УДК 612.133; 616.132

I. V. Kizub

Rho-kinase: one of the main links in Ca^{2+} sensitization in diabetes-induced vascular smooth muscle hypercontractility

SI «Institute of Pharmacology and Toxicology of the NAMS of Ukraine», Kiev

Key words: diabetes, protein kinase C, Rho-kinase, Ca^{2+} sensitization, vascular smooth muscle, vascular tone

Diabetes mellitus (DM) is a widespread syndrome that is rapidly rising in frequency throughout the world. Hyperglycemia and alterations of metabolism are the most severe components of DM [1]. Near 5–10 % of patients with DM have autoimmune type 1 insulin-dependent diabetes, whereas 90–95 % have type 2 DM (insulin-independent), which is a consequence of lifestyle patterns contributing to obesity [1]. Type 2 DM typically occurs in the context of a cluster of cardiovascular risk factors [1]. DM is known to cause multiple dysfunctions including cardiovascular diseases, which are major causes of mortality, end-stage renal disease, and blindness [2]. The macrovascular manifestations of DM, such as angiopathy, atherosclerosis, medial calcification, and arterial hypertension, have been shown to mostly locate in coronary and carotid arteries, cerebral vessels, and large peripheral arteries of the lower extremities [2]. The microvascular complications of DM include retinopathy, nephropathy, and peripheral neuropathy [2]. Increased blood flow and elevated vascular tone have also been documented for DM [2]. A growing body of evidence indicates that endothelial and smooth muscle dysfunctions are present in various regions of the vasculature in both diabetic patients and animal models of DM [3, 4]. Hyperglycemia is considered to be a key factor responsible for the development of vascular complications in DM [2]. Several hyperglycemia-associated mechanisms, including oxidative stress have been identified to contribute to the development of DM-associated vascular dysfunctions [5]. Dysfunctions in the regulation of vascular cells permeability, growth, angiogenesis, and vascular smooth muscle contractility in DM have been shown to involve Rho-associated coiled-coil kinase (Rho-kinase or ROCK) up-regulation [4, 5].

Rho-kinase

The Rho-kinases or ROCKs is a small group of serine/threonine kinases, regulatory enzymes that also play an important role in vascular function regulation [6].ROCKs are represented with two isoforms encoded by two different genes, ROCK-1 (ROCK I or ROKβ) and ROCK-2 (ROCK II or ROKα) [7]. Both isoforms are highly homologous and are expressed in vascular tissues [8]. ROCK is mainly dispersed in the cytoplasm, but is partially translocated to the peripheral membrane upon activation [7].

ROCK is activated by monomeric G proteins (small GTPases) of the Rho (Ras homolog) protein family [6, 7, 9–11]. The Rho protein family consists of at least 20 members classified into 5 groups: the Rho-like, Rac-like, Cdc42-like, Rnd, and RhoBTB subfamilies, whereas RhoD, Rif, and RhoH/TTF do not fall into any obvious grouping [11]. Proteins of the Rho family regulate a wide range of fundamental cell functions such as contraction, motility, proliferation, and apoptosis [11]. RhoA, belonging to the Rho-like subfamily, is the main upstream activator of ROCK [11]. Binding of the active GTP-bound form of RhoA to the Rho-binding domain stimulates the phosphotransferase activity of ROCK by disrupting the interaction between the catalytic and the inhibitory C-terminal regions of the enzyme [10]. However, the

stimulatory effect of RhoA on ROCK activity is limited. RhoA functions as a molecular switch which cycles between an inactive GDP-bound and active GTP-bound conformations. Activated RhoA interacts with downstream targets leading to cellular responses. Upon activation RhoA is translocated from the cytosol to the cell membrane [10]. Activation of Rho GTPases is regulated by several mechanisms including the activation of heterotrimeric G protein-coupled receptors [10]. Three types of regulators of Rho proteins have been identified. Rho guanine-nucleotide exchange factors (RhoGEF) promote the formation of the GTP-bound form of Rho proteins [11], whereas Rho GTPase activating protein (RhoGAP) accelerates the intrinsic GTPase activity resulting in the GDP-bound form [11]. Rho guanine nucleotide dissociation inhibitor (RhoGDI) binds to a subset of Rho proteins, inhibits nucleotide exchange and sequesters these proteins away from the membrane, where normally they would be active [11]. On the other hand, it has been shown that ROCK-1 activity can be inhibited by the Rho protein Rnd3/RhoE which binds to the N-terminal region of ROCK-1 and prevents RhoA binding to the Rho-binding domain [12]. Two other small G proteins of the Ras superfamily, Gem and Rad, have been shown to inhibit the ROCK function [12].

