Bachelor thesis project of **Bernd Zetsche** (2005)

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Spatial and Temporal Dynamics of Intracellular cGMP in Response to Natriuretic Peptides in Vascular Smooth Muscle Cells.

The second messenger cyclic 3', 5'-guanosine monophosphate (cGMP) plays crucial rules in several biological functions such as smooth muscle dilation, neuronal plasticity or epithelial electrolyte transport and it is therefore not surprising that this molecule is a target of medical research.

Two classes of guanylyl cyclases (GC) are able to convert guanosine triphosphate (GTP) into cGMP. The first are the soluble guanylyl cyclases (sGC) localized in the cytoplasm of certain cells, which are activated by the gaseous nitric oxide radical (NO). The second class is the particular guanylyl cyclases (pGC), a family of seven isoforms with different functions. All pGCs are bound to the plasma membrane but only three of them can be activated by the natriuretic peptides ANP (atrial natriuretic peptide), BNP (brain natriuretic peptide) and CNP (C-type natriuretic peptide).

This report focuses on the intracellular cGMP concentration changes mediated through the natriuretic peptide sensitive isoforms A, B, and C of the pGC.

Intracellular cGMP elevations were measured in rat aorta smooth muscle cells (RASMC) through genetically encoded probes using fluorescence resonance energy transfer (FRET), so called cygnets (cyclic GMP indicators using energy transfer). These novel indicators allow spatial and temporal measurements of cGMP levels in living cells via fluorescence microscopy.

The first aim was to compare the cGMP responses mediated through different natriuretic peptide. ANP and BNP were used as activators for guanylyl cyclase-A (GC-A) and CNP served as activator for the guanylyl cyclase- B (GC-B). The results showed significant differences in the responses to ANP and BNP, both elicit equal amounts of cGMP with same kinetics. The cGMP responses mediated through CNP were less than those mediated through ANB/ BNP.

The second aim was to observe cGMP levels after treating the RASMCs with different activators of protein kinase C, which leads to an unknown mechanism of phosphorylation and subsequent desensitization of the GC-A/ GC- B receptors. The hormone angiotensin II (AII) which activates PKC through the stimulation of phospholipase C-β showed no effect on ANP mediated cGMP responses. In contrast, the direct activator of PKC, Phorbol-12-myristate-13-acetate (PMA), caused a significant reduction of cGMP levels mediated through ANP or CNP.