Abstract

By relaxing vascular smooth muscle, the intracellular messenger cyclic guanosine monophosphate (cGMP) plays an important role in blood pressure regulation. cGMP is formed by guanylyl cyclases (GC) and degraded by phosphodiesterases (PDE). In smooth muscle cells, two forms of GC exist, the nitric oxide-sensitive GC (NO-GC) and the membrane-bound natriuretic peptide receptor GC A and B. Three phosphodiesterases, PDE 1, PDE 3 and PDE 5, are thought to be responsible for cGMP degradation in these cells. In this thesis, the impact of PDE 3 and PDE 5 on NO- and natriuretic peptide (NP)-induced cGMP-signals in smooth muscle cells has been investigated.

A FRET-based cGMP indicator was applied in real-time imaging to analyze cGMP-signals because conventional methods, e.g. radioimmunoassays, are not sufficient to detect physiologically occurring cGMP signals. In these experiments, NO- and NP-concentrations that induced cGMP signals in the FRET measurements were found to be sufficient to relax aortic strips in organ bath experiments as well, demonstrating the functional relevance of the signals detected by the cGMP indicator cGi-6000.

The use of specific PDE-inhibitors in the FRET measurements revealed that NO- and NP-induced cGMP signals are differentially regulated by PDE 3 and PDE 5. Whereas PDE 5 - responsible for the reduction of higher cGMP concentrations - exhibited a comparable impact on NO and NP-induced cGMP signals in smooth muscle cells, PDE 3 - involved in the degradation of lower cGMP levels - displayed a preference for NP-stimulated cGMP signals.

Interestingly, cGMP was found to be not only degraded by PDEs but also transported out of the cell by multi-drug resistance-associated protein 4 (MRP4). As this transporter also pumps cAMP out of the cell, competition between cAMP and cGMP may mediate a cross-talk between those nucleotides. The effect of cGMP export on intracellular cGMP levels was as high as that of PDE 5-mediated degradation in FRET measurements. Accordingly, inhibition of cGMP export enhanced vascular relaxation as much as inhibition of PDE5 demonstrating the functional relevance of MRP4-mediated cGMP export. Whether cGMP transport affects cGMP signals in other cells or tissues as well or whether this mechanism
is restricted to smooth muscle cells will be an interesting question for future studies.

To allow FRET measurements in freshly dissociated cells and acute slices, a cGMP indicator knock-in mouse line was generated ubiquitously expressing cGi-500. This cGi-500 indicator possesses a lower EC50 value for cGMP (500 nM) and should therefore allow the detection of lower cGMP levels. In ventricular cardiac myocytes of these mice, CNP produced measurable cGMP signals which were not detectable with cGi-6000. Application of PDE inhibitors revealed exclusive control of CNP-induced cGMP signals by PDE 2. Whether PDE 2 mediates a cross-talk between cGMP and cAMP signals needs to be clarified in further studies.

In sum, besides cGMP degradation by PDEs, export of cGMP through MRP4 has a substantial impact on cGMP signals in smooth muscle cells and possibly mediates a cross-talk between cGMP and cAMP signals. Using the newly generated cGMP indicator knock-in mouse, cGMP signals can be analysed more sensitively in freshly isolated cells or acute slices.