6 Summary

The adipose tissue derived hormone leptin plays an important role in the regulation of body weight in mammals. Almost all human patients suffering from morbid obesity however are resistant to the weight-lowering effects of leptin and thus show an impaired control of body fat mass. The underlying mechanisms are not yet completely understood but an impaired signalling of the leptin receptor is thought to contribute to this defect. It was the objective of this thesis to investigate the molecular mechanisms of leptin resistance.

The functional isoform of the leptin receptor (LEPRb) is a homo-dimeric membrane protein that is closely related to cytokine receptors of the class I family. Once the ligand has bound, these receptors activate the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway, which results in transcriptional activation of specific target genes. One of these genes encodes suppressor of cytokine signalling 3 (SOCS3) which attenuates the signalling of the LEPRb by binding to tyrosine 985 of this receptor and inhibition of the Janus kinase. In addition to this quite well established feedback inhibition, there are reports of a contribution of phosphatases like protein tyrosine phosphatase 1b (PTP1B) to the development of leptin resistance.

The negative regulation of the LEPRb was investigated by stable or transient transfection of three different cell lines (RINm5F, HIT and HepG2, respectively). All these cell lines showed a rapid downregulation of leptin-induced tyrosine phosphorylation of STAT3 within two hours of leptin stimulation. Furthermore, after a stimulation of 24 hours the cells were desensitized to further treatment with leptin. Taken together, these cell lines showed a negative regulation induced by leptin and were therefore regarded as a suitable model system for the investigation of leptin resistance under conditions of chronically elevated levels of leptin. In surface binding assays using labelled leptin I showed a slightly reduced membrane expression of LEPRb after two hours of leptin stimulation, which could not completely account for the observed downregulation. The effects of SOCS1 or SOCS3 overexpression on LEPRb signal transduction were investigated by reporter gene assays with a STAT3 responsive promoter. As expected, an inhibition of the LEPRb signal transduction by SOCS3 was observed. However, the equally potent inhibition of LEPRb signalling by SOCS1 has not been shown before. In Western blot experiments, we found a

contribution of tyrosine (Y) 1077 to the downregulation of the LEPRb and confirmed the well known role of Y 985 in this regulation. The inhibitory effects of SOCS3 and SOCS1 were investigated by overexpression in reporter gene assays. Here we found either tyrosine 985 or 1077 of the LEPRb sufficient to mediate the inhibitory effect of SOCS3. This finding corresponded very well to the requirement of either one of these tyrosines for attenuation of STAT3 phosphorylation and corroborates the suggestion that SOCS3 is mainly responsible for the downreglation of the LEPRb. In contrast, SOCS1 was only dependent on Y 985 to inhibit LEPRb signal transduction.

It was shown in this work by the use of the inhibitors of protein expression actinomycin D and cycloheximide that the downregulation of LEPRb signal transduction was dependent on active gene expression.

By Northern blot analysis SOCS3 mRNA but not the SOCS1 mRNA level was found to increase by leptin treatment of RINm5F cells. However, it cannot be ruled out that the activation of a different receptor system could lead to an increase in SOCS1 gene expression and thereby interfere with the LEPRb signal transduction. In this thesis such a „cross desensitization“ of the LEPRb could in fact be demonstrated as a result of erythropoietin receptor stimulation.

The activity of the LEPRb-associated Janus kinases was investigated in kinase assays. We demonstrated a reduced activity of these co-immunoprecipitated kinases after two hours of leptin stimulation but no change in the phosphorylation status of the kinases. This finding indicates that the JAKs are not downregulated by dephosphorylation at least in our model and argues against a function of phosphatases in the short term downregulation of leptin signalling.

Another issue of this thesis was the investigation of the specificity of the LEPRb for the associated Janus kinases. We demonstrated in fibrosarcoma cell lines specifically deficient for either JAK1 or JAK2, that the LEPRb is able to recruit JAK1 or JAK2 for signalling. This finding was also strengthened by JAK2 knockdown experiments, but is in contrast to published data that the LEPRb signal transduction was dependent on the presence of JAK2. Chimeric leptin receptors constructs containing the JAK binding region of the interleukin-6 receptor or the prolactin receptor showed greatly enhanced specifitity of JAK recruitment. These experiments defined a region of 83 amino acids which determines the specificity of JAK binding. This region of 83 amino acids length could be further investigated in these JAK specific LEPRb mutants.