® Postepy Hig Med Dosw (online), 2014; 68: 1022-1027
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2012.07.07
Accepted: 2014.05.25
Published: 2014.08.22

Rho-kinase inhibitor reduces hypersensitivity to ANG II in human mesenteric arteries retrieved and conserved under the same conditions as transplanted organs*

Inhibitor Rho-kinazy redukuje nadwrażliwość na ANG II ludzkich tętnic krezkowych pobranych i przechowywanych w takich warunkach jak przeszczepiane narządy

Rafal Szadujkis-Szadurski[1], Maciej Slupski [2], Katarzyna Szadujkis-Szadurska[1], Leszek Szadujkis-Szadurski[1], Milosz Jasinski[3,4], Grzegorz Grzesk[1], Elżbieta Grzesk[5], Aleksandra Woderska[2], Zbigniew Wlodarczyk[2],

[1] Department of Pharmacology and Therapeutics, Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Poland
[2] Department of Transplantation and General Surgery, Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Poland
[3] Department of Oncological Urology, Oncology Centre, Bydgoszcz, Poland
[4] Department of Tissue Engineering, Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Poland
[5] Department of Pediatric Hematology and Oncology, Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Polandw

Summary

Rho-kinase and GTP-ase Rho are important regulators of vascular tone and blood pressure. The aim of this study was to investigate the role of Rho-kinase in artery reactions induced by angiotensin II (ANG II) and the effects of ischemia-reperfusion injury as well as the function of intra- and extracellular calcium in these reactions.

Experiments were performed on mesenteric superior arteries procured from cadaveric organ donors and conserved under the same conditions as transplanted kidneys. The vascular contraction in reaction to ANG II was measured in the presence of Rho-kinase inhibitor Y-27632, after ischemia and reperfusion, in Ca^{2+} and Ca^{2+}-free solution.

The maximal response to ANG II was reduced after ischemia, while an increase was observed after reperfusion. Vascular contraction induced by ANG II was decreased by Y-27632. Y-27632 reduced vascular contraction after reperfusion, both in Ca^{2+} and Ca^{2+}-free solution.

Reperfusion augments vascular contraction in reaction to ANG II. The Rho-kinase inhibitor Y-27632 reduces the hypersensitivity to ANG II after reperfusion mediated by both intra- and extracellular calcium. These results confirm the role of Rho-kinase in receptor-independent function of ANG II and in reperfusion-induced hypersensitivity.

Keywords: Rho-kinase • ischemia/reperfusion injury • arteries • Y-27632

* Funding: This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1118217

Word count: 1825
Tables: –
Figures: 5
References: 39

Author's address: Maciej Slupski, M.D., Ph.D., Department of Transplantation and General Surgery, CM UMK, ul. Marii Sklodowskiej-Curie 9, 85-094 Bydgoszcz, Poland; e-mail: maciejslupski@wp.pl

Introduction

Ischemia-reperfusion injury is a major factor influencing early and late results of kidney transplantation. Many efforts to reduce this problem have been made in kidney procurement and transplantation and one of the possible ways to improve graft function is to limit the post-reperfusion no-reflow phenomenon. Rho-kinase inhibitors are among substances that can suppress this damaging effect – they should limit the influence of angiotensin II (ANG II) on reperfused arteries.

The most important and best known function of ANG II depends on activation of AT_1 receptors [1,6,20,36].

In the circulatory system these receptors are present in smooth muscle cells and take part in vessel contraction, proliferation and inflammatory processes induced by ANG II [8,12]. AT_1 receptors are metabotropic G_q-protein coupled receptors. G-protein activates phospholipase C (PLC) and synthesis of inositol trisphosphate (IP_3) and diacylglycerol (DAG) – second messengers in ANG II dependent processes. After binding to IP_3 receptors in endoplasmic reticulum, IP_3 induces release of Ca^{2+} and vascular smooth muscle contraction while DAG activates protein kinase C.

