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**DISSERTATION SUMMARIES**

# Distribution of two molecules: KCC2, which plays a role in the postsynaptic responses evoked through $GABA_A$ receptors, and δ-subunit-containing $GABA_A$ receptors, which appoint neruogliaform cells in the neuronal network that can uniquely create the long-lasting postsynaptic $GABA_A$- and $GABA_B$ receptor mediated inhibition on target neurons

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The neuron-specific potassium-chloride cotransporter 2 (KCC2) plays a crucial role in adjusting intracellular $Cl^-$ concentrations. The lack of KCC2 in the plasma membrane of the axon initial segment (AIS) of pyramidal cells contributes to variable reversal potentials for perisomatic γ-aminobutyric acid $GABA_A$ receptor-mediated postsynaptic potentials, but the distribution of KCC2 in pyramidal dendrites remains to be established. We applied high-resolution pre-embedding immunolocalization to quantify KCC2 concentrations along dendritic, somatic and axonal regions of rat hippocampal principal cells. Confirming our results on neocortical pyramidal cells, membranes of AIS of CA1 pyramidal cells and dentate granule cells contained 6.4 ± 11.9% and 6.6 ± 14.1% of somatic KCC2 concentrations, respectively. Concentrations of KCC2 in basal dendritic shafts of stratum (str.) oriens were similar to somatic levels (109.2 ± 48.8%). Along apical dendritic shafts of CA1 pyramidal cells, the concentration of KCC2 showed a complex profile: normalized to somatic levels, the density of KCC2 was 124.5 ± 15.7%, 79 ± 12.4% and 98.2 ± 33.5% in the proximal and distal part of str. radiatum and in str. lacunosum moleculare, respectively. Dendritic spines of CA1 receiving excitatory inputs contained 39.9 ± 8.5% of KCC2 concentration measured in shafts of the same dendritic segments targeted by GABAergic inputs. Dendrites of dentate granule cells, the other glutamatergic cell type in hippocampus, showed higher KCC2 concentration compared with the soma (148.9 ± 54%), but no concentration gradient was detected between proximal and distal dendrites. In conclusion, the density of KCC2 in hippocampal principal cells increases along the axo-somato-dendritic axis with cell type-specific distribution profiles within the dendritic tree.

For the localization of delta subunit of $GABA_A$ receptors ($GABA_{AD}$) we used immunofluorescent method on the neocortex. In addition to a weaker neuropil labeling in supragranular layers presumably due to dendrites of pyramidal cells, a subset of interneurons was strongly positive for the $GABA_{AD}$ The identity of strongly $GABA_{AD}$immunopositive interneurons was initially tested by multiple immunoreactions showing that α-actinin2, known to be expressed by electrophysiologically identified neocortical neurogliaform cells, were present in 65±12 % of $GABA_{AD}$receptor containing cells. However, no overlap was found with interneuron markers such as parvalbumin, somatostatin, calbindin, calretinin and vasoactive intestinal polypeptide. In addition, immunocytochemical labeling patterns in the hippocampus were in line with earlier electrophysiological data showing relatively large tonic inhibition in hippocampal granule cells and molecular layer interneurons in the dentate gyrus and weaker currents in pyramidal cells of the hippocampus. In the CA1 and CA3 regions, a subset of interneurons at the border of stratum radiatum and lacunosum moleculare and another population of interneurons close to stratum pyramidale show strong immunolabeling for $GABA_{AD}$receptors. These interneurons presumably correspond to hippocampal neurogliafrom cells and ivy cells, respectively.

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# The molecular and metabolic connections of Hox1 hydrogenase in *Thiocapsa roseopersicina*

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Hydrogen is considered as the most promising fuel of the future, since it can be produced and oxidized without emission of green house gases. Biological systems offer easily reproducible biocatalysts for production of hydrogen. Several microorganisms, including both phototrophs and non-photosynthetic heterotrophs are able to metabolize molecular hydrogen by means of their hydrogenase or nitrogenase enzymes.

Our model organism, *Thiocapsa roseopersicina* is a Gram-negative, anaerob purple sulphur photosynthetic bacterium capable to produce molecular hydrogen as a byproduct during photochemolitoautotrophic growth. The strain can grow on inorganic substrates and utilize small organic molecules such as volatile acids and glucose. It contains four NiFe hydrogenases: two of them are soluble (Hox1 and Hox2) while the other two enzymes are attached to the membrane. The strain has complex sulphur metabolism it is able to fix molecular nitrogen and

to accumulate various storage materials. The deposition of storage compounds is a widespread strategy among microbes to survive when the nutrient or energy sources are depleted.

