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# ROCKing the JAKs

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**The endocrine cytokine leptin is mainly secreted by white adipose tissue and plasma leptin levels positively correlate with body fat mass. Via its action on neurons in the hypothalamic arcuate nucleus (ARC), leptin regulates body weight by stimulating energy expenditure and inhibiting food intake. The main signaling pathway of the leptin receptor is the JAK2-STAT3 pathway. A recent publication of Huang et al. in *Nature Neuroscience* shows that leptin's hypothalamic signaling via JAK2 requires the kinase ROCK1 (Rho-associated coiled-coil-containing protein kinase 1). ROCK1 directly phosphorylates JAK2, and this phosphorylation is required for the JAK2-STAT3 pathway of the leptin receptor. Gene deletion of ROCK1 in ARC neurons targeted by leptin makes these neurons less sensitive to leptin. This is reflected by a pronounced weight gain with hyperphagia, reduced locomotor activity, and increased fat accumulation. In this article we comment on the article of Huang et al. While the mechanism of ROCK1 activation in the neurons remains uncharacterized for the moment, a literature survey suggests that the interplay between ROCK1 and a JAK kinase may be a common theme for receptors that function via a JAK2 and even for other members of the JAK kinase family.**

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Human obesity is associated with leptin resistance with increased circulating levels of leptin but a decreased sensitivity of the hypothalamus toward leptin's actions.[1] Because of this central role in energy homeostasis, the mechanisms of leptin signaling and leptin resistance have been intensively studied. The leptin receptor is a type I cytokine receptor that functions via a JAK2-STAT3 pathway. Other important pathways include the SHP2/Erk (protein-tyrosine phosphatase 2C/extracellular signal regulated kinase) and PI3K/mTor (phosphatidylinositol-3-kinase/mammalian target of rapamycin) pathway.[1] A recent paper in *Nature Neuroscience* by Huang et al.[2] now also demonstrates a critical role of the serine/threonine kinase ROCK1 in hypothalamic leptin signaling. In immortalized hypothalamic GT1–7 cells, ROCK1 phosphorylated JAK2 via a direct ROCK1-JAK2 interaction. Leptin treatment increased this ROCK1-JAK2 association and the concomitant phosphorylation. Dominant negative ROCK1 or a ROCK1 inhibitor both blocked the leptin induced phosphorylation of JAK2 and STAT3, SOCS3 (suppressor of cytokine signaling 3) expression and PI3 kinase-dependent FOXO1 (forkhead box O1) nuclear export. ROCK1 activity thus seems to be required for the leptin-induced activation of JAK2. These in cyto experiments were confirmed in vivo. In the hypothalamus of mice, ROCK1 strongly colocalized with phosphorylated STAT3 and the leptin receptor in ARC neurons. Leptin treatment induced ROCK1 activation via the leptin receptor. The role of ROCK1 in hypothalamic leptin sensitivity and body weight regulation could be further confirmed in ROCK1 gene knockout experiments. The main target site of leptin is the arcuate nucleus (ARC) in the hypothalamus, where leptin activates the anorexigenic POMC neurons and inhibits the orexigenic neurons that express agouti-related peptide and neuropeptide Y. Targeted deletion of ROCK1 in POMC

neurons induced weight gain due to hyperphagia, and the mice showed hyperleptinemia and mild insulin resistance. The ROCK1 KO POMC neurons lost the increase in neuronal activity normally induced by leptin, further confirming the important role of ROCK1 in leptin signaling. The animals showed a 50% reduction of leptin stimulated STAT3 phosphorylation in the POMC neurons, and intraperitoneal injection of high doses of leptin was unable to inhibit the increased food intake of these animals, confirming that ROCK1 deletion in the POMC neurons induces a leptin-resistant state. Targeted deletion of ROCK1 in the AgRP neurons also induced an increased body weight, and an increased body fat content. Knocking out ROCK1 in the hypothalamic ARC area via adenoviral delivery of Cre in *ROCK1*$^{loxP/loxP}$ mice increased food intake and body weight, while adenoviral overexpression of ROCK1 had the opposite effect.