The latest research suggests that Rho-kinase and GTP-ase Rho, which activates it, play a key role in regulation of vascular tone and blood pressure *in vivo* [9,27,34]. Numerous *in vivo* studies have shown that the active form of Rho-kinase mediates myosin light chain phosphorylation, activating binding of Ca^{2+} by contractile proteins and thus inducing vessel smooth muscle contraction [2,5]. Wang et al. (2001) reported that hypoxia causes a considerable, duration-dependent, increase in Rho-kinase activity and myosin light chain phosphorylation [37]. In this research, we assessed the influence of the Rho-kinase inhibitor Y-72632 on angiotensin II induced smooth muscle reaction, including the role of intra- and extracellular Ca^{2+} and effects of ischemia/reperfusion injury. Although the role of Rho-kinase and its inhibitors in ischemia/reperfusion injury have been investigated in animals in several studies, the model used in this experiment – reperfusion of human mesenteric arteries, harvested during kidney procurement from deceased donors – should more accurately reflect processes occurring in transplanted kidney vessels after reperfusion.

Material and methods:

The experiment was approved by the review board of Nicolaus Copernicus University. Superior mesenteric arteries were procured from deceased kidney donors. They were stored in UW storage solution at a temperature of 4°C for an average time of 14.8±3.6 h, the same as transplanted kidneys. After being dissected and cleared from surrounding tissue, a 15 mm long segment was cannulated and connected to the perfusion apparatus. Perfusion pressure was measured continuously using a pressure transducer (Gould Statham, type P-23ID) and universal coupler (Narco 7189) of a Narco Narcotrace 40 physiograph (Narco Bio-Systems). Perfusion flow was maintained by a peristaltic pump type 315, Zalimp (Poland). The sample was then placed in a 20 mL container filled with oxygenated normal saline at 37°C. Perfusion solution flow was gradually increased using a peristaltic pump until 1 mL/min was reached.

To estimate the influence of hypoxia on artery reaction, perfusion and oxygen supply was stopped for a set period of time (30 or 60 min), then perfusion was resumed and artery reaction was measured. After a set time of perfusion with oxygenated solution, artery reaction was measured for the second time. Drugs used in the experiment were purchased from Sigma-Aldrich, Poland.

Concentration-response curves (CRCs) for ANG II before and after addition of Rho-kinase inhibitor (1, 3 and 10 μM) and after ischemia/reperfusion were analyzed according to modified receptor theory [13,14]. EC_{50} (half maximal effective concentration) and E_{max} (maximal response) values were used to estimate changes in artery responses to ANG II.

Two models were used to estimate the role of extra- and intracellular Ca^{2+}:

• model A – solution without Ca^{2+} - EGTA – PSS (FPSS) – 71.8 mM NaCl, 4.7 mM KCl, 28.4 mM $NaHCO_3$, 2.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, 0.2 mM EGTA and 11.1 mM glucose

• model B – solution containing Ca^{2+} – EGTA – PSS (PSS)– 71 mM NaCl, 4.7 mM KCl, 1.7 mM $CaCl_2$, 28.4 mM $NaHCO_3$, 2.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, 0.2 mM EGTA and 11.1 mM glucose

To assess the effect of intracellular Ca^{2+} in vessel contraction, arteries were perfused with Ca-free Krebs solution, then examined drugs were added and the increase of perfusion pressure was measured (model A).

Afterwards, solution was replaced with fresh Ca-free Krebs solution and ANG II was added once again. Lack of vessel reaction indicated exhaustion of intracellular Ca^{2+}. 1.7 mM $CaCl_2$ was then added and the increase of perfusion pressure in response to examined drugs was measured to estimate the role of extracellular Ca^{2+} (model B).

Each experiment was repeated 12 times (n=12) or 9 times (n=9). EC_{50} and E_{max} values were estimated according to the modified receptor theory – Kenakin (2004) and Kenakin et al. (2006). Results were presented as means ±SD, and differences between means were compared using Student's t-test. Differences were considered significant at $p<0.05$. Statistical analysis was performed using the program Statistica 6.0 (StatSoft).

Results

Addition of the Rho-kinase inhibitor Y-27632 shifted the CRC for ANG II to the right and significantly reduced E_{max} to ANG II in a dose-dependant manner [Fig. 1].

