Enhanced expression of GRK2 and arrestin proteins and altered contractile signalling in arterial smooth muscle from spontaneously hypertensive rats

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G protein-coupled receptor (GPCR) signalling is regulated by G protein-coupled receptor kinases (GRKs) and arrestin proteins, leading to receptor/G-protein uncoupling, receptor internalization, and G protein-independent signalling. $P2Y_2$ and ET_A receptor desensitization in arterial smooth muscle cells (SMCs) involves GRK2 and distinct arrestins (Morris et al., 2010; 2011). Dysregulation of vasoconstrictor signalling pathways has been implicated in the development of vascular hypertrophy and hypertension. Indeed, GRK2 levels are known to be elevated in hypertensive arterial smooth muscle (Gros et al., 2000), but it is not known whether arrestin levels are altered under similar circumstances. Here we have used mesenteric arteries from 12 week-old, male spontaneously hypertensive (SHR) and control Wistar-Kyoto (WKY) rats to examine contractile responses to vasoconstrictor stimuli using wire myography and primary cell cultures from aortic and mesenteric arteries to assess $[Ca^{2+}]_i$ responses to vasoconstrictor stimulation. GRK2 levels in SHRs were significantly elevated in both aorta (1.7-fold relative to WKY expression; n=3, $p<0.05$, one-way ANOVA, Bonferroni's *post-hoc* test) and mesenteric arteries (2.4-fold relative to WKY expression; n=3; $p<0.05$). In contrast, GRK3 levels were not significantly altered in either tissue. In addition, both arrestin2 (1.9-fold; n=3, $p<0.01$) and arrestin3 (2.9-fold, n=3, $p<0.01$) were significantly elevated in SHR mesenteric arteries, relative to those from WKY rats. In aorta isolated from SHRs, arrestin2 (1.7-fold, n=3, $p<0.05$), but not arrestin3 was significantly elevated. This profile of expression was also maintained in cultured SMCs. In myography experiments, contractile responses to U46619 (500 nM) and high extracellular K^+ (60 mM) were similar in mesenteric arteries isolated from SHR and WKY rats. However, responses to UTP (100 µM), endothelin-1 (ET1, 3 nM) and angiotensin II (ATII, 100 nM) were all significantly attenuated in SHR vessels, relative to WKY arteries ($n\geq5$; $p<0.05$ (UTP, ATII), $p<0.01$ (ET1); Student's *t-test*). $[Ca^{2+}]_i$ responses to ET1 (50 nM), ATII (100 nM) and the thromboxane A_2 agonist U46619 (500 nM) were all significantly attenuated in mesenteric SMCs (MSMCs) derived from SHR relative to WKY rats ($n\geq22$; $p<0.05$ (U46619), $p<0.01$ (ATII), $p<0.001$ (ET1); Student's *t-test*). In contrast, responses to UTP were significantly elevated across a range of concentrations (1–100 µM; $p<0.001$; two-way ANOVA, Bonferroni's *post-hoc* test) in SHR compared to WKY MSMCs. A similar profile was observed in cultured aortic SMCs. In conclusion, we have demonstrated that expression of GRK2 and arrestin2 is enhanced in the arterial smooth muscle of SHRs and that vasoconstrictor signalling/contraction is significantly altered in the SHR vasculature.

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