14, 15, 18). The small guanosine triphosphate-binding protein Rho activates ROCKs, which, in turn, phosphorylate various downstream targets. Both Rho and Rho kinase have been shown to play a central role in diverse cellular functions such as smooth muscle contraction, stress fiber formation, cell migration, and cell proliferation (20). Y-27632 and fasudil are selective ROCK inhibitors that target their ATP-dependent kinase domains and therefore are equipotent in terms of inhibiting both ROCK-1 and ROCK-2 (14). ROCK-1 is highly expressed in kidney, liver, lung, spleen, and testis, whereas ROCK-2 is preferentially expressed in cardiac and brain tissues (14). ROCK may also play a role in a number of cardiovascular diseases because increased activity of ROCKs has been demonstrated in cerebral ischemia, coronary vasospasm, hypertension, vascular inflammation, atherosclerosis, erectile dysfunction, and cardiac hypertrophy (14). Recent evidence indicates that pharmacological inhibition of ROCK is protective against ischemia-reperfusion injury in liver and kidney (8, 21). These findings suggest that ROCK activation contributes to ischemia-reperfusion-induced cell death, possibly via upregulation of Bax and induction of apoptosis (3).

Sanada et al. (17) were the first to link Rho kinase to irreversible myocardial cell death when they reported that, in an in vivo dog model, intracoronary infusion of Y-27632 for 30 min after 90 min of ischemia limited infarct size, although they did not investigate or speculate about the possible mechanisms involved. Capitalizing on these observations, Hamid et al. (6) studied the role of ROCK in the development of infarct size in isolated rat hearts that were subjected to 35 min of regional myocardial ischemia. The results confirm earlier reports of a cardioprotective effect of ROCK inhibition before ischemia (1, 5), which involves phosphatidylinositol 3-kinase (PI3K)/Akt and nitric oxide (NO) synthase (NOS) activation (23), linking the ROCK to the RISK pathway. In addition, Hamid et al. (6) reported that ROCK activity increases during early reperfusion and, most importantly, that inhibition of ROCK, selectively during the reperfusion phase, limits infarct size. These observations suggest that patients undergoing interventions to restore myocardial blood flow might benefit from ROCK inhibition. However, the study by Hamid et al. (6) raises several questions that need to be addressed before ROCK inhibition can be advocated as treatment for limitation of reperfusion damage.

A concern with any pharmacological study is the selectivity of the drugs employed. Hamid et al. (6) used the ROCK inhibitors fasudil and Y-27632, which are known to inhibit both ROCK-1 and ROCK-2. Although it remains to be determined whether ROCK-1 and/or ROCK-2 are involved in the effects of fasudil and Y-27632, ROCK-2 is the most likely candidate because it is the principal isoform present in the heart. Another concern is that fasudil has been reported to be able to inhibit protein kinase A and protein kinase C, whereas Y-27632 at higher concentrations may also inhibit protein kinase C-related kinase protein kinase N and citron kinase C (16). Nevertheless, both antagonists display a high degree of selectivity for ROCK inhibition (14). Furthermore, Hamid et al. (6) showed similar infarct size limitation with fasudil and Y-27632, and showed that Y-27632 inhibited ROCK activation. Taken together, these observations suggest that infarct limitation was the result of ROCK inhibition.

Hamid et al. (6) employed a single duration of index ischemia, and, as a result, it remains unclear whether ROCK activation occurred as a specific result of reperfusion or that it was, at least in part, a time-dependent response. Thus the authors used an ischemic episode of 35 min and determined ROCK activity at the end of ischemia and after 10 min of reperfusion. They interpreted the observation that ROCK ac-
Fig. 1. Schematic overview of the Rho-associated kinase (ROCK; left) and reperfusion injury salvage kinase (RISK; right) pathways and their interaction. GPCR, G protein-coupled receptor; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; Tyk, tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; eNOS, endothelial nitric oxide kinase; GC, guanylate cyclase; PKG, protein kinase G; PKC, protein kinase C; K_{Atp}, ATP-dependent potassium channel; MPTP, mitochondrial permeability transition pore. [Modified from Hausenloy and Yellon (7a).]