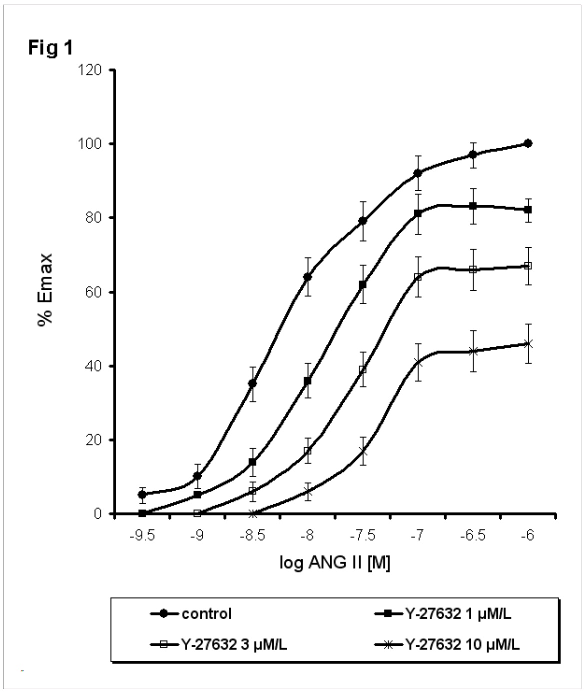

Fig. 1. Influence of increasing concentrations of Rho-kinase inhibitor Y-27632 on concentration-response curves for ANG II of perfused human mesenteric arteries. Results presented as percentage of maximal response, means ±SE, n=12, p<0.001non-significant

Thirty minutes of hypoxia reduced the reaction of arteries to ANG II and shifted the CRC to the right. Conversely, reperfusion with oxygenated Krebs solution increased E_{max} to ANG II and shifted the CRC to the left [Fig. 2].

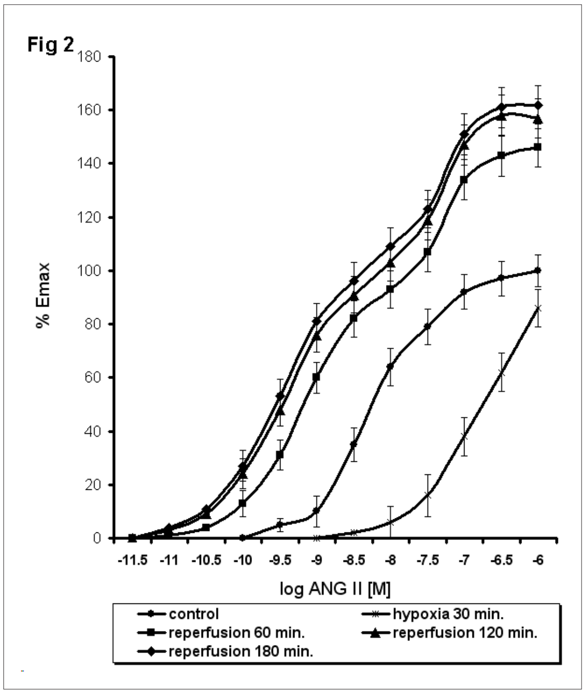

Fig. 2. Influence of 30 minutes of hypoxia and reperfusion for 60, 120 and 180 minutes on concentration-response curves for ANG II of perfused human mesenteric arteries. Results presented as percentage of maximal response, means ±SE, n=12

