Effect of forskolin on contractile responses to the $P2Y_{14}$ receptor agonists UDP-glucose and MRS2690 in porcine isolated coronary arteries

Z Abbas, W Dunn, V Ralevic. University of Nottingham, Nottingham, UK

The $P2Y_{14}$ receptor is the most recently discovered member of the P2Y receptor family. It is activated by UDP-glucose and, more potently, by MRS2690 (diphosphoric acid 1-α-D-glucopyranosyl ester 2-[(4'-methylthio)uridin-5"-yl] ester) (Abbracchio et al., 2003; Ko et al., 2007). The $P2Y_{14}$ receptor is Gi coupled and has been shown to inhibit forskolin-stimulated cAMP accumulation in neuroblastoma, astrocytoma and immune cells (Scrivens and Dickenson 2005a, 2005b, 2006). Our preliminary data have shown that UDP-glucose and MRS260 mediate contraction of porcine coronary arteries (PCA) suggesting a possible involvement of the $P2Y_{14}$ receptor (Abbas et al., 2011). The present study sought to investigate responses to UDP-glucose and MRS2690 after forskolin treatment of PCA and also to investigate the effects of suramin and PPADS, non-selective P2 receptor antagonists.

Pig hearts were obtained from breeds of the modern hybrid pig (either sex), on ice from a local abattoir. Segments of PCA were mounted for isometric tension recording in warmed, oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit buffer (Rayment et al., 2007). Tissue viability was assessed by eliciting contractions with 60mM KCl. Arteries were pre-constricted with U46619, a thromboxane A_2 mimetic to 35-75% KCl contraction. Forskolin (0.1-3 µM) was added to some arteries, which, through vasorelaxation, reversed the U46619-induced contraction; in control arteries U46619 was washed out with buffer. Cumulative concentration response curves to UDP-glucose (0.1-1000 µM) and MRS2690 (0.001-10 µM) were constructed. In separate experiments, suramin (100 µM) and PPADS (10 µM) were added at least 30 min prior to UDP-glucose or MRS2690 addition. Data were analysed by two-way ANOVA with Bonferroni post test. $P<0.05$ was taken as significant.

UDP-glucose and MRS2690 caused concentration-dependent contraction in PCA which was significantly greater in the presence of forskolin and U46619 than in their absence ($P<0.0001$) (eg 1mM UDP-glucose responses were: control, 0.37 ± 0.09 g; with forskolin 1.64 ± 0.23 g (n = 6); at 10 µM MRS2690 responses were: control 0.15 ± 0.07 g; with forskolin 1.71 ± 0.16 g (n=5-6). In the presence of forskolin and U46619, suramin attenuated UDP-glucose responses ($P<0.001$) (eg. at 1 mM UDP-glucose responses were: control, 2.91 ± 0.45 g; with suramin, 2.01 ± 0.29 g (n=7-8)). In the presence of forskolin and U46619, PPADS augmented UDP-glucose responses ($P<0.0001$) (eg. at 1 mM UDP-glucose responses were: control 2.01 ± 0.16 g; with PPADS, 2.39 ± 0.22 g (n=6).

In conclusion, forskolin augments contractile response to UDP-glucose and MRS2690 in the PCA, consistent with an action of the $P2Y_{14}$ agonists through Gi protein coupling and inhibition of cAMP; this response was partly sensitive to suramin but, perhaps surprisingly, augmented by PPADS.

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