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Poster presentation

A luminescence-based assay for sensitive nitric oxide detection

Frank Wunder*[1], Guido Buehler[1], Jörg Hüser[1], Stefan Mundt[1], Martin Bechem[2] and Bernd Kalthof[1]

Address: [1]Lead Discovery Wuppertal, Bayer HealthCare AG, D-42096 Wuppertal, Germany and [2]Cardiovascular Research, Bayer HealthCare AG, D-42096 Wuppertal, Germany

Email: Frank Wunder* - frank.wunder@bayerhealthcare.com

* Corresponding author

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Nitric oxide (NO) plays an important role in the protection against the onset and progression of various cardiovascular diseases, including hypertension and atherosclerosis, which are associated with an apparently reduced NO bioavailability. Therefore, the NO/cGMP signaling pathway has gained considerable attention and has become a target for new drug development. We have established a rapid, homogeneous, cell-based and highly sensitive nitric oxide reporter assay which is suitable for ultra-high-throughput screening. In our coculture system, endothelial nitric oxide synthase (eNOS) mediated NO generation is monitored in living cells via soluble guanylyl cyclase (sGC) activation and calcium influx through the olfactory cyclic nucleotide-gated (CNG) cation channel CNGA2, acting as the intracellular cGMP sensor [1]. Using this NO reporter assay, a fully automated high-throughput screening campaign for stimulators of NO synthesis was performed. The coculture system reflects most aspects of the natural NO/cGMP signaling pathway. Namely, Ca^{2+}-dependent and Ca^{2+}-independent regulation of eNOS activity by G protein-coupled receptor agonists, oxidative stress, phosphorylation, and cofactor availability, as well as NO-mediated stimulation of cGMP synthesis by sGC activation. The reporter assay allows the real-time detection of NO synthesis within living cells and makes it possible to identify and characterize activators and inhibitors of enzymes involved in the NO/cGMP pathway.

References

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