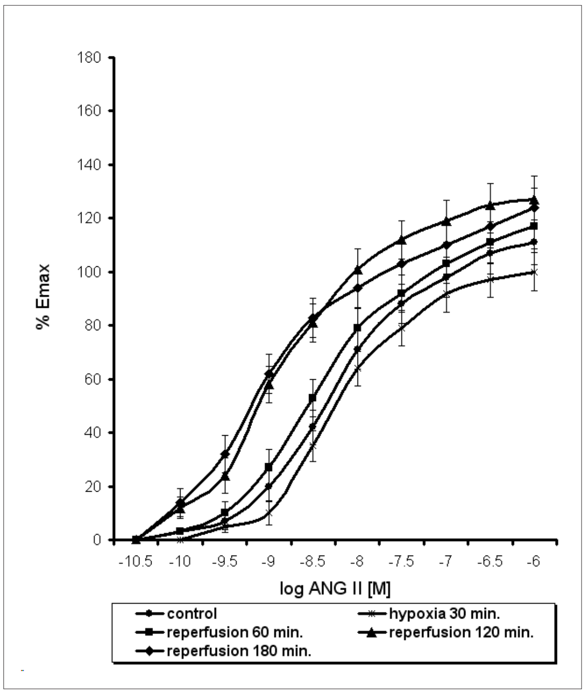

Fig. 3. Influence of Rho-kinase inhibitor Y-27632 (10 µM) on concentration-response curves for ANG II after 30 minutes of hypoxia and reperfusion for 60, 120 and 180 minutes. Results presented as percentage of maximal response, means ±SE, n=12

The Rho-kinase inhibitor Y-27632 significantly reduces the reperfusion-induced increase in the reaction of arteries to ANG II. This effect depends on the reperfusion time: in the presence of Y-27632 (10 μM/L) reperfusion shorter than 60 minutes did not cause a statistically significant shift of the CRC for ANG II to the left, although it still reduced the reperfusion-induced increase in reaction to ANG II [Fig. 3]. The CRC for ANG II was shifted to the left after 120 minutes of reperfusion.

fter hypoxia (30 and 60 minutes), perfusion pressure remained low, even in the presence of ANG II, both in FPSS and PSS. ANG II induced perfusion pressure increased with time of reperfusion with higher maximal values in experiments with PSS and in those with 60 minutes of hypoxia. The Rho-kinase inhibitor Y-27632 repressed the reperfusion-induced increase of reaction to ANG II, both in solution containing Ca^{2+} and in Ca^{2+}-free solution [Fig. 4, 5].

Discussion

The aim of this research was to investigate the role of Rho-kinase in ischemia/reperfusion induced artery hypersensitivity to ANG II by estimating the influence of Rho-kinase inhibitor on the smooth muscle reaction induced by ANG II and effects of ischemia/reperfusion injury, including the role of intra- and extracellular Ca^{2+}. The arteries used in the experiment were harvested together with organs and stored in the same conditions, thus being a model of transplanted organ arteries' reaction to ischemia/reperfusion injury. In our previous research, we have studied other aspects of ischemia/reperfusion injury [30,31] and successfully used this model [28]. Previous research suggests that ANG II increases cytosolic Ca^{2+} level by IP_3-dependent release from endoplasmic reticulum and activation of IP_3-independent membrane Ca^{2+} ion channels and influx of extracellular Ca^{2+} [32]. G-protein coupled receptor induced vascular smooth muscle contraction depends on the level of myosin light chain (MLC) phosphorylation, which is determined by the balance between activity of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) [22,29]. MLCK is activated by the Ca^{2+}-calmodulin complex. ANG II, by initiating the increase of intracellular Ca^{2+} concentration, activates MLCK, which phosphorylates myosin light chains and induces smooth muscle contraction. Inhibition of MLCP, by preventing dephosphorylation of MLC, increases the level of MLC phosphorylation for a given intracellular Ca^{2+} concentration and increases myofilament sensitivity to Ca^{2+}. The RhoA–Rho-kinase signal system is the main regulator of MLCP activity. Activated Rho-kinase inhibits MLCP and thus maintains MLC phosphorylation and contraction of smooth muscle, even at lower cytosolic Ca^{2+} levels. RhoA–Rho-kinase signal system agonists may also mediate Ca^{2+} flow into the cytoplasm [2,7,10,18,39]. Inhibition of Rho-kinase causes, as reported by Ishizaki et al. (2000), an increase in MLCP activity, dephosphorylation of MLC and relaxation of smooth muscle. Y-27632 may also cause relaxation by inhibiting agonist-induced Ca^{2+} transport and reduction of MLCK activity [11,16,19,23,24,37]. Research analyzing the influence of hypoxia and reperfu-

