5 Summary

Cholinergic neurons use the neurotransmitter acetylcholine (ACh) and are involved in basic brain functions like learning and memory formation, awareness, the sleep and awaking cycle as well as motor behavior. Cholinergic neuron loss occurs during severe neurodegenerative diseases like Alzheimer or amyotrophic lateral sklerosis.

So far immunohistochemistry or *in situ* hybridization against the cholinergic marker enzyme choline acetyltransferase (ChAT) serve to identify cholinergic neurons. However, these methods require fixation of the tissue and thus preclude *in vivo* experiments. One aim of the present work was to produce a fusion protein between ChAT and the green fluorescent protein (GFP) which will enable to visualize the cholinergic marker without fixation.

Therefore the cDNA of ChAT was isolated from mouse spinal cord and subsequently the cDNA of GFP was cloned into a unique HindIII restriction site corresponding to the N-terminal part of the ChAT protein. The ChAT-GFP protein was expressed in COS-1 cells and primary cultures of hippocampal neurons as well as in hippocampal neurons of organotypic brain slice cultures. It was shown that the enzyme activity of the recombinant ChAT-GFP fusion protein is slightly reduced compared to the wildtype enzyme. However, it is still functional and similar distributed as the wildtype protein in cultured cells. In hippocampal neurons ChAT-GFP translocates into axonal processes and it is present in the presynaptic compartments.

Since it was shown that ChAT-GFP is functional like the wildtype enzyme the cDNA of GFP was targeted to the ChAT gene of mouse embryonic stem cells by homologous recombination. These $ChAT^{(GFP/+)}$ ES cells were injected into blastozysts to produce chimeric mice. This blastozyst transfer provided 14 chimeric animals. Hetero- and homozygous mice which emerge from $ChAT^{(GFP/+)}$ ES cells will express the ChAT-GFP fusion protein in all cholinergic cells and hence these cholinergic neurons should show endogenous fluorescence. In parallel another ES cell clone was produced in which the first coding exon of ChAT is flanked by loxP sites (genotype $ChAT^{(loxP/+)}$). This “knock-out” model will allow the conditional

deletion of the ChAT gene specifically in the cholinergic nervous system during later developmental stages.

A further aim was to establish organotypic brain slice cultures in order to study the physiology, development and degeneration of cholinergic neurons. With cultures prepared from ChAT-GFP targeted mice it will be possible to transfect cholinergic neurons. To this end a new method for electroporation of individual neurons, namely single-cell electroporation (SCE), was established and the efficiency of SCE was significantly improved. Using SCE, neuronal markers were expressed as GFP-fusion proteins in pyramidal neurons of organotypic hippocampal brain slice cultures and their distribution was compared with that of ChAT-GFP. It was shown that ChAT-GFP is present in the cytosol of the soma, dendritic spines and the axon but is absent from the nucleus and is at least in part associated with synaptic vesicles.

The combination of gene targeting by homologous recombination and the direct gene transfer by SCE opens new avenues to analyze signal transduction in the cholinergic system at a molecular level.