Nitric oxide (NO) plays a crucial role in neuronal signaling in a variety of eukaryotic and prokaryotic organisms. Nitric oxide synthases (NOS) are heme-containing monooxygenases that
catalyze the oxygen dependent oxidation of L-arginine to NO and L-citrulline. The NO produced by
NOS activity is a gaseous molecule that diffuses easily through membranes and acts inter or intracellularly. NO activates metal-containing enzymes, including soluble guanylate-cyclase (sGC)
that increase levels of the messenger molecule cyclic 3,5-guanosine monophosphate (cGMP) (1, 2)
which in turn mediate various pathophysiological or physiological functions in neurons. Nevertheless,
many aspects of nitrergic neurons and NO function in the central nervous system (CNS) are unclear.
The aim of research described in this thesis was to characterize neuronal NOS, proteins metabolically linked to NOS and NO signaling pathways in the CNS of *Aplysia cali/ornica (Aplysia),*
a popular experimental model in cellular and system neuroscience. The biochemical characteristics of
*Aplysia* NOS *(AcNOSj* described here revealed its calcium-/calmodulin-(Ca/CaM) and NADPH
dependence. A representative set of inhibitors for mammalian NOS isoforms also suppressed NOS
activity in *Aplysia* Polyclonal anti-rat nNOS antibodies hybridized with a putative purified /IcNOS
(160 kDa protein) from partially purified CNS homogenates in Western blot studies. This thesis
reports /IcNOS activity about six times lower than activity detected in the mammalian cerebellum (3),
but was comparable with average values reported for the insect brain (4, 5). Basal levels of cGMP
production in *Aplysia* CNS were determined. Stimulation with NO donors and incubation with PDE
(phosphodiesterase) inhibitors significantly increased cGMP levels. A specific inhibitor of sGC
reduced basal cGMP levels by half and prevented a rise of cGMP in the presence of NO, confirming
that NO may indeed function as molluscan CNS messenger, and that cGMP is one of its effectors.
The full length gene of/IcNOS was cloned and found to contain all of the conserved sites characteristic of a functional NOS in vertebrates. Nitrergic neurons in *Aplysia* CNS were mapped and
localized; around 2% of all central neurons were shown to be nitrergic by means of *in situ* hybridization (ISH) and immunohistochemistry.
A two-color ISH protocol for whole-mount *Aplysia* ganglia was optimized for this thesis work and used to identify neurons and directly correlate functional and protein expression data The
complete topographic organization of *Aplysia* mechanosensory neurons expressing neuropeptide
sensorin-A was thus assembled.

The effect of unilateral pedal nerve crush on the level of expression of NOS mRNA in *Aplysia* pedal neurons was investigated, to look at the functional implications of NO in nerve regeneration
and neuropathic pain. ISH and densitometry showed that the number of neurons and the intensity of
neuronal staining following unilateral pedal nerve crush was significantly reduced in cells on the
injured side, whereas a concurrent and significant increase of /IcNOS mRNA was detected in pedal
nerve axoplasm by RT-PCR.

Part of the thesis work concentrated on the identification of another functionally important putative heme-containing enzyme, the *Aplysia* thyroid peroxidase gene (/4cTPO). After cloning and
localization of /IcTPO in the *Aplysia* CNS, several transcripts from the thyroid hormone (TH) signaling pathway were identified suggesting the presence of TH-like signaling in molluscs as well.

The thesis theme of heme enzymes continued with an investigation of the protein necessary for the sixth step of the heme synthesis pathway, coproporphyrinogen oxidase (CPO). CPO was
cloned from *Aplysia* and other organisms *(Chlorophlexus auranliacus, E.coli).* The cloned CPO
genes were used to optimize protein crystallization conditions, leading to the first reported crystal
structure of human CPO. The crystal structure enabled some elucidation of the catalytic mechanism
of CPO and an understanding at the molecular level of the observed decrease in CPO enzymatic
activity in certain pathological mutations of hereditary coproporphyria.