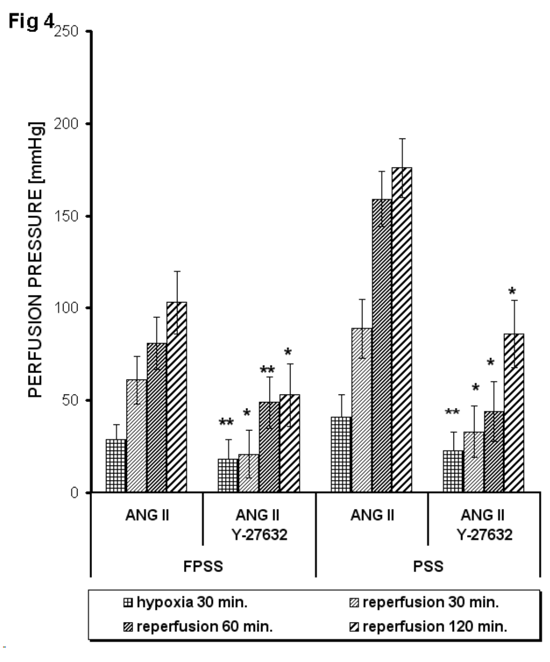

Fig. 4. Influence of Rho-kinase inhibitor Y-27632 (10 μM) on perfusion pressure after 30 minutes of hypoxia and reperfusion for 30, 60 and 120 minutes in Ca2+ and Ca2+-free solution. Results presented as means ±SE, n=9; *** $p < 0,05$; ** $p < 0,005$; * $p < 0,0001$; ns non-significant

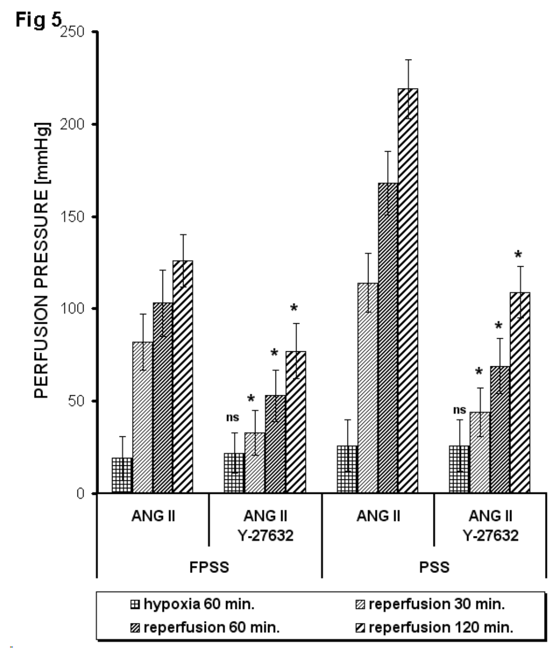

Fig. 5. Influence of Rho-kinase inhibitor Y-27632 (10 μM) on perfusion pressure after 60 minutes of hypoxia and reperfusion for 30, 60 and 120 minutes in Ca2+ and Ca2+-free solution. Results presented as means ±SE, n=9; *** $p < 0,05$; ** $p < 0,005$; * $p < 0,0001$; ns non-significant