In the study of Huang et al.,[2] the activation mechanism of ROCK1 remains unclear. ROCK1 activity in the JAK2/ROCK1 complex is upregulated by intracerebroventricular or intraperitoneal administration of high doses of leptin, but the leptin fluctuations by fasting and refeeding did not seem to affect ROCK1 activity. It is thus unclear whether physiologically relevant fluctuations in leptin levels are also able to affect the ROCK1 activation status and whether ROCK1 is a downstream target of the activated leptin receptor in a physiologically relevant context.[2] However, the study clearly shows that ROCK1 is a required positive regulator of JAK2 in hypothalamic leptin signal transduction with an important role in body weight regulation. ROCK1 may be implicated in leptin resistance and may even form a future therapeutic target for obesity treatment.[2]

Besides its central hypothalamic effects, leptin signals via the leptin receptor on numerous peripheral cell types to modulate diverse functions including bone remodeling, onset of puberty, and immune functions. The role of ROCK1 on JAK2-STAT3 and PI3K signaling probably applies to these peripheral leptin receptor signaling processes as well. Several studies report that leptin activates RhoA (Ras homolog gene family, member A)/ROCK in a JAK2-dependent way in peripheral cells. In chondrocytes, leptin induces a RhoA/ROCK/LIM domain kinase/cofilin-2 pathway that leads to cytoskeletal reorganization.[3] In colon cancer cell lines, leptin was shown to activate RhoA, and this activation is inhibited by the JAK2 inhibitor AG490, suggesting that JAK2 may be upstream of RhoA and ROCK activation in these cells.[4] Leptin induces hypertrophy of cardiomyocytes and vascular smooth muscle cells.[5,6] In these cells, leptin activates RhoA and ROCK to alter actin dynamics. In rat neonatal cardiomyocytes, this activation was shown to be dependent on JAK2, PI3K and on mTOR, and a JAK2/PI3K/p70/mTOR/RhoA/ROCK pathway was proposed for RhoA and ROCK activation.[7] These studies all support the activation of ROCK by leptin as reported by Huang et al.[2] and suggest a possible role of JAK2 itself as an upstream activator of ROCK1. The involvement of a JAK2/PI3K/p70/mTOR/RhoA/ROCK pathway in hypothalamic leptin signaling cannot be excluded. As JAK2 and ROCK1 have been placed upstream and downstream of each other, JAK2 and ROCK1 may mutually enhance each other in leptin signaling.

The effect of ROCK1 on JAK2 signal transduction may apply to other receptors that signal via JAK2, including the growth hormone and Epo receptors. Both receptor systems can activate RhoA in a JAK2-dependent way. Growth hormone was shown to activate RhoA and ROCK in NIH-3T3 cells and the RhoA activation via displacement of RhoA from its negative regulator p190RhoGAP was dependent on JAK2.[8] These RhoA and ROCK activities were shown to be required for the growth hormone induced transcription via STAT5. The Epo receptor is expressed in several cancer cell lines. In a cervical cancer cell line, Epo stimulates activation of RhoA, and this activation is blocked by the JAK2 inhibitor AG490.[9] Cooperation between RhoA and JAK2 has also been reported for systems that are apparently not directly linked to cytokine receptors. Angiotensin II is a strong vasoconstrictor that acts on the blood vessel smooth muscle cells. The angiotensin II receptor is a G protein coupled receptor that activates RhoA and ROCK.[10,11] Surprisingly, the activation of RhoA/ROCK by angiotensin is mediated by JAK2, which phosphorylates and activates the RhoA GEF ARHGEF1.[10,11] STAT3 has been reported as an imported player in the oncogenic transformation induced by RhoA.[12] RhoA can activate STAT3 in a JAK2-dependent way, and a RhoA-JAK2-STAT3 pathway was proposed for this system,[12] reminiscent of the stimulating effect of ROCK1 on JAK2 in hypothalamic leptin signaling proposed by Huang et al.[2] In summary, it seems that RhoA and ROCK can be placed upstream and downstream of JAK2 in different signal transduction systems and a reciprocal activation may be possible.

It is tempting to speculate that the reciprocal interplay with ROCK1 might apply for other JAK kinases. A direct association between the JAK kinase TYK2 and ROCK1 has been reported.[13] ROCK1 was shown to be activated by JAK1 in carcinoma associated fibroblasts.[14] In these cells, the activated Rho kinase promotes STAT3 phosphorylation, while a ROCK inhibitor reduced phosphorylation of STAT3, in line with a possible reciprocal interplay between JAK1 and RhoA/ROCK1.

In conclusion, the stimulatory effect of ROCK1 on JAK2 activation reported by Huang et al.[2] provides unexpected new insights in the mechanism of body weight regulation. This finding may very well apply to other JAK-STAT pathways as well, and further mechanistic and physiological investigations are needed to obtain a more comprehensive insight into the role of RhoA and ROCK1 in cytokine signaling. Huang et al. proposed ROCK1 as a possible therapeutic target for obesity treatment.[1] This would require direct or indirect activation of ROCK1. ROCK1 is involved in many physiological and pathophysiological processes and ROCK1 inhibitors are used or tested in treatment of cancer, heart failure, pulmonary hypertension, hemorrhage, neurodegenerative diseases, and diabetes.[15] Activation of ROCK1 for obesity treatment may therefore require a specific targeting to the correct hypothalamic areas to avoid severe side effects.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## References