The Rho/ROCK signaling pathway has been implicated in the pathogenesis of diabetes. It has been demonstrated that ROCK activity is enhanced in vascular tissues in diabetes [8, 9, 13, 14]. Recent publications demonstrated that diabetes type 1-induced vascular dysfunction in the rat aorta arises due to an upregulation of ROCK-2 isoform [15]. An increased level of ROCK-2 protein expression in corpus cavernosum of diabetic mice and retinal vessels of type 1 model diabetic rats [16] has also been reported. ROCK-1 gene and protein upregulation has also been reported from different vessels in various diabetic animal models [14]. The mRNA and protein levels of RhoA are also increased in arteries of diabetic rats and mice [9, 14, 16, 17]. The mechanism of RhoA and ROCK activation by high glucose levels remains to be defined. However ROS, thought to play a central role in the pathogenesis of vascular diabetic complications, have been shown to activate RhoA [18]. In another study, the authors reported that glucose-induced calcium-independent phospholipase $A_2\beta$ ($iPLA_2\beta$) upregulation may activate the RhoA/ROCK via 12/15-lipoxygenases in vascular smoth muscle cells (SMCs) [19]. On the other hand, it has been suggested that activation of ROCK in DM and high glucose level can be mediated by ErbB2 and Ras/Raf/extracellular signal-regulated kinase 1/2 (ERK1/2) [20]. ErbB2 is a transmembrane tyrosine kinase receptor of the epidermal growth factor receptor (ErbB/EGFR) family which downstream effector in vascular SMCs is ERK1/2 [20] (see Figure).

Mechanisms of Ca^{2+} sensitization in vascular SMCs in DM

While numerous studies have demonstrated that DM impairs vascular function by inhibiting endothelium-dependent vasodilatation [3, 4], others have shown that DM enhances vascular contractility in an endothelium-independent manner [13, 21–23]. A large number of studies suggest that vascular smooth muscle contractile responses are dramatically enhanced in diabetes [9, 13, 22–24].

It is well known that vascular smooth muscle contractile responses are primarily triggered by increased intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$). Both increased Ca^{2+} influx and release of $Ca^{2+}$from intracellular stores have been proposed to be involved in DM, contributing to the enhanced vascular reactivity [25]. There is evidence that stimulation of arteries with α_1-adrenomimetics is associated with increased Ca^{2+} influx in diabetic vessels over normal ones [24]. Other authors have also reported increased intracellular Ca^{2+} to be involved in the diabetic vascular hyperreactivity [24, 25]. However, there is a controversy here with reports of inhibition of voltage- and store-operated Ca^{2+} entry channels in vascular SMC in DM [26]. Alternatively, Ca^{2+} sensitization of the con-

tractile proteins has been proposed as a more general mechanism of the elevated DM-associated vascular contractility [9, 17, 22, 23]. One striking observation supporting this view is the enhanced contractile response to α_1-adrenomimetics by arteries from diabetic rats and mice which is not associated with $[Ca^{2+}]_i$ changes [13]. Similarly, under high glucose conditions, thromboxane A_2-induced aortic smooth muscle contraction has also been shown to increase independently from intracellular calcium concentration [27].

Phosphorylation and dephosphorylation of the 20-kDa myosin light chain (MLC) are the major regulatory mechanisms of smooth muscle contractility. Increased $[Ca^{2+}]_i$ activates calmodulin-dependent MLC kinase (MLCK) which catalyzes the phosphorylation of MLC at Thr18 and Ser19 leading to actin-myosin interaction and vascular smooth muscles contraction [28]. On top of this primary regulatory pathway, several modulatory pathways exist in smooth muscles that can alter the magnitude of the force that is developed at any given $[Ca^{2+}]_i$ [28]. ROCK-mediated [6, 10] mechanisms have been shown to be involved in elevated myofilament sensitivity to Ca^{2+}.