My study focuses on the cytoplasmic Hox1 hydrogenase (Rákhely et al. 2004) which is able to reduce protons and oxidize hydrogen *in vivo* depending on the redox status of the cell and environment. The enzyme consits of five subunits: Hox1Y and Hox1H are the small and large hydrogenase subunits, respectively; Hox1F and U are the diaphorase subunits, while Hox1E subunit is involved in the electron transport. The aim of this work is to clarify the physiological contexts of Hox1 hydrogenase; *i.e.* to examine its molecular/metabolic/redox connection to the storage materials and the bioenergetic membrane.

The Hox1 hydrogenase can produce hydrogen under illumination and in the dark. The hydrogen production requires excess electrons derived from e.g. thiosulfate under continuous illumination. On the basis of experimental and *in silico* data, it is hypothesized that the Hox1 hydrogenase is connected directly to photosynthetic membrane via the membrane-located NADH-ubiquinone oxidoreductase complex. The Hox1EFU subunits have remarkable sequence similarity to the NuoEFG subunits which are dissociable from the membrane and they have $NAD^+$ -reducing activity. According to our model, the Hox1EFU subunits can replace the NuoEFG subunits allowing Hox1 to function as a valve. When the central quinone pool is overreduced, excess electrons can be removed in the form of hydrogen by means of Hox1 hydrogenase, otherwise NADH is produced. In order to prove this model, a proteomic apporoach was chosen and affinity chromatography was used to identify interacting protein partners.

Hydrogen can also be produced in the dark through the Hox1 hydrogenase. In this case, the excess of electrons is supposed to arise from stored materials accumulated during photosynthetic growth. Depending on the nutrient supply during growth, *T. roseopersicina* can accumulate elementar sulphur, polyphosphate poly(3-hydroxyalkanoates) and glycogen. A systematic investigation of the physiology and hydrogen production of the cells indicated glycogen as a potential source of electrons for the Hox1 hydrogenase in the dark. The genome of the strain has been sequenced and genes coding for proteins involved in both glycogen synthesis and catabolism were identified. In order to confirm this metabolic connection, both the glycogen synthesis and breakdown were disrupted by genetic tools and a comparison of the hydrogen production and glycogen content of the mutant and the control strains revealed a metabolic linkage between the glycogen and hydrogen metabolism.

Rákhely G, Kovács AT, Maróti G, Fodor BD, Csanádi G, Latinovics D, Kovács KL (2004) Cyanobacterial-type, heteropentameric, NAD+-reducing NiFe hydrogenase in the purple sulfur photosynthetic bacterium Thiocapsa roseopersicina. Appl Environ Microbiol 70:722-728.

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## Diabetes-related structural, molecular and functional alterations in capillaries running in the vicinity of myenteric plexus in streptozotocin-induced diabetic rats

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It has recently been demonstrated that the nitrergic subpopulation of myenteric neurons is especially susceptible to developing neurodegenerative damage in diabetes. The nitrergic neurons located in different gut segments had different susceptibilities to diabetes. Their different responsiveness to insulin treatment had also been revealed, which suggest that the neuronal microenvironment is critical to evolving diabetic nitrergic neuropathy. Although the relationship between the presence of enteric neuropathy and impaired gastrointestinal motility in humans and also in rodent models are well documented, the impact of diabetes on capillaries within the intestinal wall has been completely overlooked until now.

Since the myenteric ganglia are not vascularizated, accordingly the mesenterial capillaries adjacent to the myenteric plexus play a key role to supply them. Therefore we supposed that diabetes-associated alterations, which influence the permeability of these capillaries, may be critical to developing enteric neuropathy observed in streptozotocin-induced diabetics. The diabetes-related endothelial dysfunction leads to decreased bioavailability of endothelial cell-derived nitric oxide and at the same time to increased amount of toxic free radicals before the clinical symptoms appear.

Therefore, the primary question of our study was whether diabetes influences the structural, molecular and functional properties of capillary endothelium closely related to the myenteric plexus.

Ten weeks after the onset of diabetes, different gut segments of control, streptozotocin-induced diabetic and insulin-treated diabetic rats were processed for electronmicroscopic and molecular studies. The thickness of basement membrane (BM) surrounding blood vessels and the size of the individual caveolar compartments were measured by electronmicroscopic morphometry. The quantitative features of blood-tissue exchange of endogenous albumin were investigated by postembedding immunohistochemistry. The quantitative changes in the expression of endothelial nitric oxide synthase (eNOS) and its negative regulatory protein, Caveolin-1 (CAV-1) were elucidated by postembedding immunohistochemistry, RT-PCR technique and western-blot analysis in the endothelium of microvessels around myenteric plexus.

Although the differences between the intestinal segments are well pronounced, region-specific thickening of BM and enlargement of caveolar compartments was demonstrated in diabetic animals. The amount of serum albumin taken up by the plasmalemmal vesicles