

**Effect of painful inflammation on proopiomelanocortin
gene expression and β -endorphin production in
lymphocytes**

**Einfluss einer schmerzhaften Entzündung auf die
Expression des Proopiomelanocortin-Gens und die
 β -Endorphin Produktion in Lymphozyten**

Dissertation

Zur Erlangung des akademischen Grades der
Doktorin der Naturwissenschaften

(Dr. rer. nat.)

eingereicht am Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

vorgelegt von

Melanie Busch

aus Berlin

April 2007

1. Gutachter: Prof. Dr. Michael Schäfer; Campus Benjamin Franklin der Charité Universitätsmedizin Berlin, Klinik für Anästhesiologie und operative Intensivmedizin
2. Gutachter: Prof. Dr. Volker Haucke; Freien Universität Berlin, Institut für Chemie und Biochemie

Disputation am 06. Juli 2007

Acknowledgements

I thank Prof. Michael Schäfer and Prof. Volker Haucke for their willingness to appraise this thesis. Furthermore, I want to thank Prof. Christoph Stein and Prof. Michael Schäfer who have given me the chance to work on this PhD project under their supervision. For the confidence they put on me I am very grateful.

I am also grateful to my first mentor Dr. Nicolle Sitte who showed enormous patience with me; her enthusiasm was always motivating. I thank all other members of the laboratory staff 'AG Prof. Stein & Prof. Schäfer'. All of them have contributed to create a warm and friendly atmosphere in the lab. Extra thanks go to Dr. Shaaban Mousa for his professional support in immunohistochemistry. The Drs. Dominika Labuz, Jana Droese, Alexander Brack, and Heike Rittner were always open for fruitful discussions. The three last mentioned moreover helped me with their expertise in flow cytometry. Thanks to our animal staff Gaby, Elfie, Francesco, and Thomas; without their help the entire project would not have been possible.

I like to thank Dr. Hans Krause (Charité Campus Benjamin Franklin, Urology) for the sequencing of a huge number of PCR products.

This PhD project was supported by the German science foundation (DFG) in the framework of the project '*Molecular mechanisms of opioid analgesia in painful inflammation*' (KFO 100/1) and by a fellowship in the course of the graduate research program '*The impact of inflammation on nervous system function*' (GRK 1258).

Special thanks to my fiancé Reyk Dienstfertig and to my mother Regina Busch for their constant support and encouragement. Thanks to my father Wolfgang Busch and to my entire family, as well as to my friends Nadine and Oliver Mohr who tolerated that I often had short time for visits but still stood to me.

Contents

0. Abbreviations.....	6
1. Introduction.....	7
POMC discovery, distribution and function.....	7
POMC mRNA expression in pituitary.....	11
POMC peptide processing in pituitary.....	13
POMC – relevance in opioid-mediated antinociception.....	15
POMC in immune cells.....	19
Objectives.....	23
2. Animals, Materials and Methods.....	24
Animals and animal housing.....	24
Materials.....	24
Bacterium.....	24
Enzymes.....	24
Radioactive labelled peptide.....	24
Antibodies.....	25
Chemicals, Kits and Media.....	25
Oligodeoxynucleotides.....	27
Plasmid vector.....	28
Technical and other materials.....	29
Methods.....	29
Induction of inflammation.....	29
Tissue preparation.....	30
Flow cytometry.....	31
Magnetic cell sorting (MACS).....	32
Total RNA preparations.....	32
RNA electrophoresis.....	33
RNA/DNA quantification and quality.....	33
PCR Primers.....	33
Two-step reverse-transcription polymerase chain reaction (RT-PCR).....	35
Rapid Amplification of cDNA Ends (RACE-PCR).....	35
Quantitative real-time RT-PCR (qRT-PCR).....	37
Agarose gel electrophoresis.....	38
Purification and ligation of PCR products.....	38
Transformation of <i>Escherichia coli</i> (<i>E. coli</i>).....	39
Isolation and purification of plasmid DNA.....	39
Sequencing.....	39
Immunofluorescence.....	40
Immunohistochemistry.....	40

Morphological identification of cell subsets.....	41
Intracellular staining of LN cells.....	41
Radioimmunoassay (RIA).....	42
<i>Ex vivo</i> LN cell stimulation experiments	43
Data processing.....	43
Statistical analysis.....	44
3. Results.....	46
Characterization of LN cell populations during inflammation.....	46
POMC mRNA expression in lymphocytes.....	48
RT-PCR and primer conditions.....	48
Expression of POMC mRNA including the signal sequence in lymphocytes.....	49
Expression of POMC mRNA in cell subsets of LN.....	50
Quantification of POMC mRNA.....	54
Expression profile of prohormone convertases in lymphocyte subsets.....	58
Identification of END-containing lymph node cells.....	59
Characterization of the polyclonal END antibody.....	59
Co-localization of ir-POMC and ir-END in lymphocytes.....	60
Magnitude of opioid peptide-expressing cell subsets in inflamed LN.....	62
Quantification of END in lymphocytes.....	65
Increased ir-END levels in regional LN cells during inflammation.....	65
CD4 ⁺ T-cells show the highest increase of ir-END during inflammation....	67
IL-1 β -induced POMC transcription and END synthesis in LN cells.....	68
4. Discussion.....	70
Expression of POMC mRNA in lymphocytes.....	70
Lymphocytes express full-length POMC mRNA during inflammation.....	70
Upregulation of signal sequence-encoding POMC mRNA.....	74
Relevance of truncated POMC mRNA.....	76
Distinct expression of prohormone convertases in lymphocyte subsets	78
END expression in LN lymphocytes during inflammation.....	80
POMC and END co-localize in T and B lymphocytes.....	80
END levels increase in lymphocyte subsets during inflammation.....	81
Future studies.....	85
5. Summary/Zusammenfassung.....	87
6. References.....	91
Curriculum vitae.....	106
Publications.....	107
Erklärung.....	108
Appendix.....	109

Abbreviations

ACTH	Adrenocorticotrope hormone
bp	Base pairs
BSA	Bovine serum albumin
CD	Cluster of differentiation
cDNA	copy DNA
CFA	Complete Freund's Adjuvant
CRH	Corticotropin releasing factor/hormone
DC	Dendritic cell
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
DTT	Dithio-1,4-threitol
END	beta-endorphin ₁₋₃₁
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
FC	Flow cytometry
FITC	Fluorescein isocyanate
Ig	Immunoglobulin
IL	Interleukin
Ir	immunoreactive
LPH	Lipotropin
LN	Lymph node(s)
M	DNA standard marker
MACS	Magnetic cell sorting
MHC	Major histocompatibility complex
MSH	Melanocyte stimulating hormone
mRNA	messenger ribonucleic acid
NC	Negative control
PBS	Phosphate buffered saline
PC	Prohormone convertase
PCR	Polymerase chain reaction
PE	R-Phycoerythrin
POMC	Proopiomelanocortin
PT	Pituitary
qRT-PCR	quantitative reverse transcriptase - polymerase chain reaction
RACE	Rapid amplification of cDNA ends
RIA	Radioimmunoassay
RNase	Ribonuclease
RPMI 1640	Roswell Park Memorial Institute 1640 medium
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase - polymerase chain reaction
SD	Standard deviation
SDS	Sodium dodecylsulfate
TBE	Tris boric acid (buffer)
TNF	Tumor necrosis factor