These pathways converge to phosphorylate an inhibitory protein CPI-17 at Thr38 [28-30] (see Figure). The CPI-17, or Protein kinase C (PKC) – dependent protein phosphatase-1 (PP1) inhibitor of 17 kDa, effects on smooth muscle myosin light chain phosphatase (MLCP) [28–30]. The MLCP holoenzyme of smooth muscles is a heterotrimer consisting of three subunits: the 130-kDa, 38-kDa, and 21-kDa subunit [31]. The 38-kDa subunit is identified as catalytic subunit PP1cδ. The 130-kDa subunit is a regulatory and myosin-bound MLCP targeting subunit 1 (MYPT1) [31]. Phosphorylated CPI-17 directly binds to MLCP and inhibits its activity [32]. Inhibition of MLCP results in a higher level of MLC phosphorylation for any given level of $[Ca^{2+}]_i$ and activity of MLCK [32]. This phenomenon, known as Ca^{2+} sensitization of smooth muscles, could thereby maintain vascular contraction [10].

ROCK-mediated SMCs Ca^{2+} sensitization in DM

Numerous studies have demonstrated that enhanced contractile responses to α-adrenomimetics, angiotensin II (ANG II), and 5-hydroxytryptamine (5-TH) in vessels from animal models of both types of DM [4, 9, 13, 20, 24, 33] are mediated by ROCK.

For increase in myofilament Ca^{2+} sensitivity in the DM ROCK-mediated mechanism have been shown [6, 9, 10, 23, 30, 31]. In this mechanism, ROCK phosphorylates CPI-17 at Thr38 [28–30] which then directly binds to PP1cδ and inhibits the activity of MLCP [32] (see Figure). In addition, ROCK directly phosphorylates the MYPT1 subunit of MLCP at Thr855 and/or Thr850, Thr695, Thr696, and Thr697 [6, 10, 30, 31, 34] that also consequently inhibits the phosphatase activity.

In smooth muscle cells from STZ-induced model of type 1 diabetes rats, phosphorylation levels of MYPT1 in aorta has been shown to be significantly elevated [15]. Similar results have been obtained from the saphenous vein of patients with DM by Matsuo and coauthors, which have shown that the hyperreactivity to 5-HT in smooth muscles of these vessels is due to higher phosphorylation of MLCP, evident from elevation of the P (Thr696)-MYPT1 to total MYPT1 ratio [34].

Our research group has shown that elevated vascular SMC contractility in rats with STZ-induced DM is associated with enhanced Ca^{2+} sensitivity of contractile myofilaments and ROCK is clearly contribute to this process in diabetic vasculature [23]. ROCK-mediated calcium sensitization has been shown to be responsible for hypertension development in type 2 diabetes as well [13, 17]. It has also been suggested recently that ROCK mediate Ca^{2+} sensitization in smooth muscles of the intrarenal interlobar artery of type 2 diabetic (*ob/ob*) mice [13]. It has been shown that α_1-adrenoceptor-mediated vasoconstriction in penile [24] and gracilis arteries [33] from rat models of prediabetes/metabolic syndrome (obese Zucker rats) also involves ROCK-dependent Ca^{2+} sensitization.

Other authors have demonstrated that ROCK-mediated CPI-17 phosphorylation increase in vascular SMCs of the type 2 diabetic *db/db* mice model and SMCs cultured in presence of high glucose concentration [9]. Both activation of the ROCK pathways that phosphorylates CPI-17 and increase in total CPI-17 protein level seem to be involved [9], and CPI-17 up-regulation/activation in type 2 diabetic *db/db* mice vasculature is associated with a significant blood pressure increase [35].

Interaction between ROCK and PKC in mechanisms of SMCs Ca^{2+} sensitization in DM

A number of papers have reported a link between the ROCK- and PKC-mediated pathways in diabetes orunder high glucose conditions. PKC is presented with a family of serine/threonine kinases, with 10 isoforms and has been reported to be also involved in mechanisms of SMCs Ca^{2+} sensitization in DM [36].

Hyperglycemia in DM results in an overproduction of reactive oxygen species (ROS) and the ensuing oxidative stress which contributes to the activation of PKC [36]. Furthermore, there is strong evidence that PKC activation is mediated, at least in part, by induction of oxidative stress and increased production of ROS [37]. In vascular tissues, PKC activation can also be mediated by diacylglycerol (DAG) [36] which has been shown to be elevated in DM [21, 27]. Hyperglycemia can enhance the amount of DAG primarily by increasing *de novo* DAG synthesis from the glycolytic intermediate dihydroxyacetone phosphate (DHAP), as well as by inducing phosphorylation of the phospholipase Cγ (PLCγ) [21, 36] (Figure).

