Exogenous Lysophosphatidolinositol Exacerbates Myocardial Tissue Injury Via A GPR55 Dependent Mechanism

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Myocardial ischaemia/reperfusion induced Ca^{2+} overload promotes cardiomyocyte death and myocardial tissue injury.[1] Data has demonstrated that lysophosphatidolinositol (LPI), the endogenous ligand of G protein coupled receptor 55 (GPR55) can increase intracellular Ca^{2+} levels[2], which may contribute to apoptosis. Therefore LPI, through an action at GPR55, may contribute to myocardial ischaemia/reperfusion injury. To examine this, 9-12 week old male/female wild-type (WT) and heterozygous GPR55 knockout ($GPR55^{+/-}$) mice (18-32g) were anaesthetized with ketamine and xylazine (120mg kg^{-1} and 16mg kg^{-1}, respectively, i.p.). Hearts were mounted onto Langendorff apparatus and perfused with Kreb's Henseleit buffer (pH7.4; 37°C; 2-2.5ml min^{-1}). A 15 minute stabilisation period preceded 30 minutes of no-flow global ischaemia (GI) and reperfusion, respectively. Hearts were treated with a bolus of vehicle (0.1% DMSO) or LPI (10µM) prior to GI or during early reperfusion. Heart sections were stained with 1% 2,3,5-Triphenyltetrazolium chloride and infarct size measured (ImageJ). In a separate study, the effect of LPI on $[Ca^{2+}]_i$ release from isolated cardiomyocytes from both WT and $GPR55^{+/-}$ mice was investigated. Male/female (10-12 week old) WT and $GPR55^{+/-}$ mice (20-25g) were anaesthetised with pentobarbital sodium (120mg kg^{-1}, i.p.). Hearts were removed and cardiomyocytes isolated via enzymatic digestion. Cardiomyocytes were subsequently incubated with the Ca^{2+} indicator Fluo-4AM (5µM) and either KCl (80mM) or LPI (5 µM and 10µM) applied directly onto cells via a picospritzer and any changes in fluorescence recorded (ImageJ). Data are expressed as mean ± S.E.M. and were compared using a one-way ANOVA with a Bonferroni post-hoc test or a Student's t-test. In vehicle treated hearts, infarct size was comparable between the WT (n=14) and $GPR55^{+/-}$ (n=3) groups (33.9±2.9% & 42.6±1.9%, respectively). In the WT group, LPI administration pre-ischaemia (n=14) increased infarct size (48±4.7 vs. 33.9±2.9%; $P<0.05$), an effect which did not occur if LPI was administered post-ischaemia (n=9). Moreover, neither pre- (n=3) nor post-ischaemic (n=3) LPI administration to $GPR55^{+/-}$ hearts significantly altered infarct size compared to controls. To investigate signalling mechanisms involved in the detrimental effect of LPI, the ROCK inhibitor, Y-27632 dihydrochloride (10 and 50µM; n=6 and 7, respectively) was administered to WT hearts. Treatment with either concentration of inhibitor, prior to LPI treatment, attenuated LPI's deleterious effect. Finally, LPI (10µM) induced comparable increases in intracellular Ca^{2+} release in cardiomyocytes from both WT (n=4) and $GPR55^{+/-}$ (n=4) mice. In conclusion, administration of exogenous LPI exacerbated myocardial tissue injury via a GPR55-ROCK dependent mechanism. As this only occurred when LPI was administered pre-ischaemia it may suggest that activation of GPR55 during ischaemia is necessary for this increase in myocardial tissue

injury. Furthermore, as LPI induced comparable changes in intracellular Ca^{2+} in cardiomyocytes from both WT and $GPR55^{+/-}$ mice, this may suggest that GPR55 mediated Ca^{2+} overload is unlikely to explain the detrimental effect of LPI.

1. Hausenloy *et al.,* (2009) *Basic Res Cardiol.* 104:189-202
2. Oka *et al.,* (2007) *Biochem Biophys Res Commun.* 362:928-934