sion on vessel contraction confirms the participation of two different signaling systems in ANG II induced increase of perfusion pressure. In our experiment, the Rho-kinase inhibitor Y-27632 caused a concentration-dependent decrease of contractile response to ANG II. The reaction of mesenteric arteries to ANG II in hypoxemic conditions was decreased and reperfusion with oxygenated solution caused a duration-dependent increase of the response to ANG II, CRCs for ANG II were shifted to the left and E_{max} values were increased – a model of hypersensitivity to ANG II occurring in transplanted organ arteries after ischemia/reperfusion. When these results are compared to ones under the same conditions, but in the presence of Y-27632 [Fig. 2, Fig. 3], the hypersensitivity, expressed as the time-dependent increase of perfusion pressure and maximal reaction, is still present (although not significant for reperfusion shorter than 60 min) but significantly reduced. In a Ca^{2+}-free environment, where influx of extracellular Ca^{2+} through membrane Ca^{2+} ion channels could not occur, the hypersensitivity to ANG II was less expressed – the perfusion pressure was lower [Fig. 4, Fig. 5], and it was also correlated with hypoxia time – higher perfusion pressure values were observed after longer hypoxia, which confirms the role of Ca^{2+} in this phenomenon. Inhibition of Rho-kinase significantly reduced hypersensitivity to ANG II caused by ischemia/reperfusion not only in Ca^{2+}-containing but also in Ca^{2+}-free solution. Inhibition of Rho-kinase and increase of MLCP activity induce relaxation of vessel smooth muscle, previously contracted in reaction to ANG II, and reduce hypersensitivity to ANG II caused by ischemia/reperfusion. Previous research has proven that Y-27632 has a similar effect on arteries isolated from human placenta [33]. ANG II activates the RhoA/Rho-kinase signaling system, activates MLCP and strengthens smooth muscle contraction. Agonists activating the RhoA/Rho-kinase signaling system may also mediate Ca^{2+} flow from the extracellular space into the cytoplasm. The presented results confirm participation of both PLC and IP_3 dependent and independent components in ischemia/reperfusion induced hypersensitivity to ANG II. The second component is also effectively inhibited by Y-27632, as was proven in the experiment with human mesenteric arteries. These experiments confirmed the role of Rho-kinase activation in reaction to ANG II and ischemia/reperfusion induced hypersensitivity. Similar results were obtained for human chorionic arteries [34]. The chain of reactions initiated by RhoA/Rho-kinase plays an important role in circulatory system pathophysiological processes, such as hypertension and arteriosclerosis [11,15,19,25,26,35]. It was proven that RhoA/Rho-kinase occupies a prominent place in the C-reactive protein signaling system, which is involved in arteriosclerosis and thrombosis, and that it is a factor sensitizing smooth muscle to Ca^{2+} as well as connected with the inflammatory cascade and initiating arteriosclerosis and vessel remodeling [3,4,17]. Inhibition of Rho-kinase causes dephosphorylation of MLC and thus reduces smooth muscle sensitivity to Ca^{2+}, which may be important in case of ischemia/reperfusion related Ca^{2+} overload. Therefore, Rho-kinase is a potentially attractive therapeutic target and inhibiting its activity may reduce the effect of ischemia/reperfusion injury in transplanted organs.

References

[1] Breitwieser G.E.: G protein-coupled receptor oligomerization: implications for G protein activation and cell signaling. Circ. Res., 2004; 94: 17-27

[2] Budzyn K., Marley P.D., Sobey C.G.: Targeting Rho and Rho-kinase in the treatment of cardiovascular disease. Trends Pharmacol. Sci., 2006; 27: 97-104

[3] Büssemaker E., Pistrosch F., Förster S., Herbrig K., Gross P., Passauer J., Brandes R.P.: Rho kinase contributes to basal vascular tone in humans: role of endothelium-derived nitric oxide. Am. J. Physiol. Heart Circ. Physiol., 2007; 293: H541-H547

[4] Chapados R., Abe K., Ihida-Stansbury K., McKean D., Gates A.T., Kern M., Merklinger S., Elliott J., Plant A., Shimokawa H., Jones P.L.: ROCK controls matrix synthesis in vascular smooth muscle cells: coupling vasoconstriction to vascular remodeling. Circ. Res., 2006; 99: 837-844

[5] Che Q., Carmines P.K.: Src family kinase involvement in rat preglomerular microvascular contractile and $[Ca^{2+}]i$ responses to ANG II. Am. J. Physiol. Renal Physiol., 2005; 288: F658-F664

[6] Drake M.T., Shenoy S.K., Lefkowitz R.J.: Trafficking of G protein-coupled receptors. Circ. Res., 2006; 99: 570-582

[7] Friel A.M., Sexton D.J., O'reilly M.W., Smith T.J., Morrison J.J.: Rho A/Rho kinase: human umbilical artery mRNA expression in normal and pre eclamptic pregnancies and functional role in isoprostane-induced vasoconstriction. Reproduction, 2006; 132: 169-176

