5. Summary

POMC is the precursor of different biologically active peptides like ACTH and END and is produced in the pituitary and in non-pituitary tissues such as immune cells. END is released from immune cells migrating into inflamed tissue which can attenuate inflammatory pain by activating opioid receptors on peripheral terminals of sensory neurons. While all biologically active POMC-derived peptides such as END are encoded by exon 3, the signal peptide required for the entry of the nascent polypeptide into the regulated secretory pathway, is encoded by exon 2 of the POMC gene. However, the expression of signal sequence-encoding POMC mRNA in lymphocytes has long been a matter of debate which is related to the predominant expression of 5`-end truncated transcripts that do not encode the signal sequence. Hypothesizing that the transcription of such POMC mRNA is enhanced in lymphocytes under pathological situations the present study investigated POMC mRNA expression and END levels in the draining LN during the development of a local painful paw inflammation in rats. Using RT- and RACE-PCR several POMC mRNA molecules were identified. Besides detection of low levels of full-length POMC mRNA comprising exons 1, 2, and 3, various 5`-end truncated transcripts were expressed in lymphocytes. In accordance with previous studies none of these truncated POMC mRNAs encoded the signal sequence. While levels of truncated POMC mRNA remained unchanged, qRT-PCR analysis showed that exon 2-3 spanning POMC mRNA levels increased in lymphocytes 2 h after induction of paw inflammation. In the pituitary, the expression level of exon 2-3 spanning POMC transcripts was unaltered, indicating that POMC gene expression was not systemically enhanced. Immunofluorescence showed co-localization of POMC and END in LN cells and flow cytometry analysis showed that opioid peptides were expressed in T helper and cytotoxic T-cells, in B-cells and in monocytes/macrophages. By cell separation signal sequence-encoding POMC transcripts were identified in both T- and B-cells. The mRNAs of the POMC processing enzymes PC1/3 cleaving POMC into ACTH and β-LPH and PC2 that converts β-LPH into END were differentially expressed in T- and B-cells. T lymphocytes expressed only PC2 while the expression of both prohormone convertases was induced in B-cells after induction of paw inflammation. Radioimmunoassay analysis revealed that the amount of END increased more than

2-fold on a cellular base within the first 12 - 24 h of inflammation. Together these findings indicate that newly synthesized lymphocytic END can be attributed to an enhanced transcription of signal sequence-encoding POMC mRNA during inflammation. This provides an important missing link in the demonstration of a classical processing of POMC into biologically active peptides in immune cells. In accordance with findings in pituitary cells, signal sequence-encoding POMC mRNA seems to be translated and processed into END within 6 - 12 h in lymphocytes.