Some investigations suggest that ROCK is upstream of PKC in diabetic bladder [38] and diabetic cardiomyocytes [39], whereas in diabetic vascular SMC ROCK has been reported to be down-

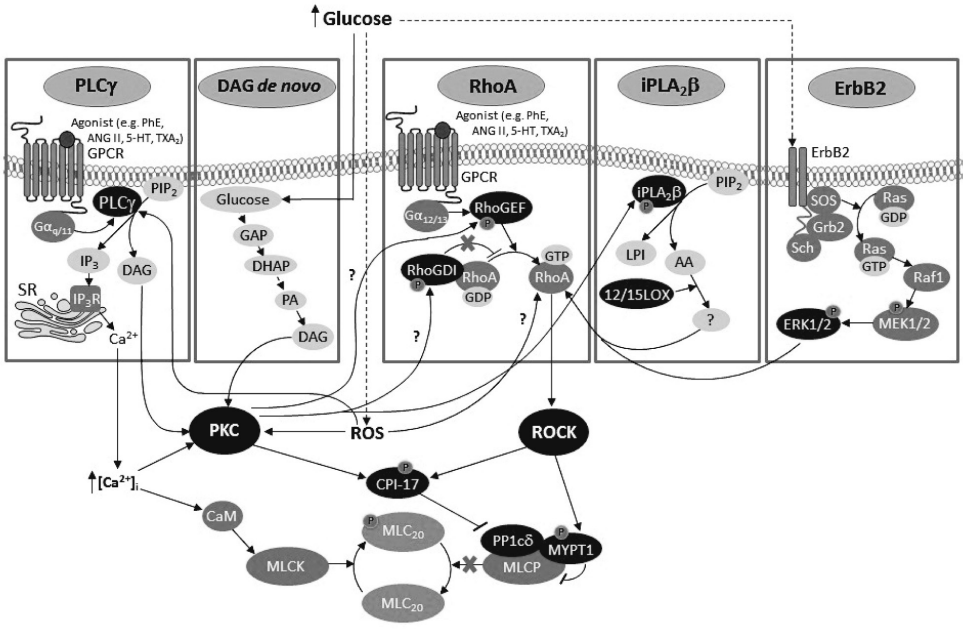

Figure. A scheme illustrating the ROCK-mediated mechanisms of contractile proteins Ca^{2+} sensitization in vascular smooth muscle cells in DM

DHAP – dihydroxyacetone phosphate; GAP – glyceraldehyde-3-phosphate; GPCR – G-protein coupled receptor; Grb-2 – growth factor receptor-bound protein 2; MEK1/2 – mitogen-activated protein kinase kinases 1 and 2; PA – phosphatidic acid; Raf1 – rapidly accelerated fibrosarcoma proto-oncogene protein kinase 1; Ras – rat sarcoma protein; Sch–Src homology 2 domain containing transforming protein; SOS –«sonof sevenless» homolog protein; TXA_2 – Thromboxane A_2. Definitions of other abbreviations see in the main text.

stream of PKC [9, 20]. Xie and coauthors have shown that PKC in vascular SMC is required for high glucose-induced ROCK activation and consequently CPI-17 phosphorylation [9]. These authors also suggest that, although PKC can directly phosphorylate CPI-17 upon some agonist stimulation in the vascular smooth muscle tissue under physiological conditions, PKC is not the kinase that directly phosphorylates CPI-17 in the presence of high glucose [9]. It has been shown that ROCK mRNA and protein levels can be upregulated through PKC and it is luckily that under diabetic conditions RhoA/ROCK and CPI-17 are downstream effectors of PKC [9]. More recently, the same group of researchers has shown that high glucose-induced activation of PKC leading to activation of iPLA2β and up-regulation of RhoA/ROCK via 12/15-lipoxygenases, thereby contributes to diabetes-associated vascular smooth muscle Ca^{2+} sensitization and hypercontractility [19]. Products of the phospholipase A_2 enzymes group include arachidonic acid which can be rapidly metabolized to a variety of mediators by lipoxygenases and other oxygenases to a number of bioactive eicosanoids [40]. However, it remains unclear how the catalytic activity of the 12/15-lipoxygenase may activate RhoA/ROCK/CPI-17. It is possible to assume, that PKC may mediate high glucose-induced activation of RhoA via phosphorylation of RhoGDI or RhoGEF [41] (Figure).