[8] Ganesan L.P., Joshi T., Fang H., Kutala V.K., Roda J., Trotta R., Lehman A., Kuppusamy P., Byrd J.C., Carson W.E., Caligiuri M.A., Tridandapani S.: FcγR-induced production of superoxide and inflammatory cytokines is differentially regulated by SHIP through its influence on PI3K and/or Ras/Erk pathways. Blood, 2006; 108: 718-725

[9] Ganguli A., Persson L., Palmer I.R., Evans I., Yang L., Smallwood R., Black R., Qwarnstrom E.E.: Distinct NF-κB regulation by shear stress through Ras-dependent IκBα oscillations: real-time analysis of flow-mediated activation in live cells. Circ. Res., 2005; 96: 626-634

[10] Hilgers R.H., Webb R.C.: Molecular aspects of arterial smooth muscle contraction: focus on Rho. Exp. Biol. Med., 2005; 230: 829-835

[11] Ishizaki T., Uehata M., Tamechika I., Keel J., Nonomura K., Maekawa M., Narumiya S.: Pharmacological properties of Y-27632 a specific inhibitor of rho-associated kinases. Mol. Pharmacol., 2000; 57: 976-983

[12] Keef K.D., Hume J.R., Zhong J.: Regulation of cardiac and smooth muscle Ca^{2+} channels (Ca_V1.2a,b) by protein kinases. Am. J. Physiol. Cell Physiol., 2001; 281: C1743-C1756

[13] Kenakin T.: Principles: receptor theory in pharmacology. Trends Pharmacol. Sci., 2004; 25: 186-192

[14] Kenakin T., Jenkinson S., Watson C.: Determining the potency and molecular mechanism of action of insurmountable antagonists. J. Pharmacol. Exp. Ther., 2006; 319: 710-723

[15] Kimura K., Ito M., Amano M., Chihara K., Fukata Y., Nakafuku M., Yamamori B., Feng J., Nakano T., Okawa K., Iwamatsu A., Kaibuchi K.: Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science, 1996; 273: 245-248

[16] Laufs U., Liao J.K.: Targeting Rho in cardiovascular disease. Circ. Res., 2000; 87: 526-528

[17] Lee D.L., Webb R.C., Jin L.: Hypertension and RhoA/Rho-kinase signaling in the vasculature: highlights from the recent literature. Hypertension, 2004; 44: 796-799

[18] Lin K., Wang D., Sadée W.: Serum response factor activation by muscarinic receptors via RhoA novel pathway specific to M1 subtype involving calmodulin calcineurin and Pyk2. J. Biol. Chem., 2002; 277: 40789-40798

[19] Loirand G., Guerin P., Pacaud P.: Rho kinases in cardiovascular physiology and pathophysiology. Circ. Res., 2006; 98: 322-334

[20] Mehta P.K., Griendling K.K.: Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am. J. Physiol. Cell. Physiol., 2007; 292: C82-C97

[21] Ohtsu H., Dempsey P.J., Eguchi S.: ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. Am. J. Physiol. Cell Physiol., 2006; 291: C1–C10

[22] Ohtsu H., Mifune M., Frank G.D., Saito S., Inagami T., Kim-Mitsuyama S., Takuwa Y., Sasaki T., Rothstein J.D., Suzuki H., Nakashima H., Woolfolk E.A., Motley E.D., Eguchi S.: Signal-crosstalk between Rho/ROCK and c-Jun NH2-terminal kinase mediates migration of vascular smooth muscle cells stimulated by angiotensin II. Arterioscler. Thromb. Vasc. Biol., 2005; 25: 1831-1836

[23] Ratz P.H., Berg K.M., Urban N.H., Miner A.S.: Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. Am. J. Physiol. Cell Physiol., 2005; 288: C769-C783

[24] Rikitake Y., Liao J.K.: ROCKs as therapeutic targets in cardiovascular diseases. Expert Rev. Cardiovasc. Ther., 2005; 3: 441-451

[25] Shiga N., Hirano K., Hirano M., Nishimura J., Nawata H., Kanaide H.: Long-term inhibition of RhoA attenuates vascular contractility by enhancing endothelial NO production in an intact rabbit mesenteric artery. Circ. Res., 2005; 96: 1014-1021

