GRK2 regulates the endothelial responsiveness to bradykinin: role in human angioedema

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Background: Bradykinin (BK) regulates vascular homeostasis through the endothelial Gq protein coupled receptors (B1-B2), using Ca2+ as second messenger. Several kinases are involved in the regulation of BK signaling, such as CamKII; also GRK2 could be involved as it is able to phosphorylate BK receptors but with unknown biological effects. Purpose: to verify the role of GRK2 in regulation of BK signaling in physiological and pathological conditions. Methods: We used Bovine Aortic Endothelial cells (BAEC) for in vitro study where we determined GRK2 modulation by western blot and ubiquitination test, Ca2+ release and Nitric Oxide (NO) production using probes based techniques, and cell permeability through a vascular permeability assay. In mice with endothelial GRK2 Knock out (Tie2-CRE/GRK2fl+/fl-) we performed a Miles assay to test vascular permeability. In PBMCs from patients with ACE-inhibitor-related Angioedema (ACEi-A) and C1- deficit related Angioedema (C1-inh-HA) we evaluated GRK2 levels by western blot analysis. Results: At 5min, BAEC stimulation with BK(100nM) induced an increase of GRK2, which reverberates in all cellular compartments returning to baseline levels at 15min. BK induced-GRK2 accumulation is proteasome dependent, since GRK2 ubiquitination was significantly reduced post BK stimulation and the interaction between GRK2 and E3 ligase mdm2, decreased. We hypothesized that CamKII activated upon BK stimulation, can regulate GRK2 accumulation. Indeed, GRK2 and CamKII interaction increased in response to BK and the accumulation of GRK2 does not occur after CamKII inhibition (C17) supporting the involvement of the Kinase in GRK2 recruitment. Ca2+cytosolic accumulation induced by BK was sensitive to GRK2 activity, as it was enhanced by inhibition of the kinase with KRXC7. Accordingly, permeabilization and NO induced vasodilation, typically endothelial responses to BK, were enhanced when GRK2 was inhibited. To test in vivo the involvement of GRK2 in the regulation of BK-dependent endothelial responses we evaluated BK-induced vascular permeability in Tie2-CRE/GRK2fl+/fl- mice. Interestingly, these mice showed an increased vascular permeability already in basal condition and an increased response to BK respect to wt mice. Since GRK2 regulates the sensitivity of endothelium to BK, we speculated that GRK2 could have a role in BK-mediated Human Angioedema. We evaluated GRK2 levels in ACEi-A and C1inh-HA patients. Interestingly, we evidenced that in both populations, patients with reduced GRK2 levels showed a more severe phenotype of Angioedema. Conclusions: Through CamKII, BK is able to activate GRK2 which in turn inhibits BK signalling; indeed, in vitro end in vivo results evidenced GRK2 as endogenous inhibitor of BK signalling. Consistently, patients with severe Angioedema have reduced levels of GRK2, suggesting that GRK2 can contribute to BK-dependent pathological response of endothelium during Angioedema