Conclusion

The presented data suggest that ROCK in vascular SMC highly contribute to enhanced vascular tone and arterial hypercontractility in diabetes by mediating SMC Ca^{2+}-sensitization. Our review shows that ROCK are potential therapeutic targets for treating vascular diabetic complications. Developing innovative pharmacological approaches that could modulate ROCK activity is highly important for new strategies that might prove clinical relevancy in preventing the development and/or retarding the progression of diabetes-associated vascular complications.

1. *Unwin N.* The IDF diabetes atlas: providing evidence, raising awareness and promoting action / N. Unwin, D. Gan, D. Whiting // Diabetes Res. Clin. Pract. – 2010. – V. 87. – P. 2–3.
2. *Madonna R.* Cellular and molecular mechanisms of vascular injury in diabetes - part I: pathways of vascular disease in diabetes / R. Madonna, R. De Caterina // Vasc.Pharmacol. – 2011. – V. 54. – P. 68–74.
3. *Kolluru G. K.* Endothelial dysfunction and diabetes: Effects on angiogenesis, vascular remodeling, and wound healing / G. K. Kolluru, S. C. Bir, C. G. Kevil // Int. J. Vasc. Med. – 2012. – P. 1–30.
4. Acute Rho-kinase inhibition improves coronary dysfunction *in vivo*, in the early diabetic microcirculation / J. T. Pearson, M. J. Jenkins, A. J. Edgley [et al.] // Cardiovasc. Diabetol. – 2013. – V. 12. – P. 111.
5. *Geraldes P.* Activation of protein kinase C isoforms and its impact on diabetic complications / P. Geraldes, G. L. King // Circ. Res. – 2010. – V. 106. – P. 1319–1331.
6. *Schofield A. V.* Rho-associated coiled-coil kinase (ROCK) signaling and disease / A. V. Schofield, O. Bernard// Crit. Rev. Biochem. Mol. Biol. – 2013. – V. 48, № 4. – P.301–316.
7. Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho / T. Matsui, M. Amano, T. Yamamoto [et al.] // EMBO J. – 1996. – V. 15. – P. 2208–2216.
8. *Nuno D. W.* Sex-dependent differences in Rho activation contribute to contractile dysfunction in type 2 diabetic mice / D. W. Nuno, J. S. Harrod, K. G. Lamping // Am. J. Physiol. Heart Circ. Physiol. – 2009. – V. 297, № 4. – P. H1469–H1477.
9. Up-regulation of CPI-17 phosphorylation in diabetic vasculature and high glucose cultured vascular smooth muscle cells / Z. Xie, W. Su, Z Guo [et al.] // Cardiovasc. Res. – 2006. – V. 69. – P. 491–501.
10. *Loirand G.* Rho kinases in cardiovascular physiology and pathophysiology / G. Loirand, P. Guerin, P. Pacaud // Circ. Res. – 2006. – V. 98. – P. 322–334.
11. *Burridge K.* Rho and Ractake center stage / K. Burridge, K. Wennerberg // Cell. – 2006. – V. 116. – P. 167–179.
12. The GTP binding proteins Gem and Rad are negative regulators of the Rho-Rho kinase pathway / Y. Ward, S. F. Yap, V. Ravichandran [et al.] // J. Cell Biol. – 2002. – V. 157. – P. 291–302.
13. Two types of over-contraction are involved in intrarenal artery dysfunction in type II diabetic mouse / K. Nobe, Y. Takenouchi, K. Kasono [et al.] // J. Pharmacol. Exp. Ther. – 2014. – published online before print.
14. RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: a mechanism for diabetes-associated erectile dysfunction / T. J. Bivalacqua, H. C. Champion, M. F. Usta [et al.] // Proc. Natl. Acad. Sci. USA. – 2004. – V. 101. – P. 9121–9126.

