Z603 Identification and cDNA Cloning of the Long Form of the Leptin Receptor (OB-Rb) from Rat Splenocytes

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The *ob* gene product, leptin, is a small non-glycosylated protein primarily involved in food intake and energy expenditure. In the rat, 6 different isoforms of leptin receptors (Ob-R) have been described, of which the receptor with the long cytoplasmic tail, OB-Rb, is believed to be responsible for the transduction of leptin-mediated signals. While OB-Rb expression in the brain has been repeatedly confirmed, so far, its distribution in the periphery is not definitively established. Since recent reports indicate a possible role of leptin also in the immune system, it was questioned whether OB-Rb expression is also detectable in rat spleen. To prove this, RT-PCR was performed, and, for the first time, the OB-Rb cDNA was physically cloned from splenocytes. Nucleotide sequence analysis of the cloned cDNA and further search for OB-Rb expressing cells in the spleen revealed that leptin might indeed serve as a potential immunomodulatory molecule.

Z604 Recombinant Expression of Biologically Active Rat Leptin in *Escherichia coli*

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Leptin is a 16-kDa non-glycosylated hormone, which acts primarily in the hypothalamus to reduce food intake and body weight. While the rat is a representative laboratory animal model in obesity research, so far recombinant rat leptin was scarcely available. In the present study, rat leptin was recombinantly expressed and purified to provide a further tool for its functional analysis. For this, leptin cDNA was cloned by RT-PCR, and overexpression was achieved by cloning the leptin cDNA into the pET-29a vector, which resulted in an S-tagged fusion protein. Since the fusion proteins were expressed in inclusion bodies, after purification, leptin proteins were refolded by sequential dialysis into physiological buffers. The biological activitiy of this recombinant protein was confirmed in a proliferation assay using leptin sensitive cells, and the specific binding of the S-tagged leptin to these cells were further shown by flow cytometric analysis.