Review

Metabolic actions of Rho-kinase in periphery and brain

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Obesity has increased at an alarming rate in recent years and is now a worldwide public health problem. Elucidating the mechanisms behind the metabolic dysfunctions associated with obesity is of high priority. The metabolic function of Rho-kinase (Rho-associated coiled-coil-containing kinase; ROCK) has been the subject of a great deal of investigation in metabolic-related diseases. It appears that inhibition of ROCK activity is beneficial for the treatment of a wide range of cardiovascular-related diseases. However, recent studies with genetic models of ROCK demonstrate that ROCK plays a positive role in insulin and leptin signaling. Here we discuss the newly identified functions of ROCK in regulating glucose and energy metabolism, with particular emphasis on metabolic actions of insulin and leptin.

ROCK: key player in energy homeostasis

Obesity has reached epidemic levels in the United States and worldwide, and poses an increasingly severe economic burden [1,2]. Obesity is a major risk factor for developing insulin resistance, impaired glucose tolerance, type 2 diabetes, dyslipidemia, and hypertension, all of which are predispose patients to cardiovascular comorbidities [3,4]. This cluster of medical disorders is known as the metabolic syndrome [5,6]. Data emerging from several laboratories over the past decade indicate that the ROCK signaling pathway plays a pivotal role in various metabolic syndrome-related disorders including cardiovascular disease (CVD) [7,8]. Recent studies demonstrate that ROCK is an important regulator of insulin and leptin action in the context of glucose and energy homeostasis [9–12]. In this article we review recent work identifying the physiological roles of ROCK isoforms in controlling glucose homeostasis and insulin sensitivity, underlining the complex and context-dependent nature of this regulation. We also outline recently described ROCK-specific functions in hypothalamic neurons in regulating feeding behavior and energy balance, and highlight emerging evidence that ROCK is a molecular mediator underlying the etiopathogenesis of obesity.

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ROCK isoforms are increasingly important members of the AGC protein kinase family

ROCK belongs to the protein kinase A/G/C (AGC) subfamily of serine/threonine protein kinases and is a major downstream effector of small GTPase RhoA [13]. Two isoforms of this enzyme, ROCK1 (also known as ROKβ) and ROCK2 (also known as ROKα), have been identified [14–16]. Each isoform has a kinase domain at its N-terminus, a central coiled-coil domain, and, at its C-terminus, a pleckstrin-homology (PH) domain split by a cysteine-rich region. The two ROCK isoforms share 65% overall amino acid homology, with their kinase domains exhibiting 92% identity [17]. The Rho-binding domain of ROCK lies within the C-terminus of the coiled-coil domain [17]. ROCK isoforms have been implicated in variety of cellular functions, including smooth-muscle contraction, actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expression [18].

Owing to their important function in regulating numerous cellular activities, several ROCK inhibitors have been developed [19]. The ROCK inhibitor Y-27632 inhibits the kinase activity of both ROCK1 and ROCK2 with equal potency, by competing with ATP for binding to the catalytic site [20]. Fasudil (HA1077), originally developed as a Ca^{2+} antagonist and vasodilator, also inhibits both ROCK isoforms [21], but it is less selective for ROCK isoforms than Y-27632. Both Y-27632 and fasudil also have limited capacity to inhibit other AGC kinase subfamily members, including protein kinases A, G, and C [22]. Importantly, fasudil has a proven efficacy and safety profile in humans [19,23]. Animal and human studies with ROCK inhibitors have been of fundamental importance in elucidating ROCK physiologic functions [7,8,19]. However, interpretation of studies with ROCK inhibitors is limited due to lack of isoform selectivity and incomplete understanding of their respective specificities.

The Rho family of small GTPases are crucial regulators of ROCK activity. Interaction of ROCK with RhoA, -B, and -C through the Rho-binding domain of ROCK increases ROCK catalytic activity [13]. The 22 known Rho family members are classified into four main subfamilies: Rho, Rac, Cdc42, and others that lack GTPase activity [24]. In the Rho subfamilies, RhoA, RhoB, and RhoC share 85% amino acid sequence identity [25]. Until now, 11 Rho-binding proteins or Rho effectors have been identified,

including ROCK1 and ROCK2 [25]. Although RhoA has been thought to be the main upstream mediator of ROCK [18,26], protein interaction studies have revealed that ROCK has a higher affinity for RhoC compared to RhoA and RhoB [15,25,27–29]. In fact, RhoC appears to have a stronger ability to activate ROCK in epithelial cells [30].

Aberrant ROCK activity is associated with diverse aspects of the metabolic syndrome

Abnormalities in RhoA/ROCK expression and/or activity are associated with several metabolic syndrome-related disorders, including CVD, obesity, insulin resistance, and diabetes and its complications [31–34]. In rodents, elevated RhoA/ROCK signaling is observed in heart [35], aorta [31], and kidney [32,33] of insulin-resistant, obese, or diabetic animal models. Moreover, in patients with the metabolic syndrome, ROCK activity is increased in circulating leukocytes [36]. Furthermore, treatment of hypertensive or diabetic nephropathic animal models with ROCK inhibitor fasudil effectively lowers blood pressure and reduces albuminuria, respectively [37,38]. Consistent with this, in patients with angina pectoris, vasospastic angina, pulmonary hypertension, heart failure, or stroke, treatment with ROCK inhibitor fasudil ameliorates CVD risk factors [7,8,19]. By contrast, insulin-stimulated ROCK1 activity is impaired in skeletal muscle of insulin-resistant humans with obesity and type 2 diabetes [39]. Of note, insulin-induced ROCK1 activity is positively correlated with glucose disposal rate in lean subjects but not in obese type 2 diabetic subjects [39]. Collectively, these data suggest that abnormalities in ROCK activation contribute to the development and progression of diverse aspects of the metabolic syndrome.