1. Wauman J, Tavernier J. Leptin receptor signaling: pathways to leptin resistance. Front Biosci 2011; 16:2771-93; PMID:21622208; http://dx.doi.org/10.2741/3885
2. Huang H, Kong D, Byun KH, Ye C, Koda S, Lee DH, et al. Rho-kinase regulates energy balance by targeting hypothalamic leptin receptor signaling. Nat Neurosci 2012; 15:1391-8; PMID:22941110; http://dx.doi.org/10.1038/nn.3207
3. Liang J, Feng J, Wu WK, Xiao J, Wu Z, Han D, et al. Leptin-mediated cytoskeletal remodeling in chondrocytes occurs via the RhoA/ROCK pathway. J Orthop Res 2011; 29:369-74; PMID:20886658; http://dx.doi.org/10.1002/jor.21257
4. Jaffe T, Schwartz B. Leptin promotes motility and invasiveness in human colon cancer cells by activating multiple signal-transduction pathways. Int J Cancer 2008; 123:2543-56; PMID:18767036; http://dx.doi.org/10.1002/ijc.23821
5. Zeidan A, Javadov S, Karmazyn M. Essential role of Rho/ROCK-dependent processes and actin dynamics in mediating leptin-induced hypertrophy in rat neonatal ventricular myocytes. Cardiovasc Res 2006; 72:101-11; PMID:16901475; http://dx.doi.org/10.1016/j.cardiores.2006.06.024
6. Zeidan A, Paylor B, Steinhoff KJ, Javadov S, Rajapurohitam V, Chakrabarti S, et al. Actin cytoskeleton dynamics promotes leptin-induced vascular smooth muscle hypertrophy via RhoA/ROCK- and phosphatidylinositol 3-kinase/protein kinase B-dependent pathways. J Pharmacol Exp Ther 2007; 322:1110-6; PMID:17562852; http://dx.doi.org/10.1124/jpet.107.122440
7. Zeidan A, Hunter JC, Javadov S, Karmazyn M. mTOR mediates RhoA-dependent leptin-induced cardiomyocyte hypertrophy. Mol Cell Biochem 2011; 352:99-108; PMID:21318349; http://dx.doi.org/10.1007/s11010-011-0744-2
8. Ling L, Lobie PE. RhoA/ROCK activation by growth hormone abrogates p300/histone deacetylase 6 repression of Stat5-mediated transcription. J Biol Chem 2004; 279:32737-50; PMID:15102857; http://dx.doi.org/10.1074/jbc.M400601200
9. Hamadmad SN, Hohl RJ. Erythropoietin stimulates cancer cell migration and activates RhoA protein through a mitogen-activated protein kinase/extracellular signal-regulated kinase-dependent mechanism. J Pharmacol Exp Ther 2008; 324:1227-33; PMID:18079357; http://dx.doi.org/10.1124/jpet.107.129643
10. Guilluy C, Brégeon J, Toumaniantz G, Rolli-Derkinderen M, Retailleau K, Loufrani L, et al. The Rho exchange factor Arhgef1 mediates the effects of angiotensin II on vascular tone and blood pressure. Nat Med 2010; 16:183-90; PMID:20098430; http://dx.doi.org/10.1038/nm.2079
11. Bernstein KE, Fuchs S. Angiotensin II and JAK2 put on the pressure. Nat Med 2010; 16:165-6; PMID:20134469; http://dx.doi.org/10.1038/nm0210-165
12. Aznar S, Valerón PF, del Rincon SV, Pérez LF, Perona R, Lacal JC. Simultaneous tyrosine and serine phosphorylation of STAT3 transcription factor is involved in Rho A GTPase oncogenic transformation. Mol Biol Cell 2001; 12:3282-94; PMID:11598209.
13. Kiian I, Tkachuk N, Haller H, Dumler I. Urokinase-induced migration of human vascular smooth muscle cells requires coupling of the small GTPases RhoA and Rac1 to the Tyk2/PI3-K signalling pathway. Thromb Haemost 2003; 89:904-14; PMID:12719789.
14. Sanz-Moreno V, Gaggioli C, Yeo M, Albrengues J, Wallberg F, Viros A, et al. ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. Cancer Cell 2011; 20:229-45; PMID:21840487; http://dx.doi.org/10.1016/j.ccr.2011.06.018
15. Zhou H, Li YJ. Rho kinase inhibitors: potential treatments for diabetes and diabetic complications. Curr Pharm Des 2012; 18:2964-73; PMID:22571664; http://dx.doi.org/10.2174/138161212800672688