REGULATION OF IL-6-SIGNALING AND INFLAMMATORY RESPONSE -ROLE OF INSULIN, FOXO1 AND PKCδ

Emelie Wallerstedt

The Lundberg Laboratory for Diabetes Research
The Sahlgrenska Academy at University of Gothenburg

The aim of this thesis was to investigate if and how insulin antagonizes interleukin-6 (IL-6)-signaling and action using both 3T3-L1 mouse adipocytes (*Paper I*) and HepG2 human hepatocytes (*Paper III*). We also investigated the importance of PKCδ in 3T3-L1 adipocytes and mouse embryonic fibroblasts (MEFs) lacking protein kinase C-δ (PKCδ-/-) (*Paper II*).

Obesity is associated with a low grade chronic inflammation in the adipose tissue as enlarged adipocytes and macrophage infiltration increase the secretion of inflammatory molecules, such as IL-6, which further enhance the inflammatory response in the adipose tissue and liver.

Insulin was found to exert an anti-inflammatory effect on IL-6-signaling in 3T3-L1 adipocytes by reducing the tyrosine phosphorylation of the transcription factor STAT3, increasing the serine phosphorylation of STAT3 and, furthermore, reducing the nuclear translocation and the transcriptional activity of STAT3. In addition, we found that insulin both induced activation of the phosphatase SHP2, which dephosphorylates STAT3, and synergistically increased gene expression of the suppressor of cytokine signaling (*Socs3)* and thus, impairing IL-6-signaling. These effects also reduced IL-6-induced gene expression of inflammatory genes such as serum amyloid A 3 *(Saa3)* and haptoglobin *(Hp)*. The effect of insulin was mediated through a MEK-mitogen-activated protein kinase (MAPK) pathway since PD98059 (MEK-inhibitor) reduced the inhibitory effects of insulin (*Paper I*).

The anti-inflammatory effect of insulin was also observed in HepG2 hepatocytes. Insulin reduced the IL-6-induced transcription of *SAA1*, *SAA2*, *HP*, plasmin activator inhibitor 1 (*PAI-1)* and *orosomucoid 1 (ORM1)*. However, the signaling mechanism for how insulin exerts its anti-inflammatory effect in HepG2 hepatocytes differed from that of 3T3-L1 adipocytes as insulin also stimulates nuclear exit of forkhead box O1 (FOXO1); a co-activator of STAT3 (*Paper III*).

Furthermore, the tyrosine and serine phosphorylation of STAT3 was found to be dependent on the serine and threonine kinase PKCδ, as the specific PKCδ inhibitor rottlerin reduced these phosphorylations in 3T3-L1 adipocytes. Consequently, the nuclear translocation of STAT3, the IL-6-induced gene transcription of *Socs3*, *Il-6*, *Saa3* and *Hp* as well as the protein secretion of SAA3, were all reduced. Furthermore, PKCδ was found to translocate to the nucleus following IL-6 and this was reduced by rottlerin. In agreement with the effect of rottlerin, PKCδ-/- MEFs also displayed a markedly reduced ability of IL-6 to activate the gene transcription of *Saa3*, *Hp, Socs3* and *Il-6* genes when compared to wild type (wt) MEFs. These results associated with a reduced nuclear translocation and phosphorylations of STAT3 (*Paper II*).

In conclusion, we have found that insulin exerts anti-inflammatory effects by antagonizing IL-6-signaling and action in both 3T3-L1 adipocytes and HepG2 heptocytes. PKCδ was also found to play an important role in STAT3 activation and for IL-6-induced inflammation in 3T3-L1 adipocytes while FOXO1 seems of importance as a co-activator in HepG2 cells. Future studies should be focused on the interplay between PKCδ and FOXO1, which can increase our knowledge of cytokine-induced inflammation and development of new anti-inflammatory treatments.

Keywords: Type 2-Diabetes, inflammation, obesity, IL-6, STAT3, PKCδ, FOXO1

ISBN: 978-91-628-8040-8 Göteborg 2010

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AKADEMISK AVHANDLING

Som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs universitet kommer att offentligheten försvaras i Hörsal 2118, Hus 2, Hälsovetarbacken, Arvid Wallgrens Backe, Göteborg

Fredagen den 16 april 2010, kl.13.00

av

EMELIE WALLERSTEDT

Fakultetsopponent: Professor Giorgio Sesti
Department of Experimental and Clinical Medicine
University Magna Graecia of Catanzaro, Catanzaro, Italy

Avhandlingen baseras på följande arbeten:

I. Andersson CX, Sopasakis VR, Wallerstedt E, Smith U. **Insulin antagonizes interleukin-6 signaling and is anti-inflammatory in 3T3-L1 adipocytes**. J Biol Chem. 2007 Mar 30;282(13):9430-5.

II. Wallerstedt E, Smith U, Andersson CX. **Protein kinase C-δ is involved in the inflammatory effect of IL-6 in mouse adipose cells**. Diabetologia. 2010 Feb 12. [Epub ahead of print].

III. Wallerstedt E, Sandqvist M, Smith U, Andersson CX. **Anti-inflammatory effect of insulin in the human hepatoma cell line HepG2**. Submitted 2010.

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