[26] Shimokawa H., Takeshita A.: Rho-kinase is an important therapeutic target in cardiovascular medicine. Arterioscler. Thromb. Vasc. Biol., 2005; 25: 1767-1775

[27] Slupski M., Szadujkis-Szadurski L., Grześk G., Szadujkis-Szadurski R., Szadujkis-Szadurska K., Wlodarczyk Z., Masztalerz M., Piotrowiak I., Jasiński M.: Guanylate cyclase activators influence reactivity of human mesenteric superior arteries retrieved and preserved in the same conditions as transplanted kidneys. Transplant. Proc., 2007; 39: 1350-1353

[28] Somlyo A.P., Somlyo A.V.: Ca^{2+}-sensitivity of smooth and non-muscle myosin II: modulation by G proteins kinases and myosin phosphatase. Physiol. Rev., 2003; 83: 1325-1358

[29] Szadujkis-Szadurska K., Slupski M., Szadujkis-Szadurski R., Jasinski M., Grześk G., Matusiak G.: Modulation of the reaction of vascular smooth muscle cells to angiotensin II induced by catalase and aminotriasol during ischemia-reperfusion. Transplant. Proc., 2010; 42: 1614-1617

[30] Szadujkis-Szadurska K., Slupski M., Szadujkis-Szadurski R., Szadujkis-Szadurski L., Jasiñski M., Kolodziejska R.: The role of the endothelium in the regulation of vascular smooth muscle cell contractions induced by angiotensin II after ischemia and reperfusion. Arch. Pharm. Res., 2010; 33: 1019-1024

[31] Szadujkis-Szadurska K., Szadujkis-Szadurski L., Szadujkis-Szadurski R., Grześk G.: Modulation of the reactivity of the vascular smooth muscle cells on angiotensin II (ANG II) during ischemia/reperfusion (I/R). Eur. J. Clin. Invest., 2006; 36, suppl. 1: 4

[32] Szadujkis-Szadurski L., Szadujkis-Szadurski R., Szadujkis-Szadurska K., Skublicki S., Szymański W.: Nitric oxide induces dilation of human chorionic via inhibition of Rho-kinase signalling. Fund. Clin. Pharmacol., 2004; 18, suppl. 1: 77

[33] Szadujkis-Szadurski R., Szadujkis-Szadurska K., Szadujkis-Szadurski L., Skublicki S., Szymański W.: Angiotensin II-evoked Ca^{2+} release and influx responses are inhibited by nitric oxide in human chorionic arteries. Pol. J. Pharmacol., 2004; 56, suppl.: 199

[34] Thompson-Torgerson C.S., Holowatz L.A., Flavahan N.A., Kenney W.L.: Cold-induced cutaneous vasoconstriction is mediated by Rho kinase in vivo in human skin. Am. J. Physiol. Heart Circ. Physiol., 2007; 292: H1700-H1705

[35] Uehata M., Ishizaki T., Satoh H., Ono T., Kawahara T., Morishita T., Tamakawa H., Yamagami K., Inui J., Maekawa M., Narumiya S.: Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature, 1997; 389: 990-994

[36] Volpe M., Tocci G., Savoia C.: Angiotensin II receptor blockers and coronary artery disease: 'presumed innocents'. Eur. Heart J., 2006; 27: 1506-1507

[37] Wang Q.S., Zheng Y.M., Dong L., Ho Y.S., Guo Z., Wang Y.X.: Role of mitochondrial reactive oxygen species in hypoxia-dependent increase in intracellular calcium in pulmonary artery myocytes. Free Radic. Biol. Med., 2007; 42: 642-653

[38] Wang Z., Jin N., Ganguli S., Swartz D.R., Li L., Rhoades R.A.: Rho-kinase activation is involved in hypoxia-induced pulmonary vasoconstriction. Am. J. Respir. Cell Mol. Biol., 2001; 25: 628-635

[39] Yin J., Jin L., Ying Z., Webb R.C.: Activation of NADPH oxidase contributes to RhoA/Rho-kinase dependent contraction induced by angiotensin II. Hypertension, 2004; 44: 560

The authors have no potential conflicts of interest to declare.