15. *Cicek F. A.* Role of ROCK upregulation in endothelial and smooth muscle vascular functions in diabetic rat aorta / F. A. Cicek, H. B. Kandilci, B. Turan // Cardiovasc. Diabetol. – 2013. – V. 12. – P. 51.
16. Activated Rho kinase mediates diabetes-induced elevation of vascular arginase activation and contributes to impaired corpora cavernosa relaxation: Possible involvement of p38 MAPK activation / H. A. Toque, K. P. Nunes, L. Yao [et al.] // J. Sex. Med. – 2013. – V. 10, № 6. – P. 1502–1515.
17. *Sandu O. A.* Diabetes in the Goto-Kakizaki rat is accompanied by impaired insulin- mediated myosinbound phosphatase activation and vascular smooth muscle cell relaxation / O. A. Sandu, L. Ragolia, N. Begum // Diabetes. – 2000. – V. 49, № 12. –P. 2178–2189.
18. *Knock G. A.* Redox regulation of protein kinases as a modulator of vascular function / G. A. Knock, J. P.T. Ward //Antioxid. Redox. Signal. – 2011. – V. 15, № 6. – P. 1531–1547.
19. Role of calcium-independent phospholipase A2beta in high glucose-induced activation of RhoA, Rho kinase, and CPI-17 in cultured vascular smooth muscle cells and vascular smooth muscle hypercontractility in diabetic animals / Z. Xie, M. C. Gong, W. Su [et al.] // J. Biol. Chem. – 2010. – V. 285. – P. 8628–8638.
20. Activation of ErbB2 and downstream signalling via Rho kinases and ERK1/2 contributes to diabetes-induced vascular dysfunction / S. Akhtar, M. H. M. Yousif, G. [et al.] // PLoS One. – 2013. – V. 8, № 6. – P. e67813.
21. Novel diacylglycerol kinase inhibitor selectively suppressed an U46619-induced enhancement of mouse portal vein contraction under high glucose conditions / K. Nobe, M. Miyatake, H. Nobe [et al.] // Brit. J. Pharmacol. – 2004. – V. 143. – P. 166–178.
22. *Mueed I*. Role of the PKC/CPI-17 pathway in enhanced contractile responses of mesenteric arteries from diabetic rats to α-adrenoceptor stimulation / I. Mueed, L. Zhang, K. M. MacLeod // Brit. J. Pharmacol. – 2005. – V. 146. – P. 972–982.
23. Rho kinase and protein kinase C involvement in vascular smooth muscle myofilament calcium sensitization in arteries from diabetic rats / I. V. Kizub, O. O. Pavlova, C. D. Johnson [et al.] // Brit. J. Pharmacol. – 2010. – V. 159, № 8. – P.1724–1731.
24. Impaired Ca^{2+} handling in penile arteries from prediabetic Zucker rats: involvement of Rho kinase / N. Villalba, C. Contreras, M. Hernández [et al.] // Am. J. Physiol. Heart. Circ. Physiol. – 2011.– V. 300. – P. H2044–H2053.
25. Evidence for mechanistic alterations of Ca^{2+} homeostasis in type 2 diabetes mellitus / M. Balasubramanyam, R. A. Balaji, B. Subashini, V. Mohan // Int. J. Exp. Diabetes Res.– 2001. – V.1. – P.275–287.
26. Altered l-type Ca^{2+} channel currents in vascular smooth muscle cells from experimental diabetic rats / R. Wang, Y. Wu, G. Tang [et al.] // Am. J. Physiol. Heart. Circ. Physiol. – 2000. – V. 278. – P.H714–H722.
27. High-glucose enhances a thromboxane A_2-induced aortic contraction mediated by an alteration of phosphatidylinositol turnover / K. Nobe, H. Suzuki, H. Nobe [et al.] // J. Pharmacol. Sci. – 2003. – V. 92. – P. 267–282.
28. *Schubert R.* The emerging role of Ca^{2+} sensitivity regulation in promoting myogenic vasoconstriction / R. Schubert, D. Lidington, S. S. Bolz // Cardiovasc. Res. – 2008. – V. 77. – P. 8–18.
29. Senba S. Identification of trimeric myosin phosphatase (PP1M) as a target for a novel PKC-potentiated protein phosphatase-1 inhibitory protein (CPI17) in porcine aorta smooth muscle / S. Senba, M. Eto, M. Yazawa // J. Biochem. (Tokyo). – 1999. – V. 125. – P. 354–362.
30. *Swärd K.* The role of RhoA and Rho-associated kinase in vascular smooth muscle contraction / K. Swärd, M. Mita, D. P. Wilson [et al.] // Curr. Hypertens. Rep. – 2003. – V. 5, № 1. – P. 66–72.
31. Ichikawa K. Phosphorylation of the large regulatory subunit of myosin phosphatase and inhibition of phosphatase activity / K. Ichikawa, M. Ito, D. J. Hartshorne // J. Biol. Chem. – 1996. – V. 271. – P. 4733–4740.
32. *Eto M.* Regulation of cellular protein phosphatase-1 (PP1) by phosphorylation of the CPI-17 family, C-kinase-activated PP1 inhibitors / M. Eto // J. Biol. Chem. – 2009. – V. 284. – P. 35273–35277.
33. Didion S. P. Cerebral vascular dysfunction in TallyHo mice: a new model of Type II diabetes / S. P. Didion, C. M. Lynch, F. M. Faraci // Am. J. Physiol. Heart. Circ. Physiol. – 2007. – V. 292. – P. H1579–H1583.
34. The defective protein level of myosin light chain phosphatase (MLCP) in the isolated saphenous vein, as a vascular conduit in coronary artery bypass grafting (CABG), harvested from patients with diabetes mellitus (DM) / Y. Matsuo, M. Kuwabara, N. Tanaka-Totoribe [et al.] // Biochem. Biophys. Res. Commun. – 2011. – V. 412. – P. 323–327.
35. Hypertension and disrupted blood pressure circadian rhythm in Type 2 diabetic db/db mice / W. Su, Z. Guo, D. C. Randall [et al.] // Am. J. Physiol. Heart Circ. Physiol. – 2008. – V.2 95. – P. H1634–H1641.
36. *Kizub I. V.* Protein kinase C in enhanced vascular tone in diabetes mellitus / I. V. Kizub, K. I. Klymenko, A. I. Soloviev // Int. J. Cardiol. – 2014. – V. 174. – P. 230–242.
37. Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation / T. Inoguchi, R. Battan, E. Handler [et al.] // Proc. Natl. Acad. Sci. USA. – 1992. – V. 89. – P. 11059–11063.