ROCK regulation of glucose metabolism and insulin sensitivity is conditional and complex

A fundamental mechanism for the maintenance of glucose homeostasis is the rapid action of insulin to stimulate glucose uptake in muscle and adipocytes. Insulin action is initiated by binding of insulin to its receptor, which results in tyrosine phosphorylation of insulin receptor substrates (IRSs) including IRS1, IRS2, IRS3, IRS4, and Gab1 [40,41]. Binding of IRSs to the regulatory subunit of phosphoinositide 3-kinase (PI3K) via Src homology 2 (SH2) domains results in activation of PI3K. This then activates Akt/protein kinase B (PKB), which phosphorylates its substrate of 160 kDa (AS160), leading to the translocation of insulin-mediated glucose transporter 4 (Glut4) from intracellular stores to the cell surface (Figure 1). Defects in insulin-mediated glucose uptake result in insulin resistance, which is a major pathogenic factor in the development of type 2 diabetes [42].

Unlike the effects of ROCK inhibitors on other aspects of the metabolic syndrome, the reported effects of ROCK inhibitors on glucose homeostasis have been conflicting. Studies with obese Zucker animals have revealed that 4 weeks of treatment with the ROCK inhibitor fasudil (20 mg/kg/day for 4 weeks) decreases blood pressure and improves glucose tolerance [43]. By contrast, acute treatment with ROCK inhibitor Y-27632 (0.25 mg/kg/h for 4 h) results in insulin resistance *in vivo* by reducing insulin-mediated glucose uptake in skeletal muscle of normal mice [9]. Moreover, treatment of *db*/*db* mice with ROCK fasudil inhibitor (10 mg/kg/day for 16 weeks) has no effects on circulating glucose concentration [33]. Although the mechanisms underlying these conflicting findings remain unclear, the use of different inhibitors, doses, treatment times, and animal models in these *in vivo* animal studies complicates understanding of the specific roles of ROCK in regulating glucose homeostasis and insulin sensitivity *in vivo*.

Evidence demonstrates that genetic deletion of ROCK1 globally leads to whole-body insulin resistance and impaired skeletal-muscle insulin signaling [10]. These effects are independent of changes in adiposity. In skeletal muscle, insulin action is impaired as evidence by a reduction in insulin-stimulated PI3K activity associated with IRS-1, although tyrosine phosphorylation of the insulin receptor is unaltered [10]. Concurrently, Akt and AS160 phosphorylation, both of which are required for insulin-dependent GLUT4 translocation [44,45], are markedly impaired in skeletal muscle of ROCK1-deficient mice, suggesting impaired glucose transport into muscle. Supporting these studies in animals, in humans, *in vivo* administration of insulin stimulates ROCK1 activity in skeletal muscle [39]. Moreover, the ability of insulin to activate ROCK1 *in vivo* in skeletal muscle of type 2 diabetic subjects is significantly impaired compared to lean subjects [39]. Concurrently, defects in insulin-induced IRS-1-associated PI3K activity are also found in skeletal muscle of type 2 diabetic humans [46]. Together, these data identify ROCK1 as a novel regulator of glucose homeostasis and insulin sensitivity *in vivo*, and the emergence of ROCK1 as a potentially important step in the pathogenesis of insulin resistance could lead to new treatment approaches for obesity and type 2 diabetes. Future studies will need to determine the specific role of ROCK isoforms in the development of insulin resistance and type 2 diabetes.

ROCK targets IRS-1 to regulate insulin signal transduction

Tyrosine phosphorylation of IRS-1 is a crucial step because it permits this docking protein to interact with signaling proteins that promote insulin action [40]. Multiple studies have shown that ROCK isoforms regulate insulin signaling, both positively and negatively, either directly via IRS-1 serine phosphorylation, or indirectly by affecting IRS-1 tyrosine phosphorylation [9,47,48]. Mass spectrometry analysis identified $Ser^{632/635}$, Ser^{936}, and Ser^{972} of IRS-1 as ROCK substrates *in vitro* [9]. Recent evidence by Chun *et al.* reveals that siRNA-mediated suppression of each ROCK isoform decreases insulin-dependent IRS-1 $Ser^{632/635}$ phosphorylation in 3T3-L1 adipocytes [11], indicating that these serine sites are regulated via a ROCK-dependent mechanism. Overexpression of the IRS-1 $Ser^{632/635}$ active mutant significantly increased insulin-stimulated glucose transport in 3T3-L1 adipocytes, whereas overexpression of the IRS-1 $Ser^{632/635}$ inactive mutant decreased this [11]. Moreover, the ability of insulin to increase IRS-1 tyrosine phosphorylation and PI3K activity was impaired in adipocytes expressing the inactive IRS-1 $Ser^{632/635}$ mutant compared to adipocytes expressing wild type IRS-1