MEDinPROT main topic:

**„The role of signaling proteins in inflammation and cancer”**

Title of the research project:

**„The regulation of interactions between calmodulin and the important vasomodulator enzimes eNOS and MLCK via sphingolipid mediators”**

Cardiovascular disorders, presenting serious public health problem in both the economically developed countries and the developing world, especially atherosclerosis and vascular complications in type-two diabetes can be considered as diseases initiated and progressed by inflammation, based on research of the last decade. During this period, research in physiology and pathophysiology resulted in the recognition of sphingolipid mediators among the important regulatory molecules of the immune and inflammatory processes. One of them, sphingosine-1-phosphate (S1P) acts primarily on cell-surface G protein-coupled receptors, while sphingosine, ceramide, and ceramide-1-phosphate particularly modulate cellular functions *via* interactions with intracellular signaling proteins. The results of preliminary experiments done by the applying research group of RCNS-HAS show that sphingosine inhibits endothelial nitric oxide synthetase (eNOS), a key enzyme regulating the tone and permeability of vasculature, due to its binding to calmodulin (CaM), thereby preventing the interaction of the two proteins, which is the basis for the activation of eNOS. Nitrogen monoxide (NO), the product of eNOS enzyme, under normal physiologic conditions is a vasorelaxant and attenuates inflammation and thrombus formation, while in certain pathologic conditions its increased production leads to the initiation and progression of inflammation. We assume, that a portion of the vascular effects of sphingosine and related sphingolipid mediators in inflammation is due to their direct influence on the activity of Ca2+-CaM-dependent enzymes. In the MedInProt project, we plan to investigate the molecular mechanisms of regulations elicited by the sphingolipids sphingosine, sphinganine, C2-cerimde, and C16-ceramide on two of such enzymes, eNOS and myosin light-chain kinase (MLCK), which controls the contraction of vascular smooth muscle cells. The synergy of research and methodology of the two research groups help us to verify the *in vivo* relevance of the *in vitro* identified inhibitory effect of sphingosine on CaM, to explore the regulatory effects of physiologically relevant sphingolipid relatives on the vascular tone, and finally to study the mechanisms of action of the vasoactive compounds.