38. Glucose-dependent enhancement of diabetic bladder contraction is associated with a rho kinase-regulated protein kinase C pathway / K. Nobe, T. Yamazaki, N. Tsumita [et al.] // J. Pharmacol. Exp. Ther. – 2009. – V. 328. – P. 940–950.
39. Diabetes-induced increased oxidative stress in cardiomyocytes is sustained by a positive feedback loop involving Rho-kinase and PKCβ2 / H. Soliman, A. Gador, Y.-H. Lu [et al.] //Am. J. Physiol. Heart Circ. Physiol. – 2012. – V. 303, № 8. – P. H989–H1000.
40. *Dennis E. A.* Diversity of group types, regulation, and function of phospholipase A2 / E. A. Dennis // J. Biol. Chem. – 1994. –V. 269, № 18. – P. 13057–13060.
41. Protein kinase C alpha-induced p115RhoGEF phosphorylation signals endothelial cytoskeletal rearrangement / M. Holinstat, D. Mehta, T. Kozasa [et al.] // J. Biol. Chem. – 2003. – V. 278. – P. 28793–28798.

І. В. Кізуб

Rho-кіназа: одна з головних ланок у Ca^{2+}-сенситизації за індукованої діабетом гіперскоротливості судинних гладеньких м'язів

Цукровий діабет є комплексним синдромом, що призводить до чисельних порушень, у тому числі судинних дисфункцій. Rho-залежна кіназа (RhoK) є важливим регуляторним ферментом, що опосередковує передачу сигналів у реалізації багатьох судинних функцій, у тому числі скоротливості судинних гладеньких м'язів. Чисельні дослідження показали, що скоротливі відповіді судинних гладеньких м'язів значною мірою підвищені за умов цукрового діабету. RhoK робить суттєвий внесок у цей процес, опосередковуючи зростання чутливості скоротливих білків до іонів Ca^{2+} (або Ca^{2+}-сенситизацію) у судинних гладеньком'язових клітинах. За умов цукрового діабету опосередковані RhoK внутрішньоклітинні сигнальні шляхи спрямовані на фосфорилювання інгібіторного білка CPI-17, який зв'язуючись із фосфатазою легких ланцюгів міозину (ФЛЛМ) призводить до пригнічення її активності. Окрім того, RhoK викликає пригнічення активності ФЛЛМ за рахунок фосфорилювання її таргетної субодиниці 1 (MYPT1). Наслідком пригнічення ФЛЛМ є зростання фосфорилювання легких ланцюгів міозину незалежно від внутрішньоклітинної концентрації Ca^{2+}та рівня активності кінази легких ланцюгів міозину. Ca^{2+}-сенситизація гладеньких м'язів, таким чином, може суттєво підвищувати скоротливість судинної стінки за умов цукрового діабету. Багато досліджень також показали зв'язок між RhoK- та протеїн кіназа С (ПКС)-опосередкованими сигнальними шляхами за умов діабету. При цьому встановлено, що в стінці діабетичних судин RhoK може бути активована ПКС. Таким чином, проаналізовані дані показують, що RhoK є важливою фармакологічною мішенню для лікування судинних ускладнень, що пов'язані з цукровим діабетом.