activity was unchanged at 35 min of ischemia but had increased 1.7-fold at 10 min of reperfusion as evidence that ROCK activation occurs selectively during reperfusion, which was supported by the observation that administration of the ROCK inhibitor Y-27632 just before reperfusion was similarly effective in limiting infarct size as compared with administration before ischemia. However, there is evidence that ROCK can already become activated during ischemia. Sanada et al. (17) previously reported a 2.7-fold increase in Rho activity after 60 min of ischemia without reperfusion. Unfortunately, Hamid et al. (6) employed only a single duration of index ischemia and did not investigate whether ROCK becomes activated after 45 min of ischemia in their experimental model. Since the observations by Sanada et al. (17) suggest that ROCK activation may also contribute to ischemic damage produced by longer periods of ischemia, it is clear that future studies are of great importance to determine the therapeutic potential of ROCK inhibition during early reperfusion following longer (≥60 min) coronary artery occlusions. This is particularly important since the time to reperfusion treatment in patients with an acute myocardial infarction often exceeds 2 h. With such an extended coronary artery occlusion, it is likely that a major part of the irreversible damage arises from ischemic rather than reperfusion damage, which is also suggested by a recent study from our own laboratory. Thus, in an in vivo rat model of regional myocardial ischemia, we observed that postconditioning limited myocardial infarct size produced by index ischemia periods of 45 min and 60 min in duration but failed to afford significant cardioprotection when the index ischemia was extended to 90 min and 120 min (12). Hence it is of eminent importance that future studies evaluate the therapeutic potential of ROCK inhibition in models of varying durations of index ischemia.

Another important issue that needs to be addressed in future studies is the interaction of ROCK inhibition with the protection afforded by other therapeutic modalities, such as ischemic preconditioning and postconditioning. Demiryurek et al. (4) have shown in anesthetized rats that fasudil in a high dose (10 mg/kg iv bolus) limited myocardial infarct size produced by 30 min of myocardial ischemia to a similar extent as ischemic preconditioning by 5 min of myocardial ischemia (4). However, fasudil in lower doses (0.3–1.0 mg/kg iv bolus), which had no effect on infarct size per se, prevented the limitation of infarct size by ischemic preconditioning produced by a single 5-min period of regional myocardial ischemia (4). Since these authors used only a single preconditioning stimulus, it remains to be determined whether this effect of fasudil is dependent on the employed ischemic preconditioning stimulus. This is important since it has been shown that there are adenosine-dependent and adenosine-independent pathways involved in ischemic preconditioning (11). Nevertheless, the study by Demiryurek et al. (4) suggests that ROCK activation may play a dual role in ischemia-reperfusion damage and underscores the importance of additional studies to determine the appropriate doses of ROCK inhibitors to prevent underdosing, which may actually increase infarct size in those patients who have been preconditioned by preinfarct angina. Demiryurek et al. (4) also reported that the combination of a high dose of fasudil and preconditioning did not appear to result in greater protection than either treatment alone (4), which is reminiscent of the reported lack of additional cardioprotection by postconditioning in preconditioned hearts (2). Because this supports the notion that ROCK inhibition, preconditioning, and postconditioning act via similar signaling pathways (e.g., PI3K/Akt/endothelial NOS) and target similar intracellular end points (7), it will be interesting to investigate whether ROCK inhibition can still afford protection against infarction in hearts that have become tolerant to an employed ischemic preconditioning stimulus (10).

In conclusion, ischemic heart disease is a major cause of heart failure, and interventions aimed at limiting infarct size remain critical. Over the past 20 years, research into the characteristics and mechanism(s) of pre- and postconditioning has greatly increased our understanding of irreversible myocardial damage produced by ischemia-reperfusion and continues to yield new targets for pharmacotherapy against ischemia-
reperfusion damage. To fully benefit from our insight into these endogenous protective mechanisms, it is important to recognize that the concept of both pre- and postconditioning is not limited to one stimulus but can encompass a multitude of ischemic and nonischemic stimuli, which may employ different signaling pathways yet exhibit significant cross talk. An improved understanding of all the cellular pathways and agents that trigger these pathways in a time-dependent manner continues to be essential for the development of novel strategies to limit ischemia-reperfusion damage. The study by Hamid et al. (6) shows that ROCK activation poses a “RISK” for irreversible myocardial damage and that inhibitors of ROCK during early reperfusion can limit infarct size by activating the RISK pathway. However, rigorous testing of these promising compounds requires evaluation of these drugs 1) over a wide dose range, 2) during varying index ischemia periods, and 3) in vivo animal models mimicking the clinical setting more closely. A lack thereof has likely contributed to previously failed attempts to translate promising results obtained in animal models into the clinical setting.

GRANTS
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