Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol14Issue2abst017P.pdf

A potential novel mechanism by which G protein-coupled receptor kinases regulate vascular β-adrenoceptor-stimulated adenylyl cyclase activity

Vascular smooth muscle (VSM) relaxation is a fundamental process to prevent excessive vasoconstrictor-stimulated vessel contraction and hypertension. Activation of G protein-coupled receptors (GPCR), such as β-adrenoceptors (βAR), stimulates adenylyl cyclase (AC) to synthesize cAMP and plays a crucial role in mediating VSM relaxation. GPCR signalling often is regulated by G protein-coupled receptor kinases (GRKs) and previous evidence from model cell systems indicates that GRK2 is the key negative regulator of βAR function[1]. Hypertension is linked to increased VSM GRK2 expression, suggesting that this GRK isoenzyme might enhance βAR desensitization and exacerbate the hypertensive phenotype. However, no studies have directly examined whether GRK2, or indeed any other GRK isoenzyme, is able to desensitize endogenous βARs in VSM cells. Here, we have utilized RNAi techniques specifically to deplete individual GRK isoenzymes to identify their roles in regulating βAR signalling and desensitization in adult male Wistar rat aortic smooth muscle cells (ASMC).

ASMC were transfected with siRNAs targeting GRKs 2, 5 or 6 or a negative-control (NC) siRNA. After 48 h ASMC were stimulated with the βAR agonist isoprenaline (ISO, 1 μM, 15 min) or vehicle to act as a desensitizing stimulus. Next, ASMC were washed 3 times with Krebs buffer containing the non-selective phosphodiesterase 3-isobutyl-1-methylxanthine (IMBX; 300 μM) and re-stimulated with ISO (1 μM, 10 min) in the presence of IBMX. Following acid extraction, samples were processed and cAMP concentrations determined as previously described[2]. βAR desensitization was determined as a percentage response of ISO pre-treated cells, when compared to vehicle pre-treated cells.

Transfection with siRNAs decreased expression of the targeted GRK by ≥80% when compared to NC transfected cells. In NC transfected cells, ISO (1 μM) caused a 12 fold increase in cAMP (basal 26 ± 6; ISO-treated 315 ± 37 pmol/mg protein; mean ± SEM, n=6). In GRK2, GRK5 or GRK6 depleted cells the basal level of cAMP production was similar to that in NC-treated cells. However, in ASMCs depleted of GRK2 or GRK5, but not GRK6, ISO-stimulated cAMP accumulation was significantly (p<0.01 two-way ANOVA; Tukey's *post hoc* test) increased (GRK2 knockdown, 465 ± 27; GRK5 knockdown, 524 ± 27 compared to NC, 315 ± 37 pmol/mg protein; n=6). Following ISO (1 μM; 15 min) pre-treatment cAMP accumulation was significantly (p<0.01 one-way ANOVA; Sidak's *post hoc* test) reduced in NC siRNA treated cells (vehicle pre-treatment, 315 ± 37; ISO pre-treatment, 179 ± 23 pmol/mg protein; n=6), being equivalent to 55% βAR desensitization. The extent of βAR desensitization was unaffected following GRK2 (53 ± 2%; n=6), GRK5 (56 ± 6%; n=6) or GRK6 (54 ± 3%; n=6) knockdown.

These data suggest that knockdown of GRK2, GRK5 or GRK6 in ASMC fails to prevent agonist-stimulated βAR desensitization, at least when observed at the level of cAMP accumulation. Both GRK2 and GRK5 appear to exert a tonic inhibitory effect on acute ISO-stimulated AC activity, indicating a potentially novel mechanism by which GRKs can regulate G_s-coupled GPCR activity and vessel relaxation.

We gratefully acknowledge the support of the British Heart Foundation (Grant No. PG11/60/29007) for funding this work.

1. Fredericks Z.L. (1996) *J Biol Chem* **271**:13796-803.
2. Brown B.L. (1971) *Biochem J* **121**:561-562.