Ключові слова: діабет, Rho-кіназа, Ca^{2+}-чутливість, судинні гладенькі м'язи, судинний тонус

И. В. Кизуб

Rho-киназа: одно из главных звеньев в Ca^{2+}-сенситизации при индуцированной диабетом гиперсократимости сосудистых гладких мышц

Сахарный диабет является комплексным синдромом, приводящим к многочисленным нарушениям, включая сосудистые дисфункции. Rho-зависимая киназа (RhoK) – важный регуляторный фермент, опосредующий передачу сигналов в реализации многих сосудистых функций, включая сократимость гладких мышц сосудов. Многочисленные исследования показали, что сократительные ответы гладких мышц сосудов в значительной степени увеличены в условиях сахарного диабета. RhoK вносит значительный вклад в этот процесс, опосредуя повышение чувствительности сократительных белков к ионам Ca^{2+} (или Ca^{2+}-сенситизацию) в гладкомышечных клетках сосудов. При сахарном диабете опосредованные RhoK внутриклеточные сигнальные пути направлены на фосфорилирование ингибиторного белка CPI-17, который, связываясь с фосфатазой легких цепей миозина (ФЛЦМ), приводит к угнетению ее активности. Кроме того, RhoK вызывает угнетение активности ФЛЦМ за счет фосфорилирования ее таргетной субъединицы 1 (MYPT1). Следствием угнетения ФЛЦМ является повышение фосфорилирования легких цепей миозина независимо от внутриклеточной концентрации Ca^{2+} и уровня активности киназы легких цепей миозина. Ca^{2+}-сенситизация гладких мышц, таким образом, может существенно повышать сократимость сосудистой стенки при сахарном диабете. Многие исследования также показали связь между RhoK- и протеин киназа С (ПКС) – опосредованными сигнальными путями при диабете. При этом установлено, что в стенке диабетических сосудов RhoK может быть активирована под действием ПКС. Таким образом, проанализированные данные показывают, что RhoK является важной фармакологической мишенью для лечения сосудистых осложнений, связанных с сахарным диабетом.

Ключевые слова: диабет, Rho-киназа, Ca^{2+}-чувствительность, сосудистые гладкие мышцы, сосудистый тонус

I. V. Kizub

Rho-kinase: one of the main links in Ca^{2+} sensitization in diabetes-induced vascular smooth muscle hypercontractility

Diabetes is a complex syndrome which leads to multiple dysfunctions including vascular disorders. Rho-associated coiled-coil kinase (ROCK) is important regulatory enzyme mediating signal transduction in a number of vascular functions, including vascular smooth muscle contractility. Many studies have shown that vascular smooth muscle contractile responses are dramatically enhanced in diabetes. ROCK significantly contributes to this process by mediating elevation in sensitivity of contractile proteins to Ca^{2+}ions (or Ca^{2+} sensitization)in vascular smooth muscle cells. In diabetes mellitus ROCK-mediated pathways converge to phosphorylate the inhibitory protein CPI-17 which binds to myosin light chain phosphatase (MLCP) and inhibits its activity. Besides, ROCK phosphorylates myosin light chain phosphatase targeting subunit 1 (MYPT1) which also inhibits the phosphatase activity. Inhibition of MLCP results in a higher level of myosin light chain phosphorylation for any given intracellular level of Ca^{2+} and activity of the myosin light chain kinase. Ca^{2+} sensitization of smooth muscle, thus, could potentially maintain vascular contraction in diabetes. A link between the ROCK- and protein kinase C (PKC)-mediated pathways in diabetic vascular smooth muscle cells has been shown, assuming that the ROCK is downstream effectors of PKC. The data analyzed suggest that ROCK isan important therapeutic targetfor treating diabetes-related vascular complications.

Key words: diabetes, Rho-kinase, Ca^{2+} sensitization, vascular smooth muscle, vascular tone

Надійшла: 17.11.2014 р.

Контактна особа: Кізуб Ігор Володимирович, старший науковий співробітник, канд. біол. наук, відділ експериментальної терапії, ДУ «Інститут фармакології та токсикології НАМН України», буд. 14, вул. Е. Потьє, м. Київ, 03680. Тел.: + 38 0 44 456 02 88.
Електронна пошта: buzzmann@ukr.net