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Oral presentation

NO-insensitive sGCbeta1 H105F knockin mice: if NO has no place to go

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Background

Soluble guanylate cyclase (sGC) is a heterodimer, consisting of an alpha1- or alpha2-subunit and a beta1-subunit. Activation of sGC by NO/CO critically depends on the presence of a prosthetic ferrous heme group, linked to the axial ligand His-105 of the beta1-subunit. Removal of this heme moiety as well as its oxidation abolishes any NO-induced enzyme activation. To differentiate between sGC-dependent and sGC-independent functions of NO, and to differentiate between heme-dependent and heme-independent functions of sGC, we generated heme-deficient sGCbeta1^{H105F} knockin (KI) mice, in which sGC retains its basal activity, but can no longer be activated by NO.

Methods

sGCbeta1^{H105F} knockin mice were generated using a classical approach by which the sGCbeta1 allele was replaced with a mutated allele by means of homologous recombination. As such, the codon for the His-105 residue of the sGCbeta1-subunit was replaced by a codon for Phe. Initially, mice were phenotyped on a mixed background of 129S6xC57Bl/6J. Non-invasive basal systolic blood pressure (SBP) and heart rate (HR) measurements were performed in male and female wild-type (WT) and sGCbeta1^{H105F} knockin mice with a tail-cuff pressure-recording device (Visitech BP-2000/Hatteras MC4000) and with telemetry in free living animals (DSI).

Results

sGCbeta1^{H105F} knockin mice showed a reduced life span, gastro-intestinal tract abnormalities and growth retardation. Basal SBP was higher in sGCbeta1^{H105F} knockin mice than in WT mice, while HR was lower in sGCbeta1^{H105F} knockin compared to their WT littermates. Moreover the blood pressure response to NO-donors (DETA-NO, SNP) and L-NAME was abolished, while the action of the heme-independent sGC activator Bay 58-2667 was preserved. Relaxation of precontracted aortic rings with NO-donors was completely impaired and in addition the concentration-response curve after the heme-dependent stimulator Bay 41-22172 strongly shifted to the right in KIKI aorta's vs. WT (IC50 830 nM vs 34 nM). In contrary the curve after Bay 58-2667 shifted to the left (IC50 0.26 nM vs 1.23 nM). This corroborates the hypothesis that Bay 58-2667 preferably activates the heme-free form of sGC both *in vitro* and *in vivo*.

Conclusion

The NO-activated state of sGC is necessary for the normal function of a number of important physiological proc-

esses in the body, such as the control of blood pressure and heart rate, normal gastro-intestinal tract function and development, and normal growth and viability.