Downregulation of vascular smooth muscle GRK2 expression promotes functional and biochemical alterations in a mouse model of hypertension

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Purpose: G protein-coupled receptors (GPCRs) are signaling proteins that regulate a variety of cardiovascular functions including vascular tone. GPCR kinase 2 (GRK2) modulates multiple cellular responses through GPCR desensitization and downregulation and alterations in GRK2 expression and activity have been associated with hypertension. To define the mechanism mediating the vascular effects of GRK2, we have created a transgenic mouse strain of decreased GRK2 expression and its vascular profile was assessed at functional and cellular levels.

Methods: To derive a line of viable mouse model, small hairpin interfering RNA (shRNA) involving a U6 mouse polymerase III promoter as a transgene was used to universally knockdown, but not knock-out the GRK2 protein expression in vivo. The cardiovascular function of shGRK2 transgenic mice was evaluated by means of CODA tail-cuff blood pressure monitoring system. To measure alterations at the cellular level, vascular smooth muscle cells (VSMCs) derived from GRK2 knockdown and age-matched C57Bl/6 mice were isolated from murine aortas by enzymatic digestion, maintained in primary cultures and then subjected to biochemical and functional assays.

Results: We observed that shGRK2 transgenic mice became spontaneously hypertensive at three months of age when compared to wild-type controls (150/120 ± 6/7 vs. 103/73 ± 5/4 mmHg, p < 0.05). Moreover, blood pressure and myogenic tone in response to phenylephrine were found exaggerated in three-month old shGRK2 transgenic mice. Therefore, we hypothesized that the reduced GRK2 expression in VSMCs may lead to a selective sensitization of GPCR/Gαq/11 protein-mediated signal transduction that underlies the hypertensive phenotype of shGRK2 transgenic mice. Since Ca2+ mobilization is a downstream effector of Gαq/11 signaling, intracellular Ca2+ was measured by Fura-2 fluorescent imaging analysis in response to different agonists and a four-fold increase was noticed (n = 12, p < 0.001). To analyze the functional responses of shGRK2-VSMCs, a transwell migration assay was performed and a two-fold increase in the number of migrated cells was observed (n = 3, p < 0.005). Furthermore, a two-fold increase in the proliferation rate of shGRK2-VSMCs (BrdU uptake) was noticed (n = 4, p < 0.003). We also observed a predominant activation in ERK1/2 phosphorylation for Gαq/11-mediated signaling in shGRK2-VSMCs.

Conclusion: Our data suggest that Gαq/11-mediated pathway is impaired in shGRK2-VSMCs and thus constitutes a mechanism that is potentially responsible for the hypertensive phenotype of shGRK2 transgenic mice.