7 Summary

Calcium channel modulators of dihydropyridine type increase the endothelial NO release
Development of a sensitive method for nitrite detection in biological samples

Most of the existing methods to measure NO are not sensitive enough to measure the permanent, basal NO formation taking place at the vascular endothelium. Therefore, this work presents how a procedure was developed and established which is sensitive enough to record the basal NO formation.
After the endothelial formation the short half-life of NO leads directly to a stoichiometric transformation to nitrite and in a further oxidative step (stoichiometric) to nitrate, so that nitrite and nitrate can cumulate in the incubation medium. The concentration of free NO remains below the detection level. With the use of a nitrate reductase nitrate is converted back to nitrite, which in turn is reduced in an acidic iodide solution to NO and measured with the ISO-NO electrode. Due to the stoichiometry of the reactions no NO is lost, so that a cumulative measurement of formed nitrate/nitrite, determines the formed NO quantity.

It was shown that this method of determinating the NO amount is sensitive (detection limit 0,1 µmol/l nitrite) and stable in comperison to other methods. No disturbances arise from proteins (≤ 1 mg/ml) or SH connections (≤ 1 mmol/l) as in griess-assay.

Basal NO formation rates for pig coronaries were determined at 350 pmol/10 mg tissue/30 min and for porcine aortic endothelial cells (PAEC) at 186 pmol/3x10^5 cells/30 min. Under influence of the nonspecific NO synthase (NOS) inhibitor N-methyl-l-arginine (L-NMMA) the nitrate/nitrite formation is significantly inhibited.

All calcium channel modulators of the dihydropyridine type (DHP) (Amlodipin, Bay K 8644, Bay O 5572, Bay W 9798, Nifedipin, Nisoldipin, Nitrendipin) examined in this study increased the basal nitrate/nitrite formation from the vascular endothelium. Amlodipin is effective in a concentration of 0,1 µmol/l, whereas the other DHP become effective at a 10-fold higher concentration only L-NMMA again inhibits the effect of Nifedipin (as a representative of the DHP).

Under influence of shear stress the basal nitrate/nitrite release is increased 3-4 fold both in the native blood vessel and in the endothelium cell culture. This increase is repressed by

means of L-NMMA. Nifedipin increases the nitrate/nitrite release under shear stress, whereby the relationship from basal release to stimulated release is not influenced.

The efficiency of the measurement of basal NO formation and NO formation under influence of shear stress as well as its application in different biological systems (e.g. including measurement of NO in the effluent of Langendorff-perused hearts) as described in this work offer a good alternative to the procedures used so far.