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Poster presentation



# Targeting the NO – cGMP pathway: phenotyping of NO-insensitive sGCbeta1 H105F knockin mice

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## Introduction

Nitric oxide is a key signalling molecule that is involved in the regulation of a variety of biological and physiological processes. Soluble Guanylate Cyclase (sGC) is considered as the main receptor for this gaseous molecule, and catalyses the conversion of GTP to cyclic GMP. sGC is a heterodimer, consisting of an alpha1 or alpha2 subunit and a beta1 subunit. This beta1 subunit contains an evolutionary conserved N-terminal heme-binding domain. A prosthetic ferrous heme group, which is crucial for the sensing of NO, is positioned in this domain via its interaction with the axial ligand histidine-105 and a number of anchoring residues. Binding of NO to the $Fe^{2+}$ ion of the penta-coordinated histidyl-heme complex, eventually leads to cleavage of the heme-histidine bond. This cleavage is the molecular switch that leads to a ~200-fold activation of sGC. Removal of this heme moiety as well as its oxidation abolishes any NO-induced enzyme activation. To differentiate between the sGC-dependent and sGC-independent functions of NO, and to differentiate between heme-dependent and heme-independent activities of sGC, we generated heme-deficient sGCbeta1 H105F knock in (KI) mice in which sGC retains its basal activity, but can no longer be activated by NO. These mice might furthermore form a model for sGC with a non-functional heme group as in oxidative stress.

## Materials and methods

Full sGCbeta1 H105F knock in mice were generated using a classical approach by which the sGCbeta1 allele was replaced by a mutated allele by means of homologous recombination. As such, the codon for the histidine-105 residue of the sGCbeta1 subunit was replaced by a codon for a fenylalanine. Initially, mice were phenotyped on a mixed background of 129S6 × C57Bl/6J or 129S6 × CD1. Non-invasive basal systolic blood pressure (SBP) and heart rate (HR) measurements were performed in male and female wild-type (WT) and sGC beta1 H105F knock in mice with a tail-cuff pressure-recording device (Visitech BP-2000).

## Results

sGCbeta1 H105F knock in mice showed a reduced life span, gastro-intestinal tract abnormalities, and growth retardation. Basal SBP was higher in sGCbeta1 KI mice than in WT mice (142 ± 15 vs. 113 ± 8 mmHg, $P < 0.001$), while HR was lower in sGCbeta1 KI compared to their WT littermates (513 ± 53 vs. 639 ± 62 bpm, $P < 0.001$). Relaxation of precontracted aortic rings was impaired.

## Conclusion

The NO-activated state of sGC is necessary for the normal function of a number of important physiological processes in the body. It is important for the regulation of cardiovascular homeostasis, such as the control of blood pressure and heart rate. It is necessary for normal gastrointestinal tract function and development, and essential for normal growth and viability.